Diterpenoid Alkaloids from the Aerial Parts of *Aconitum flavum* Hand.-Mazz

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Received: 8 March 2021 / Accepted: 31 March 2021 / Published online: 16 April 2021
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Abstract

Sixteen diterpenoid alkaloids (DAs), including six aconitine-type alkaloids (**5** and **9–13**), seven 7,17-*seco*-aconitine-type alkaloids (**1–4**, **6–8**), two napelline-type alkaloids (**14** and **15**) as well as one veatchine-type alkaloid (**16**), were isolated from the aerial parts of *Aconitum flavum* Hand.-Mazz. In which, flavumolines A–D (**1–4**) were four new ones, and flavumoline E (**5**) was reported as natural compound for the first time. Their chemical structures were elucidated by the analysis of extensive spectroscopic data. The inhibitory activities of these isolates on Ca_v3.1 low voltage-gated Ca²⁺ channel, NO production in LPS-activated RAW264.7 cells, five human tumor cell lines, as well as acetylcholinesterase (AChE) were tested.

Keywords *Aconitum flavum* Hand.-Mazz · Diterpenoid alkaloids · Bioactivity

1 Introduction

Aconitum species represent a large genus in the Ranunculaceae family [1]. It is estimated that there are more than 350 species of *Aconitum* all over the world, which are widely distributed in the northern temperate zone, of which 173 species are endemic to mainland China [1]. Previously chemical and pharmacological studies shown that the diterpenoid alkaloids (DAs) were the main pharmacological constituents of this genus [2]. So far, around 1300 natural DAs, categorized into C₂₀-, C₁₉-, and C₁₈- families depending on the number of contiguous carbon atoms, have been reported [3], [4], [5]. Meanwhile, they have been the targets of medicinal chemists for a broad range of confirmed pharmacological properties including analgesic, antiarrhythmic,

anti-inflammatory, hypotensive, neuroprotective and so on [6].

Aconitum flavum Hand.-Mazz, known as a perennial herb, is mainly distributed in Qinghai, Gansu, and other northwest places in China [7]. As one of the most vital Chinese traditional ethnic minority folk medicine, the roots were commonly used for the treatment of traumatic injuries, rheumatic arthritis, and some other inflammations [7]. Previously phytochemical investigations of this plant have resulted in the isolation of more than 20 DAs from the roots, such as aconitine, mesaconitine, and deoxyaconitine, which were not only considered as the predominant toxic components of this folk medicine, but simultaneously, the significant active constituents [8]. In this study, sixteen DAs including four new 7,17-*seco*-aconitine-type DAs (flavumolines A–D, **1–4**), one new natural compound (**5**), together with 11 known analogues (**6–16**) were identified. These compounds could be divided into four different types: six aconitines (**5** and **9–13**), seven 7,17-*seco*-aconitines (**1–4** and **6–8**), two napellines (**14** and **15**) as well as one veatchine-type alkaloid (**16**). All of these isolated compounds were evaluated for their inhibitory effects on Ca_v3.1 low voltage-gated Ca²⁺ channel, NO production in LPS-activated RAW264.7 cells, five human tumor cell lines, and acetylcholinesterase (AChE). Reported herein, the isolation, structural determination, and biological activity of these compounds were thoroughly described.

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2 Results and Discussion

The MeOH extract was subjected to repeated column chromatography to yield four new ones (flavumolines A–D, **1**–**4**), one new natural compound (**5**), together with 11 known analogues 13-hydroxyfranchetine (**6**) [9], vilmosine (**7**) [10], franchetine (**8**) [11], 3-acetylaconitine (**9**) [12], 15-dehydroxydecludine A (**10**) [13], pubescensine (**11**) [14], 3-O-acetyl-20-deethyl-20-formylaconitine (**12**) [15], 14 α -benzoyloxy-*N*-ethyl-3 α ,13 β ,15 α -trihydroxy-1 α ,6 α ,8 β ,16 β ,18-pentamethoxyaconitane (**13**) [16], 15-acetylsongoramine (**14**) [17], dehydrolucidusculine (**15**) [18], and veatchine azomethine (**16**) [19].

Flavumoline A (**1**) was obtained as a white amorphous powder with the molecule formula as C₂₉H₃₅NO₈ determined on the basis of HRESIMS at m/z 548.2259 [M + Na]⁺ (calcd for C₂₉H₃₅NO₈Na, 548.2255). The presence of hydroxyl (3431 cm⁻¹) and carbonyl (1672 cm⁻¹) units was deduced from the IR spectrum. The ¹³C NMR and DEPT spectra displayed 29 carbons signals, which were divided into five methylenes, nine methines, and five quaternary carbons as well as the signals for a benzoyl and three methoxy groups. Detailly, the characterized signals for a franchetine-type C₁₉-DA core could be distinguished as follows: one representative 6,17-*epoxy* unit (consisted of two oxygenated methines at δ_C 83.1, C-17 and δ_C 76.2, C-6), two quaternary carbons at δ_C 48.9 (C-4) and 51.0 (C-11), and three methines at δ_C 46.5 (C-5), 44.3 (C-9), and 47.1 (C-10), together with the typical C-7/C-8 trisubstituted double bond (δ_C 128.0, d, C-7 and δ_C 137.4, s, C-8) [3]. The ¹H NMR spectrum of **1** also verified resonances assignable to two oxygenated methines at δ_H 4.62 (d, J = 4.6 Hz, H-17) and δ_H 4.71 (d, J = 6.1 Hz, H-6), and a singlet at δ_H 5.78 (d, J = 5.8 Hz, H-7). These evidences, conjugated with the fact that a number of DAs have been reported as the major constituents of this genus, suggested that compound **1** was assigned to be a typical franchetine-type C₁₉-DA [20]. This deduction could be further confirmed by the HMBC correlated signals of H-5 with C-4/C-7/C-10/C-11/C-17/C-18/C-19, H-9 with C-12/C-13/C-14, and H-10 with C-1/C-5/C-9/C-11/C-12/C-17. Furthermore, the 6,17-*epoxy* unit was confirmed by the correlations from H-17 to C-6/C-11 and H-6 to C-5/C-7/C-8/C-11/C-17. In addition, the correlations of H-3, H-5, H-17, and H-18 with C-19, combined with correlations from N–H (δ_H 6.29, d, J = 4.7 Hz) to C-4/C-11/C-19/C-17 confirmed the amide moiety between nitrogen atom and C-19 (Fig. 2).

Moreover, the benzoyl group was placed at C-14 according to the HMBC correlation from H-14 (δ_H 5.07, s) to carbonyl (δ_C 166.7, s). Three methoxy units were located at C-1, C-16, and C-18 on the basis of the HMBC

correlated signals from OCH₃-1 (δ_H 3.41, s) to C-1 (δ_C 85.2, d), from OCH₃-16 (δ_H 3.50, s) to C-16 (δ_C 85.8, d), and from OCH₃-18 (δ_H 3.31, s) to C-18 (δ_C 74.1, t), respectively. Besides, a hydroxyl group should be located at C-13 corresponding to the HMBC correlations from OH to C-12/C-13/C-16.

Biogenetically, the configurations of H-5, 9, 10, could be ascribed to β -orientation whereas H-17 to α -orientation, which could be confirmed by ROESY correlations of H-5/9/10/12 β and H-17/12 α , respectively [21]. Then, the α -orientation of the benzoyl group at C-14 was determined on the ROESY correlations between H-14 and H-9. And the α -orientation of the OCH₃-1 was identified by the ROESY correlations between H-1/H-10. In addition, the correlations of H-17/H-12 α /H-16 and 13-OH/H-14 demonstrated the β -orientation of OCH₃-16 and 13-OH (Fig. 3, the dashed lines and solid lines to represent α -orientation and β -orientation, respectively). Therefore, the structure of **1** was determined and named flavumoline A.

The molecular formula of compounds **2** (C₃₀H₃₇NO₈) and **3** (C₃₁H₄₁NO₈) were established on the analysis of HRESIMS and NMR data. The NMR data of **2** showed the presence of six methylenes, eight methines, three quaternary carbons, and a characteristic trisubstituted intra-annular double bond, in addition to a benzoyl, an aldehyde group and three methoxy groups. All of these spectroscopic data suggested that **2** was an analogue of 13-hydroxyfranchetine (**6**) [9]. The main difference was the presence of a *N*-CHO moiety in compound **2** instead of *N*-ethyl group in **6**, which was verified by 1D NMR data and HMBC correlations. The HMBC correlations (Fig. 2) from the singlet for aldehyde group (δ_H 7.95, s) to C-19 and C-17 suggested that the aldehyde group was placed at *N*-atom. On the analysis of ROESY data, the correlations of H-14/H-9, H-16/H-12 α /H-17, H-1/H-10 confirmed the α -orientation of benzoyl group at C-14, β -orientation of methoxy at C-16, and α -orientation of methoxy at C-1, respectively. Compared with **2**, compound **3** possessed a typical *N*-ethyl moiety, which were elucidated by the HMBC correlations from H-21 to C-17, C-19. A hydroxyl located at C-3 (δ_C 71.0, d) confirmed by the ¹H-¹H COSY correlation of H-2/H-3 and HMBC correlations of H-3 with C-2/5/19. Additionally, the ROESY correlations of H-3/H-1/H-5 confirmed α -orientation of 3-OH. Therefore, the structures of flavumoline B (**2**) and flavumoline C (**3**) were confirmed as shown above (Fig. 1).

Flavumoline D (**4**) was a colorless oily liquid with molecule formula of C₃₁H₄₁NO₈ as deduced by analysis of the ion peak at m/z 556.6689 [M + H]⁺ in the HRESIMS (calcd for C₃₁H₄₂NO₈, 556.6702), which indicated 10 degrees of unsaturation. The ¹³C NMR and DEPT data showed six methylenes, nine methines, four quaternary carbons, as well as the signals for a benzoyl group, three methoxy groups, and a typical *N*-ethyl group. Side by side, comparison of

Fig. 1 Structures of compounds 1–16 from *A. flavum* Hand.-Mazz

its NMR data with those of **6**, a known franchetine-type DA isolated previously, indicated that the structure of **4** was similar with that of **6** [9], except that the C-7/C-8 double bond was replaced by 7,8-*epoxy* unit (δ_{C} 64.0, C-7 and δ_{C} 59.0, C-8). The HMBC correlations of H-5/H-15 with C-7 and H-7/H-9/H-10/H-15 with C-8 confirmed the 7,8-*epoxy* unit. In addition, the remained three methoxy groups, one hydroxyl, and one benzoyl were elucidated to be attached on C-1, C-16, C-18, C-13, and C-14, respectively, as deduced by the HMBC correlations of OCH_3 -1 (δ_{H} 3.35, s) with C-1 (δ_{C} 86.0, d), OCH_3 -16 (δ_{H} 3.42, s) with C-16 (δ_{C} 82.6, d), OCH_3 -18 (δ_{H} 3.30, s) with C-18 (δ_{C} 79.0, t), hydroxyl group (δ_{H} 3.92, s) with C-12/C-13/C-16, and H-14 (δ_{H} 5.11, d,

$J = 3.0$ Hz) with the carbonyl (δ_{C} 166.7, s) of the benzoyl group (Fig. 2).

The relative configuration of **4** was confirmed by the analysis of ROESY spectrum. At first, the correlation from H-14 to H-9 implied the benzoyl group at C-14 was α -oriented. The configurations of methoxyl groups were confirmed as $1\alpha\text{-OCH}_3$ and $16\beta\text{-OCH}_3$ on account of the correlations of H-1/H-10 and H-16/H-12 α /H-17. Then, the remained hydroxyl group at C-13 was established as β -oriented according to its correlation with 16-OCH_3 . On the basis of correlations of H-7/H-15 α /H-16, the configuration of 7,8-*epoxy* unit was identified as β -orientation (Fig. 3). Hence, the structure of **4** was established as above.

Fig. 2 Key ^1H - ^1H COSY and HMBC correlations of compounds **1**–**5**

Fig. 3 Key ROESY correlations of compounds **1**–**5**

Flavumoline E (**5**), was isolated as white amorphous powder. Its molecular formula was established as $\text{C}_{33}\text{H}_{41}\text{NO}_{10}$ on the basis of HRESIMS at m/z 612.2799 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{33}\text{H}_{42}\text{NO}_{10}$, 612.2803), in combination with

NMR spectroscopic data. The IR spectrum of **5** showed absorptions due to the hydroxyl (3487 cm^{-1}) and carbonyl (1676 cm^{-1}) functionalities. The ^{13}C NMR and DEPT spectra of **5** displayed 33 carbon resonances including five

quaternary carbons (one carbonyl at δ_C 200.5, s, C-3, two oxygenated carbons at δ_C 92.1, C-8 and δ_C 74.3, C-13), eleven methines, three methylenes, and 14 other signals attributable to a benzoyl, a typical *N*-ethyl group, an acetoxy, and three methoxy groups. By carefully analyzing the characteristic resonances of a *N*-ethyl group at δ_C 49.2, t (C-21) and δ_C 13.2, q (C-22), a nitrogen-bearing methine and methylene at δ_C 60.8, d (C-17) and δ_C 51.0, t (C-19), an α,β -unsaturated ketone moiety at δ_H 6.27, 6.46 (each 1H, d, J = 10.3 Hz) together with the C-atom signals at δ_C 132.3, 147.3, and 200.5 were clearly observed. The 1H NMR spectrum also verified resonances assignable to a nitrogen-bearing methine and methylene at δ_H 2.76 (s, H-17), δ_H 2.62 (d, J = 11.4 Hz, H-19a) and 2.38 (overlap, H-19b), as well as a representative *N*-ethyl group at δ_H 2.39, 2.69 (m, H-21) and δ_H 1.00 (t, J = 7.1 Hz, H-22). These evidences suggested that **5** should be a typical aconitine-type C_{19} -DA [22]. This deduction could be further confirmed by the HMBC correlations of H-17 with C-5/C-6/C-8/C-10/C-11/C-19, H-19 with C-3/C-4/C-5/C-17/C-18/C-21, H-5 with C-4/C-7/C-10/C-11/C-17/C-18/C-19, H-9 with C-8/C-12/C-13/C-14/C-15, H-10 with C-8/C-9/C-12/C-13/C-17, together with the connection of C-17, C-19, and C-21 to the same nitrogen atom evidenced by the HMBC correlations from H-19 to C-17 and C-21, from H-21 to C-17 and C-19 (Fig. 2).

According to the HMBC spectrum, the correlations between one OH group (δ_H 4.01, s) and C-12/C-13/C-16, another OH group (δ_H 4.47, d, J = 3.0 Hz) and C-13/C-15/C-16, OAc (δ_H 1.45, s) and C-8, H-14 (δ_H 4.98, d, J = 5.0 Hz) and carbonyl of benzoyl group, identified the location of these substituent groups at C-13, C-15, C-8, and C-14, respectively. Additionally, the substitutions of 6-OCH₃, 16-OCH₃, and 18-OCH₃ were identified by the correlations from OCH₃-6 to C-6, OCH₃-16 to C-16, OCH₃-18 to C-18. The HMBC correlated resonances between H-1 and C-10/C-17, H-2 and C-4/C-11, H-18a,b/H-19a,b and C-3, demonstrated that the C = C bond was located between C-1 and C-2 and the carbonyl group at C-3 (200.5, s).

Biosynthetically, the configurations of H-5, 9, 10, 8-OAc, 13-OH could be ascribed to β -orientation and H-17 to α -orientation, respectively, due to the caged core of aconitine-type C_{19} -DA [23]. Then, the configurations of two methoxy groups and one benzoyl at C-6, 16, and 14 were confirmed as α , β , and α , respectively, according to the correlations between H-6/H-5, H-16/H-12 α /H-17 and H-14/H-9 (Fig. 3). However, the configuration of 15-OH has not been confirmed yet because of the simultaneous correlations of H-15 with H-7/H-16/16-OCH₃ and 15-OH with H-7/H-16/16-OCH₃. Eventually, the structure of **5** was identified as shown in Fig. 1, which was reported as a natural compound for the first time according to the literature on synthesis [24].

Since diterpenoid alkaloids in *Aconitum* were reported to commonly treat traumatic injury, arrhythmia, and

rheumatism, in which ion channels or inflammation were involved in the pathophysiological process and inhibitors of ion channels or NO release were considered as potential agents for the treatment of these diseases [25], these isolated compounds were evaluated for their inhibitory effects on T-type ion channels using the whole-cell recording patch clamp method, NO production in LPS-activated RAW264.7 cells using Griess assay [26], on five human tumor cell lines [27], as well as acetylcholinesterase (AChE) [28]. As a result, compound **8** (30 μ M) exhibited 64.5% inhibitory rate on Ca_v3.1 low voltage-gated Ca²⁺ channel. Compounds **3**, **4**, **5**, **6**, **7**, and **11** showed potential inhibitory effects on NO production ranging from 20% to 32% at 50 μ M. Additionally, compound **5** showed potential inhibitory effects on four human tumor cell lines HL-60, A-549, SMMC-7721, MCF-7 with IC₅₀ value as 16.88, 33.11, 23.97, 24.21 μ M in vitro, and no compound showed inhibitory effect on AChE.

3 Experimental

3.1 General Experimental Procedures

Optical rotations were measured on a Jasco P-1020 polarimeter. UV spectra were detected on a Shimadzu UV-2401PC spectrometer. IR spectra were determined on a Bruker FT-IR Tensor-27 infrared spectrophotometer with KBr disks. All 1D and 2D NMR spectra were recorded on Bruker DRX-600 spectrometers using TMS as an internal standard. Unless otherwise specified, chemical shifts (δ) were expressed in ppm with reference to the solvent signals. ESIMS and HRESIMS analysis were carried out on Waters Xevo TQS and Agilent G6230 TOF mass spectrometers, respectively. Semi-preparative HPLC was performed on a Waters 2695 HPLC with a 5C₁₈-MS-II column [4.6 $\times$ 150 mm]. Silica gel (100–200, 200–300 mesh, Qingdao Marine Chemical Co., Ltd., People's Republic of China), and MCI gel (75–150 μ m, Mitsubishi Chemical Corporation, Tokyo, Japan) were used for column chromatography. Fractions were monitored by TLC (GF 254, Qingdao Marine Chemical Co., Ltd.), and spots were visualized under a UV lamp at 254 nm or by spraying the Dragendorff reagent and heating silica gel plates sprayed with 10% H₂SO₄ in EtOH.

3.2 Plant Material

The plants of *A. flavum* were collected in June 2019 from the Sanjiangyuan Nature Reserve of Guoluo, Qinghai Province, People's Republic of China. Plant identity was verified by prof. Li-Xin Wei. A voucher specimen (No. 201901H19) was deposited in the Kunming Institute of Botany.

3.3 Extraction and Isolation

Air-dried and powdered plant material (aerial parts) of *A. flavum* (45 kg) was extracted three times (3×150 L) with MeOH at room temperature and then concentrated to 3.88 kg under reduce pressure. The crude extract was suspended in 1% HCl followed by basification with 10% aqueous NH_4OH (pH 9–10) and subsequently, extracted with ethyl acetate to afford crude alkaloids (808 g). The total alkaloids fraction was separated on a silica gel column ($\text{CHCl}_3/\text{CH}_3\text{OH}$, 100:1–1:1) to yield eight fractions (Fr. 1–8). Then Fr. 1 (35 g) was separated on a silica gel column (PE/EtOAc/DEA, 50:1:1–3:1:1) to yield six sub-fractions (Fr. 1a–1f). Fr. 1a (2.3 g) was separated on a silica gel column (PE/EtOAc, 80:1–3:1) and then semi-preparative HPLC (Waters 2695 C18, i.d. 150×10 mm, $5 \mu\text{m}$, 3.0 ml/min, UV 210 nm) using MeOH/ H_2O (85/15, v/v) as the mobile phase afforded **6** (57 mg), **7** (10 mg) and **8** (23 mg). Fr. 1b (2.7 g) was separated on a silica gel column (PE/EtOAc, 60:1–3:1) followed by preparative HPLC using MeOH/ H_2O (75/25, v/v) to yield **4** (128 mg), **14** (15 mg), **15** (9.7 mg) and **16** (6 mg). Fr. 1c (3 g) was separated on a silica gel column (PE/EtOAc, 50:1–3:1), followed by semi-preparative HPLC using MeOH/ H_2O (70/30, v/v) as the mobile phase to afford **10** (8 mg), **11** (3 mg) and then yield **9** (230 mg) by recrystallization. Fr. 1d (7 g) was separated on a silica gel column (PE/EtOAc, 50:1–1:1) yielded **12** (50 mg) and then on semi-preparative HPLC using MeOH/ H_2O (70/30, v/v) as the mobile phase to afford **13** (10 mg). Fr. 1e (5.5 g) yielded **2** (7.3 mg) and **3** (20.1 mg) by preparative HPLC using MeOH/ H_2O (70/25, v/v) as mobile phase, subsequently, afforded **5** (4.7 mg) and **1** (2 mg) isolated by Sephadex II column using acetone.

3.3.1 Flavumoline A (1)

White powder; $[\alpha]_{\text{D}}^{19} -4.9$ (c 0.175, MeOH); IR (KBr) ν_{max} : 3431, 2975, 2933, 2828, 1718, 1672, 1282, 1099 cm^{-1} ; For ^{13}C NMR data (see Table 1) and ^1H NMR data (see Table 2). ESIMS m/z 526 $[\text{M} + \text{H}]^+$; HRESIMS m/z : 548.2259 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{29}\text{H}_{35}\text{NO}_8\text{Na}$, 548.2255).

3.3.2 Flavumoline B (2)

White powder; $[\alpha]_{\text{D}}^{18} -12.5$ (c 0.100, MeOH); IR (KBr) ν_{max} : 3447, 2938, 2882, 2828, 1717, 1670, 1280, 1099 cm^{-1} ; For ^{13}C NMR data (see Table 1) and ^1H NMR data (see Table 2). ESIMS m/z 540 $[\text{M} + \text{H}]^+$; HRESIMS m/z : 562.2408 $[\text{M} + \text{Na}]^+$ (calcd for $\text{C}_{30}\text{H}_{37}\text{NO}_8\text{Na}$, 562.2411).

Table 1 ^{13}C NMR (150 MHz) spectroscopic data for compounds **1–5** (in CDCl_3)

No	1	2	3	4	5
1	85.2	85.1	83.9	86.0	147.3
2	25.1	23.6	33.0	24.2	132.3
3	31.0	32.3	71.0	32.4	200.5
4	48.9	36.5	42.0	36.9	49.3
5	46.5	46.8	45.2	43.0	48.6
6	76.2	76.5	74.6	76.0	82.5
7	128.0	127.9	128.9	64.0	45.1
8	137.4	137.4	136.0	59.0	92.1
9	44.3	44.0	43.9	44.3	43.3
10	47.1	47.6	46.6	44.4	37.6
11	51.0	51.3	50.3	49.1	51.1
12	38.6	38.8	38.8	38.5	38.2
13	77.3	77.2	77.3	76.8	74.3
14	83.0	83.0	83.4	82.8	79.1
15	39.1	39.0	39.0	36.9	78.9
16	85.8	85.7	85.9	82.6	90.2
17	83.1	86.9	92.1	94.4	60.8
18	74.1	77.6	75.8	79.0	72.0
19	174.9	41.9	45.5	52.2	51.0
21	–	160.7	49.0	49.0	49.2
22	–	–	13.0	13.0	13.2
1-OCH ₃	57.3	57.0	57.1	57.1	–
6-OCH ₃	–	–	–	–	58.5
16-OCH ₃	58.2	58.1	58.1	58.1	61.6
18-OCH ₃	59.5	59.5	59.5	59.5	59.3
-OBz	166.7	166.7	166.7	166.7	166.1
1'	130.1	130.0	130.2	130.0	129.7
2',6'	129.8	129.8	129.8	129.8	129.8
3',5'	128.5	128.5	128.5	128.4	129.0
4'	133.2	133.2	133.1	133.2	133.7
8-OAc	–	–	–	–	172.6
	–	–	–	–	21.6

3.3.3 Flavumoline C (3)

White powder; $[\alpha]_{\text{D}}^{18} -9.7$ (c 0.103, MeOH); IR (KBr) ν_{max} : 3534, 3402, 2974, 2824, 1717, 1280, 1103 cm^{-1} ; For ^{13}C NMR data (see Table 1) and ^1H NMR data (see Table 2). ESIMS m/z 556 $[\text{M} + \text{H}]^+$; HRESIMS m/z : 556.2905 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{31}\text{H}_{42}\text{NO}_8\text{Na}$, 556.2905).

3.3.4 Flavumoline D (4)

Colorless oily liquid; $[\alpha]_{\text{D}}^{19} -3.9$ (c 0.101, MeOH); IR (KBr) ν_{max} : 3437, 2973, 2932, 2826, 1719, 1279, 1101 cm^{-1} ; For ^{13}C NMR data (see Table 1) and ^1H NMR data (see Table 2). ESIMS m/z 556 $[\text{M} + \text{H}]^+$; HRESIMS m/z : 556.6689 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{31}\text{H}_{42}\text{NO}_8$, 556.6702).

Table 2 ^1H NMR (600 MHz) spectroscopic data for compounds **1–5** (in CDCl_3)

No	1 (<i>J</i> in Hz)	2 (<i>J</i> in Hz)	3 (<i>J</i> in Hz)	4 (<i>J</i> in Hz)	5 (<i>J</i> in Hz)
1	3.58 m	3.52 m	3.38 m	3.21 m	6.46 d (10.3)
2	2.20 m 1.74 m	2.05 m 1.52 m	2.52 m 2.22 m	2.38 m 1.93 m	6.27 d (10.3)
3	1.83 m 1.78 m	1.79 m 1.60 m	3.85 dd (4.9 12.3)	1.80 m 1.44 m	–
4	–	–	–	–	–
5	2.68 m	2.47 s	2.19 s	1.82 s	3.11 d (6.4)
6	4.71 d (6.1)	4.69 d (6.0)	4.39 d (6.1)	4.58 d (4.5)	4.11 d (6.4)
7	5.78 d (5.8)	5.77 d (5.76)	5.76 d (5.8)	2.90 d (4.2)	3.04 br s
8	–	–	–	–	–
9	3.28 br s	3.25 br s	3.14 br s	2.70 m	2.98 m
10	2.73 m	2.71 m	2.60 m	2.56 m	2.37 m
11	–	–	–	–	–
12	2.14 m 2.07 m	2.20 m 2.00 m	2.08 m 1.99 m	2.58 overlap 2.21 m	2.44 m 1.74 m
13	–	–	–	–	–
14	5.07 s	5.06 br s	5.01 br s	5.11 d (3.0)	4.98 d (5.0)
15	2.99 m 2.70 m	2.98 m 2.69 overlap	2.92 m 2.62 m	2.78 m 1.68 m	4.54 dd (2.7 5.3)
16	3.35 m	3.37 m	3.31 m	3.46 m	3.31 d (5.4)
17	4.62 d (4.6)	4.96 s	4.35 s	4.49 br s	2.76 s
18	3.68 d (9.7) 3.60 d (9.7)	3.22 d (9.4) 3.10 d (9.4)	3.32 overlap 3.17 d (9.4)	3.09 br s	3.91 d (8.5) 3.65 d (8.5)
19	– –	3.74 d (13.7) 2.66 overlap	2.87 d (11.3) 1.73 d (11.3)	2.52 overlap 2.11 d (11.3)	2.62 d (11.4) 2.38 overlap
21	–	7.95 s	2.57 m 2.38 m	2.65 m 2.48 m	2.69 m 2.39 overlap
22	–	–	1.01 t (7.3)	1.05 t (7.3)	1.00 t (7.1)
1-OCH ₃	3.41 s	3.33 s	3.34 s	3.35 s	–
6-OCH ₃	–	–	–	–	3.22 s
16-OCH ₃	3.50 s	3.51 s	3.44 s	3.42 s	3.77 s
18-OCH ₃	3.31 s	3.28 s	3.28 s	3.30 s	3.27 s
2',6'	8.10 d (7.6)	8.08 d (7.8)	8.05 d (7.8)	8.08 d (7.6)	8.04 d (7.4)
3',5'	7.47 t (7.6)	7.47 t (7.8)	7.43 t (7.8)	7.48 t (7.6)	7.48 t (7.4)
4'	7.59 t (7.6)	7.59 t (7.8)	7.55 t (7.8)	7.58 t (7.6)	7.60 t (7.4)
15-OH	–	–	–	–	4.47 d (3.0)
13-OH	3.95 s	3.93 s	3.89 s	3.92 s	4.01 s
N–H	6.29 d (4.7)	–	–	–	–
CHO	–	7.95 s	–	–	–
8-OAc	–	–	–	–	1.45 s

3.3.5 Flavumoline E (5)

White powder; $[\alpha]_{\text{D}}^{18} + 4.4$ (*c* 0.122, MeOH); IR (KBr) ν_{max} : 3487, 2973, 2936, 1721, 1676, 1280 cm^{-1} ; For ^{13}C NMR data (see Table 1) and ^1H NMR data (see Table 2). ESIMS m/z 612 $[\text{M} + \text{H}]^+$; HRESIMS m/z 612.2799 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{33}\text{H}_{42}\text{NO}_{10}$, 612.2803).

4 Bioassays

4.1 T-Type Ion Channel Inhibitory Activity Assay

All experiments were performed at room temperature ($\sim 22^\circ\text{C}$). Pipettes were fabricated from borosilicate glass

(World Precision Instruments) using a micropipette puller (P-1000, Sutter Instrument), and were fire-polished to resistances of 2–4 M for whole-cell recording. Whole-cell currents were elicited by 150 ms depolarization to –40 mV at 4 s intervals from a holding potential (HP) of –100 mV. Currents were amplified by Axopatch 200B and digitized by Digidata 1440A (Molecular Devices). Currents were low-pass filtered at 2 kHz and sampled at 10 kHz. pCLAMP 10 software (Molecular Devices) was used for data acquisition and analysis. The extracellular solutions contained (in mM) 142 CsCl, 1 MgCl₂, 2 CaCl₂, 10 Glucose and 10 HEPES (pH 7.4 adjusted with CsOH). The intracellular solutions contained (in mM) 127 Cs-methanesulphonate, 2MgCl₂, 2Na₂ATP, 10 HEPES and 11 EGTA (pH 7.4 adjusted with CsOH). The tested compounds (30 μ M) were added.

4.2 Anti-Inflammatory Activity Assay

The murine macrophage cell line RAW264.7 was obtained from Cell Bank of Chinese Academy of Sciences. RAW264.7 cells were seeded in 96-well cell culture plates (1.5×10^5 cells/well) and treated with serial dilutions of the compounds with a maximum concentration of 50 μ M in triplicate, followed by stimulation with 1 μ g/mL LPS (Sigma) for 18 h. NO production in the supernatant was assessed by Griess reagents (Sigma). The absorbance at 570 nm was measured with a microplate reader (Thermo, Waltham, MA, USA). N^G-Methyl-L-arginine acetate salt (L-NMMA, Sigma) was used as a positive control [29]. The viability of RAW264.7 cells was evaluated by the MTS assay simultaneously to exclude the interference of the cytotoxicity of the test compounds.

4.3 Cytotoxicity Assay

The human tumor cell lines HL-60, SMMC-7721, A-549, MCF-7, and SW-480 were obtained from ATCC (Manassas, VA, USA). Cells were cultured in RPMI-1640 or DMEM medium (Biological Industries, Kibbutz Beit-Haemek, Israel) supplemented with 10% fetal bovine serum (Biological Industries) at 37 °C in a humidified atmosphere with 5% CO₂. The cytotoxicity assay was evaluated by the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) (Promega, Madison, WI, USA) assay [30]. Briefly, cells were seeded into each well of a 96-well cell culture plate. After 12 h of incubation at 37 °C, the test compound (40 μ M) was added. After incubated for 48 h, cells were subjected to the MTS assay. Compounds with a growth inhibition rate of 50% were further evaluated at concentrations of 0.064, 0.32, 1.6, 8, and 40 μ M in triplicate, with cisplatin and paclitaxel (Sigma, St. Louis, MO, USA) as positive controls. The

IC₅₀ value of each compound was calculated with Reed and Muench's method [31].

4.4 AChE Inhibitory Activity Assay

Acetylcholinesterase (AChE) inhibitory activity of the compounds isolated was assayed by the spectrophotometric method developed by Ellman et al. [32] with slightly modification. *S*-Acetylthiocholine iodide, *S*-butyrylthiocholine iodide, 5,5'-dithio-bis-(2-nitrobenzoic) acid (DTNB, Ellman's reagent), acetylcholinesterase derived from human erythrocytes were purchased from Sigma Chemical. Compounds were dissolved in DMSO. The reaction mixture (totally 200 μ L) containing phosphate buffer (pH 8.0), test compound (50 μ M), and acetyl cholinesterase (0.02U/mL), was incubated for 20 min (37 °C). Then, the reaction was initiated by the addition of 40 μ L of solution containing DTNB (0.625 mM) and acetylthiocholine iodide (0.625 mM) for AChE inhibitory activity assay, respectively. The hydrolysis of acetylthiocholine was monitored at 405 nm every 30 s for one hour. Tacrine was used as positive control with final concentration of 0.333 μ M. All the reactions were performed in triplicate. The percentage inhibition was calculated as follows: (%) inhibition = (E–S)/E $\times$ 100 (E is the activity of the enzyme without test compound and S is the activity of enzyme with test compound).

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13659-021-00302-3>.

Acknowledgements The work was financially supported by the Second Tibetan Plateau Scientific Expedition and Research (STEP) program (2019QZKK0502), State Key Laboratory of Phytochemistry and Plant Resources in West China (P2017-KF02 and P2019-ZZ05), and the Natural Sciences Foundation of Yunnan Province (2019FA003).

Declarations

Conflict of interest All authors declare no conflict of interest.

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