Amides, Isoquinoline Alkaloids and Dipeptides from the Aerial Parts of Piper mullesua

Meng-Yuan Xia¹,² · Jun Yang¹,² · Pan-Hua Zhang¹ · Xiao-Nian Li³ · Ji-Feng Luo¹ · Chun-Lin Long⁴,⁵ · Yue-Hu Wang¹,²

Received: 3 June 2018 / Accepted: 3 July 2018 / Published online: 2 August 2018
© The Author(s) 2018

Abstract
One undescribed amide, pipermullesine A, two undescribed isoquinoline alkaloids, pipermullesines B and C, and six undescribed dipeptides, pipermullamides A–F, along with 28 known compounds, were isolated from the aerial parts of Piper mullesua. The structures of the undescribed compounds were elucidated based on the analysis of 1D and 2D NMR and MS data. Furthermore, the structures of pipermullesines A–C were confirmed by single crystal X-ray diffraction analysis. All isolates were evaluated for inhibitory activity against platelet aggregation induced by thrombin (IIa) or platelet-activating factor (PAF). (-)-Mangochinine, pellitorine, and (2E,4E)-N-isobutyl-2,4-dodecadienamide showed weak inhibitory activity against rabbit platelet aggregation induced by PAF, with IC₅₀ values of 470.3 µg/mL, 614.9 µg/mL, and 579.7 µg/mL, respectively.

Graphical Abstract

Meng-Yuan Xia and Jun Yang have contributed equally to this work.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13659-018-0180-z) contains supplementary material, which is available to authorized users.

¹ Key Laboratory of Economic Plants and Biotechnology and the Yunnan Key Laboratory for Wild Plant Resources, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People’s Republic of China
² Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences, Yezin, Nay Pyi Taw 05282, Myanmar
³ State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming 650201, People’s Republic of China
⁴ College of Life and Environmental Sciences, Minzu University of China, Beijing 100081, People’s Republic of China
Keywords Piper mullesua · Piperaceae · Antiplatelet · Amides · Isoquinoline alkaloids

1 Introduction

Traditional Chinese medicines with the functions of promoting blood circulation (“Huoxue” in Chinese) and/or removing blood stasis (“Huayu” in Chinese) are claimed to be useful in antiplatelet therapies and the treatment of thrombotic diseases [1]. For example, antiplatelet compounds have been found in a Huoxue herb Selaginella moellendorffii Hieron. (Selaginellaceae) [2, 3].

The genus Piper (Piperaceae) is a medicinally important group of plants consisting of approximately 2000 species worldwide. There are approximately 60 species distributed in the tropical areas of the People’s Republic of China, of which approximately 30 species have been used as traditional Chinese medicines [4]. Some Piper species are used for promoting blood circulation, while Piper mullesua Buch.-Ham. ex D. Don and P. yunnanense Tseng are used for removing blood stasis [5]. As a folk medicine in China with the Chinese name of Duan-Jv (短蒟), the whole plants of P. mullesua are also used to treat bleeding, bone fractures, injuries from falls, rheumatoid arthritis, rheumatic arthralgia, acroanesthesia, asthma, colds, stomach aches, abdominal pain, toothaches, swelling and pain of furuncles, dysmenorrhea, menoxenia, empyrosis, and snake and insect bites [5, 6].

Alcoholic extracts of P. mullesua showed the activity against rabbit platelet aggregation induced by 7.2 nM of the platelet-activating factor (PAF) with an IC50 value of 64.43 μg/mL [7]. Amides including retrofractamide A, chingchenganamide A [6], N-isobutyl-16-phenylhexadeca-2,4E,6E-dienamide, and N-isobutyldeca-2E,4E,6E-dienamide [8], lignans including (-)-nectandrin A, nectandrin B, galgravin [6], asarinin, fargesin, and sesamin with antifeedant activity [8, 9], a phenylpropanoid myristicin with insecticidal activity [9, 10], and several arylalkenyl carboxylic acid esters [10, 11] have been isolated from the plants. However, the active constituents of P. mullesua responsible for the antiplatelet aggregation remain unclear. In continuing efforts to search for antiplatelet compounds from Piper plants [12, 13], we herein present the results of the analysis of compounds from the aerial parts of P. mullesua and the bioactivity of these compounds.

2 Results and Discussion

2.1 Structure Elucidation

Nine undescribed compounds (1–9, Fig. 1) and 28 known ones (10–37) were isolated from the methanolic extracts of P. mullesua by silica gel, D101 resin and Sephadex LH-20 column chromatography and semipreparative HPLC. Pipermullesine A (1) had the molecular formula C15H13NO4 based on 13C NMR (Table 1) and HREIMS data. Its IR spectrum showed absorption peaks for a tertiary amide at 1643 cm−1 and a phenyl ring at 1595, 1513, and 1461 cm−1. The 1H NMR data (Table 1) indicated a 1,2,4-trisubstituted phenyl ring [δH 7.12 (1H, dd, J=8.3, 1.8 Hz), 7.01 (1H, d, J=1.8 Hz), and 6.86 (1H, d, J=8.3 Hz)], an E double bond [δH 7.67 (1H, d, J=15.3, 1.8 Hz) and 6.49 (1H, d, J=15.3 Hz)], a 1,4-oxazine ring [δH 6.61 (1H, dd, J=5.1, 1.9 Hz), 6.10 (1H, dd J=5.1, 1.9 Hz), 5.84 (1H, d, J=5.1 Hz), and 5.70 (1H, d, J=5.1 Hz)] [14], and two methoxy groups [δH 3.92 (3H, s) and 3.91 (3H, s)]. The above NMR characteristic signals implied that compound 1 might be a cinnamamide derivative.

According to the 1H–1H COSY and HMBC correlations of compound 1 (Fig. 2), (E)-3,4-dimethoxycinnamamoyl and 1,4-oxazine groups were confirmed. Although the correlations from H-1″ and H-4″ to C-1 were not observed in the HMBC spectrum, the structure of 1 was finally determined as (E)-3-(3,4-dimethoxyphenyl)-1-(4H,1,4-oxazin-4-yl) prop-2-en-1-one by a single-crystal X-ray diffraction analysis (Fig. 3).

The molecular formula of pipermullesine B (2), C14H22N2O3, was determined by 13C NMR data (Table 2) and an HREIMS ion at m/z 290.1620 [M]+ (calced for C14H22N2O3 290.1630) and required 7 indices of hydrogen deficiency. The 1H NMR data (Table 2) indicated a tetrasubstituted phenyl ring [δH 7.15 (1H, s) and 6.52 (1H, s)], one methoxy group [δH 3.85 (3H, s)], and one acetyl group [δH 1.91 (3H, s)]. The 13C NMR data (Table 2) exhibited 15 signals. However, according to its HREIMS data, compound 2 should have 16 carbon atoms. The disappeared signal for C-9 (δC 33.0) was detected by the HMBC correlation (Fig. 2) from H2-11 to C-9.

1H–1H COSY correlations (Fig. 2) exhibited two partial structures comprising C-2 to C-3 and C-10 to C-12. On the basis of the HMBC correlations from H2-3 to C-1 and C-4a, H2-4 to C-5 and C-8a, H-5 to C-7 and C-8a, H-8 to C-1, C-4a, and C-6, and 7-OMe to C-7, a 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline fragment with a substituent group at C-1 was confirmed. The group at C-1 was deduced as 4-acetamidobutyl by the HMBC correlations.
from H2-10 to C-1, H2-11 to C-9 and C-1', and H3-2' to C-1'. Thus, the structure of 2 was determined as 1-(4-acetamidobutyl)-6-hydroxy-3,4-dihydroisoquinoline and given the common name pipermullesine B.

The crystals for pipermullesine B trifluoroacetate (2a) were obtained from methanol. The NMR data of 2a (Table 2) and the result of its single-crystal X-ray diffraction analysis (Fig. 3) further supported the structure elucidation of 2.

Pipermullesine C (3) yielded a molecular formula of C22H30N4O4 with 10 degrees of unsaturation, as deduced by 13C NMR (Table 3) and the HREIMS data. A comparison of the NMR data (Tables 2, 3) of 3 with those of 2 indicated that there were signals for one additional 4-acetamidobutyl group [δC 173.2 (C), 40.1 (CH2), 29.7 (CH2), 28.9 (CH2), 25.2 (CH2), and 22.6 (CH3)] and one more imine (δC 168.8) in 3.

On the basis of 2D NMR correlations (Fig. 2), a 1-(4-acetamidobutyl)-6-hydroxy-3,4-dihydroisoquinoline moiety was determined. One more ring is needed to meet the unsaturation, and the ring was deduced as an oxazole ring attached to C-7 and C-8 by comparison of the NMR data with those of benzoazoles in the literature [15, 16]. The additional 4-acetamidobutyl group was located at C-1 by the HMBC correlation from H-3 to C-2. Thus, the structure of 3 (pipermullesine C) was determined.

Fortunately, the crystals for pipermullesine C trifluoroacetate (3a) were also obtained from methanol. The NMR data of 3a (Table 3) and the result of its single-crystal X-ray diffraction analysis (Fig. 3) confirmed the chemical structure of 3.

The molecular formula of pipermullamide A (4), C19H28N2O3, was determined by 13C NMR data (Table 4) and an HREIMS ion at 320.2102 [M]+ (calcd for
C_{18}H_{28}N_{2}O_{3}, 320.2100), indicating 6 degrees of unsaturation. The 1H NMR data (Table 2) indicated one mono-substituted phenyl ring [$\delta_{H}$ 7.31 (2H, d, $J=7.4$ Hz), 7.25 (2H, dd, $J=7.4, 7.4$ Hz), and 7.17 (1H, dd, $J=7.4, 7.4$ Hz)], three N-methyl groups [$\delta_{H}$ 2.85 (9H, s)], and two methyl groups [$\delta_{H}$ 0.98 (3H, d, $J=5.9$ Hz) and 0.95 (3H, d, $J=6.3$ Hz)]. By comparing the NMR data of 4 with those of phenylalanine and leucine trimethylbetaine [17, 18], compound 4 might comprise the two fragments, which was confirmed through its 1H–1H COSY and HMBC correlations (Fig. 2). The amino of phenylalanine was acylated by the carboxyl group of leucine trimethylbetaine according to the HMBC correlation from H-8′ to C-1.

Natural amino acids generally have an L configuration. Compound 10 from the plant is also a derivative of L-phenylalanine. Accordingly, compound 4 (pipermullamide A) was elucidated as L-(N,N,N-trimethyl)leucyl-L-phenylalanine.

The molecular formulae of pipermullamides B to F (5–9) were determined as C_{18}H_{28}N_{2}O_{3}, C_{17}H_{26}N_{2}O_{3}, C_{20}H_{29}N_{2}O_{3}, C_{20}H_{29}N_{3}O_{3}, and C_{19}H_{27}N_{3}O_{3}, respectively, by 13C NMR data (Tables 4, 5) and HRMS analysis. According to 1H–1H COSY and HMBC correlations (Fig. 2), compounds 5–9 were determined as L-(N,N,N-trimethyl)isoleucyl-L-phenylalanine (pipermullamide B, 5), L-(N,N,N-trimethyl)valyl-L-phenylalanine (pipermullamide C, 6), L-(N,N,N-trimethyl)leucyl-L-tryptophan (pipermullamide D, 7), L-(N,N,N-trimethyl)isoleucyl-L-tryptophan (pipermullamide E, 8), and L-(N,N,N-trimethyl)valyl-L-tryptophan (pipermullamide F, 9), respectively.

The known compounds (+)-phenylalanine betaine (10) [19], (-)-mangochinine (11) [20], xylopine (12) [21], (-)-oblongine (13) [22], pellitorine (14) [23], (2E,4E)-N-isobutyl-2,4-dodecadienamide (15) [24], retrofractamide A (16) [23], guineensine (17) [23], brachystamide B (18) [25], retrofractamide C (19) [26], sarmentine (20) [27], 3-(3,4-dimethoxyphenyl)propanoylpyrrole (21) [28], N-trans-feruloyltyramine (22) [29], (-)-machilusin (23) [30], galgravin (24) [31], (-)-nactadrin A (25) [32], methyl 3-(3,4-dimethoxyphenyl)propanoate (26) [33], piperic acid (27) [34], methyl piperate (28) [34], methyl (2E,4E)-7-(1,3-benzodioxol-5-yl)hepta-2,4-dienoate (29) [35], (-)-bluemol B (30) [36], (-)-T-muurolol (31) [37], trans-phytol (32) [38], $\alpha$-tocopherolquinone (33) [39], $\gamma$-tocopherol (34) [40], stigmaster-4-ene-3,6-dione (35) [41], (22E)-stigmastera-4,22-diene-3,6-dione (36) [42], and (22E)-stigmastera-4,6,8-
(14,22-tetraen-3-one (37) [43] were determined by comparing the NMR data of 10–37 and the optical rotation values of 10–13, 23–25, 30, and 31 with those reported in the literature.

2.2 In Vitro Platelet Aggregation Assay

All isolates (1–37) were evaluated for inhibitory activity against platelet aggregation induced by thrombin (IIa) or PAF. As shown in Tables 6 and 7, compounds 2, 3, 5, 14, 27, 33, and 34 possessed weak inhibitory effects on the aggregation of rabbit platelets induced by thrombin (IIa) (1 U/mL) with inhibition rates from 11.5 to 22.2% at a concentration of 300 µg/mL. Compounds 11, 14, 15, 20, and 25 showed weak inhibitory activity against the rabbit platelet aggregation induced by PAF (0.4 µg/mL) with inhibition rates from 16.8% to 36.4% at a concentration of 300 µg/mL. Compounds 11, 14, 15, 20, and 25 showed weak inhibitory activity against the rabbit platelet aggregation induced by arachidonic acid (IC50 = 53.0 µg/mL) [45], antituberculosis activity (MIC=25 µg/mL) [46], antifungal activity against Cryptococcus neoformans (IC50=7.7 µg/mL) [47], and α-glucosidase-I enzyme inhibitory activity (IC50=34.39 µg/mL) [48]. Although its in vitro activity against platelet aggregation is weak, the amide shows strong in vivo anticoagulant activities at a dose of 4.5 µg/mouse or 9.0 µg/mouse [49]. It is worthwhile to conduct further in vivo antithrombotic studies of pellitorine along with (-)-mangochinine and (2E,4E)-N-isobutyl-2,4-dodecadienamide.

3 Experimental Section

3.1 General Experimental Procedures

The instruments and materials for isolation and identification of compounds from the herb were presented in Supplementary Material.
3.2 Plant Material

The aerial parts of *Piper mullesua* Buch.-Ham. ex D. Don (Piperaceae) were collected from Mengyuan Village (E101°22′01″, N21°45′22″), Guanlei Town, Mengla County, Xishuangbanna of Yunnan Province, People’s Republic of China, in July 2014, and identified by one of the authors (C.-L.L.). A voucher specimen (No. 201401) was deposited at the Key Laboratory of Economic Plants and Biotechnology, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and Isolation

The air-dried, powdered *P. mullesua* plant (1.8 kg) was exhaustively extracted with MeOH (4×10 L) at room temperature. The MeOH extracts (92.5 g) were suspended in H2O and further partitioned with petroleum ether and CHCl3. The petroleum ether-soluble part (31.2 g) and CHCl3-soluble part (4.8 g) were combined (36.0 g, part B) according to the testing results of thin-layer chromatography. The water phase was partitioned by D101 resin column chromatography to obtain the water-eluted part (discarded) and 95% EtOH-eluted part (7.2 g, part A).

### Table 2

| No. | ω1 (J in Hz)a | ω1, typeb | ω1 (J in Hz)c | ω1, typeb |
|-----|---------------|-----------|---------------|-----------|
| 1   | 174.4, C      | 179.1, C  |               |           |
| 3   | 3.63, t (7.7) | 42.4, CH2 | 3.77, t (7.9) | 42.3, CH2 |
| 4   | 2.87, t (7.7) | 27.1, CH2 | 3.01, t (7.9) | 26.0, CH2 |
| 4a  | 137.6, C      | 136.4, C  |               |           |
| 5   | 6.52, s       | 118.2, CH | 6.85, s       | 116.7, CH |
| 6   | 167.6, C      | 158.3, C  |               |           |
| 7   | 151.1, C      |           | 149.2, C      |           |
| 8   | 7.15, s       | 112.2, CH | 7.42, s       | 114.1, CH |
| 8a  | 112.3, C      |           | 116.9, C      |           |
| 9   | Disappeared   | 33.0c     | Disappeared   | 33.1c     |
| 10  | 1.72, m       | 26.9, CH2 | 1.73, m       | 26.3, CH2 |
| 11  | 1.60, m       | 29.9, CH2 | 1.61, m       | 29.9, CH2 |
| 12  | 3.20, t (6.9) | 40.0, CH2 | 3.20, t (6.9) | 39.6, CH2 |
| 1″  | 173.3, C      | 173.4, C  |               |           |
| 2″  | 1.91, s       | 22.6, CH3 | 1.90, s       | 22.5, CH3 |
| 7-OMe| 3.85, s       | 56.4, CH3 | 3.95, s       | 57.0, CH3 |

*Measured at 600 MHz
*Measured at 150 MHz
*Measured at 800 MHz
*Measured at 200 MHz
*Detected by HMBC

### Table 3

| No. | ω1 (J in Hz)a | ω1, typeb | ω1 (J in Hz)c | ω1, typeb |
|-----|---------------|-----------|---------------|-----------|
| 1   | 173.0, C      |           |               |           |
| 3   | 3.62, t (7.5) | 41.9, CH2 | 3.80 dd 7.6 7.6 | 42.1, CH2 |
| 4   | 2.95, m       | 28.4, CH2 | 3.13 dd 7.6 7.6 | 27.1, CH2 |
| 4a  | 141.1, C      |           | 140.4, C      |           |
| 5   | 6.30, s       | 117.8, CH | 6.78, s       | 113.5, CH |
| 6   | 167.5, C      | 153.8e    |               |           |
| 7   | 144.1, C      |           | 140.8, C      |           |
| 8   | 145.9, C      |           | 146.4, C      |           |
| 8a  | 101.8, C      | 107.9, C  |               |           |
| 9   | 3.31, overlapped | 34.7, CH2 | 3.48 dd 7.6 7.6 | 35.6, CH2 |
| 10  | 1.73, m       | 27.3, CH2 | 1.75, m       | 26.6, CH2 |
| 11  | 1.63, m       | 30.2, CH2 | 1.65, m       | 30.1, CH2 |
| 12  | 3.21, m       | 40.0, CH2 | 3.20 dd 7.1 7.1 | 39.9, CH2 |
| 1″  | 173.2, C      | 173.3, C  |               |           |
| 2″  | 1.90, s       | 22.6, CH3 | 1.92, s       | 22.6, CH3 |
| 1″″ | 168.8, C      | 171.3, C  |               |           |
| 2″″ | 2.98, m       | 28.9, CH2 | 3.06 dd 7.4 7.4 | 28.9, CH2 |
| 3″  | 1.90, overlapped | 25.2, CH2 | 1.94, m       | 24.9, CH2 |
| 4″  | 1.63, m       | 29.7, CH2 | 1.65, m       | 29.8, CH2 |
| 5″  | 3.21, m       | 40.1, CH2 | 3.23 dd 7.1 7.1 | 39.9, CH2 |
| 2″″ | 173.2, C      | 173.3, C  |               |           |
| 2″″″| 1.93, s       | 22.6, CH3 | 1.89, s       | 22.6, CH3 |

*Measured at 500 MHz
*Measured at 125 MHz
*Measured at 600 MHz
*Measured at 150 MHz
*Detected by HMBC

Part A was subjected to column chromatography (silica gel; CHCl3/MeOH, 10:1→0:1, v/v) to yield three fractions (A1–A3). Fraction A1 was separated on an RP-18 silica gel column eluted with MeOH/H2O (10%→100%). The 40% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) and semi-preparative HPLC (Agilent Zorbax SB-C18 column, 10×250 mm, 2 mL/min) to obtain 3 (6.5 mg, MeOH/H2O, 80:20, tR = 12.150 min) and 11 (6.9 mg, MeCN/H2O, 15:85, tR = 5.190 min).

Fraction A2 was separated on an RP-18 silica gel column eluted with MeOH/H2O (10%→100%) to yield two main subfractions. The 5% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) to yield two main subfractions (A2-1-1 and A2-1-2). Subfraction A2-1-1 was performed on preparative TLC (CHCl3/MeOH, 3:1) to obtain 2 (4.3 mg). Subfraction A2-1-2 was recrystallized from MeOH to obtain 10 (23.5 mg).
The 30% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) and semipreparative HPLC [Welch Ultimate AQ-C18 column, 5.0 μm, φ 4.6 × 300 mm, MeCN/H2O (containing 0.05% TFA), 20:80, 1 mL/min] to obtain 13 (2.0 mg, tR=5.796 min).

Fraction A3 was separated on an RP-18 silica gel column eluted with MeOH/H2O (10%→100%) to yield two main subfractions. The 15% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) and semipreparative HPLC (Aligent Zorbax SB-C18 column, 10 μm, φ 4.6 × 250 mm, MeOH/H2O, 20:80, 2 mL/min) to obtain 7 (4.5 mg, tR=14.369 min), 6 (8.0 mg, tR=15.398 min), 5 (24.2 mg, tR=17.255 min), and 8 (8.5 mg, tR=22.038 min). The 35% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) and semipreparative HPLC [Welch Ultimate AQ-C18 column, 5.0 μm, φ 4.6 × 300 mm, MeCN/H2O (containing 0.05% TFA), 20:80, 1 mL/min] to obtain 12 (3.6 mg, tR=9.300 min).

Table 4 1H and 13C NMR Data of 4–6 in CD3OD

| No. | δH (J in Hz)a | δC, typeb | δH (J in Hz)a | δC, typeb | δH (J in Hz)a | δC, typec |
|-----|---------------|------------|---------------|------------|---------------|------------|
| 1   | 166.2, C      | 75.1, CH   | 164.9, C      | 51.8, CH   | 165.0, C      | 52.0, CH   |
| 2   | 3.82, dd (12.4, 2.1) | 7.15, CH | 7.12, dd (12.4, 2.1) | 7.13, CH | 7.14, dd (12.4, 2.1) | 7.15, CH |
| 3   | 1.96, m       | 36.2, CH2  | 34.1, CH      | 2.42, m    | 27.8, CH      |            |
| 4   | 1.56, m       | 25.9, CH   | 30.6, CH2     | 1.03, d (6.7) | 20.2, CH3     |            |
| 5   | 0.95, d (6.3) | 24.2, CH3  | 1.01, dd (7.3, 7.3) | 12.2, CH3 | 1.23, d (7.0) | 23.6, CH3 |
| 6   | 0.98, d (5.9) | 21.5, CH3  | 1.01, d (6.7) | 17.6, CH3  | 79.1, CH      |            |
| 1'  | 140.0, C      |            | 139.9, C      | 79.3, CH   | 139.9, C      |            |
| 2,6' | 7.3, d (7.4) | 130.6, CH  | 7.29, d (7.4) | 130.8, CH | 7.29, d (7.4) | 130.7, CH |
| 3,5' | 7.25, dd (7.4, 7.4) | 129.4, CH | 7.23, dd (7.4, 7.4) | 129.2, CH | 7.24, dd (7.4, 7.4) | 129.2, CH |
| 4'  | 7.17, dd (7.4, 7.4) | 127.6, CH | 7.15, dd (7.4, 7.4) | 127.4, CH | 7.15, dd (7.4, 7.4) | 127.4, CH |
| 7'  | 3.41, dd (14.1, 4.4) | 39.8, CH2 | 3.36, dd (13.9, 4.4) | 40.2, CH2 | 3.38, dd (13.9, 4.3) | 40.1, CH2 |
| 8'  | 2.91, dd (14.1, 10.7) | 57.6, CH | 4.68, dd (10.2, 4.4) | 57.2, CH | 4.70, dd (10.3, 4.3) | 57.1, CH |
| 9'  | 4.71, dd (10.7, 4.4) | 177.2, C | 2.98, s        | 52.8, CH3 | 2.96, s        | 52.9, CH3 |
| NMe | 2.85, s       | 52.4, CH3  |              |            |              |            |

aMeasured at 600 MHz
bMeasured at 150 MHz
cMeasured at 400 MHz
dMeasured at 100 MHz

Amides, Isoquinoline Alkaloids and Dipeptides from the Aerial Parts of Piper mullesua
The 90% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) and semipreparative HPLC (Agilent Zorbax SB-C18 column, 10×250 mm, MeCN/H2O, 99:1, 2 mL/min) to obtain 36 (3.4 mg, t_R=37.378 min), 35 (4.0 mg, t_R=42.351 min), and 33 (5.0 mg, t_R=45.179 min).

Fraction B3 was separated on an RP-18 silica gel column eluted with MeOH/H2O (10%→100%) to yield two main subfractions. The 60% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) to obtain 17 (32.6 mg) and 24 (105.7 mg) recrystallized from MeOH. The 70% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) and semipreparative HPLC (Agilent Zorbax SB-C18 column, 10×250 mm, MeOH/H2O, 85:15, 2 mL/min) to obtain 15 (10.3 mg, t_R=19.983 min).

Fraction B4 was separated on an RP-18 silica gel column eluted with MeOH/H2O (10%→100%) to yield four main subfractions. The 60% MeOH-eluted portion was purified by Sephadex LH-20 column chromatography (MeOH) to obtain 1 (169.9 mg) recrystallized from MeOH.

The inhibitory effect of all compounds on aggregation of rabbit platelet induced by Thrombin (IIa) (1 U/mL) is shown in Table 6. The inhibition of other tested compounds (1, 4, 6–13, 15–26, 28–32, and 35–37) was less than 10% at the concentration of 300 µg/mL.

| Compound | Concentration (µg/mL) | Inhibition (%) |
|----------|-----------------------|----------------|
| 2        | 300                   | 19.0±9.6       |
| 3        | 300                   | 17.0±3.2       |
| 5        | 300                   | 11.5±7.9       |
| 14       | 300                   | 22.2±12.1      |
| 27       | 300                   | 21.9±11.3      |
| 33       | 300                   | 14.8±8.5       |
| 34       | 300                   | 12.8±9.4       |
| Bivalirudinb | 50                   | 98.8±1.0       |

The inhibition of other tested compounds (1, 4, 6–13, 15–26, 28–32, and 35–37) was less than 10% at the concentration of 300 µg/mL.

Table 5 1H and 13C NMR Data of 7–9 in CD3OD

| No. | δH (J in Hz)a | δC, typeb | δH (J in Hz)a | δC, typec | δH (J in Hz)d | δC, typec |
|-----|---------------|-----------|---------------|-----------|---------------|-----------|
| 7   | 166.0, C      |           | 164.8, C      |           | 164.9, C      |           |
| 2   | 3.71, dd (11.8, 1.8) | 75.1, CH | 3.59, br s | 79.3, CH     | 3.59, d (2.8) | 80.6, CH   |
| 3   | 1.93, m       | 36.1, CH2 | 2.08, m      | 34.1, CH   | 2.36, m       | 27.8, CH   |
| 4   | 1.54, m       | 25.9, CH  | 1.58, m      | 30.6, CH2  | 1.01, d (6.7) | 20.2, CH   |
| 5   | 0.93, d (6.0) | 24.1, CH3 | 0.99, overlapped | 12.1, CH3 | 1.20, d (7.0) | 23.5, CH3 |
| 6   | 0.95, d (5.8) | 21.6, CH3 | 0.99, overlapped | 17.4, CH3 | 7.14, s      | 124.5, CH  |
| 2'  | 7.15, s       | 124.5, CH | 7.13, s      | 124.5, CH | 112.4, C      | 112.4, C   |
| 3'  | 112.4, C      | 129.1, C  | 129.4, C     | 129.3, C  | 129.3, C      | 129.3, C   |
| 3'a | 1.01, d (6.7) | 20.2, CH3 | 124.5, CH | 7.14, s | 124.5, CH | 112.4, C |
| 3'b | 1.54, m       | 25.9, CH  | 1.58, m      | 30.6, CH2  | 1.01, d (6.7) | 20.2, CH   |
| 4'  | 7.66, br d (7.9) | 119.8, CH | 7.66, br d (7.9) | 119.8, CH | 7.67, br d (7.8) | 119.8, CH |
| 5'  | 7.01, ddd (7.9, 7.0, 0.8) | 119.7, CH | 7.00, ddd (7.9, 6.9, 0.7) | 119.6, CH | 7.00, ddd (7.8, 7.5, 1.0) | 119.6, CH |
| 6'  | 7.07, ddd (8.1, 7.0, 0.8) | 122.4, CH | 7.06, ddd (8.1, 6.9, 0.7) | 122.2, CH | 7.06, ddd (8.0, 7.5, 1.0) | 122.3, CH |
| 7'  | 7.30, br d (8.1) | 112.2, CH | 7.28, br d (8.1) | 112.1, CH | 7.28, br d (8.0) | 112.1, CH |
| 7'a | 137.8, C      |           | 137.8, C     |           | 137.8, C      |           |
| 8'  | 3.51, dd (15.0, 4.6) | 29.3, CH2 | 3.48, dd (14.9, 4.4) | 29.6, CH2 | 3.49, dd (14.8, 4.3) | 29.6, CH2 |
| 3.15, dd (15.0, 9.9) | 112, CH | 7.28, br d (8.1) | 112.1, CH | 7.28, br d (8.0) | 112.1, CH |
| 9'  | 4.80, dd (9.9, 4.6) | 57.2, CH | 4.75, dd (9.5, 4.4) | 56.9, CH | 4.77, dd (9.8, 4.3) | 56.9, CH |
| 10' | 177.8, C      |           | 177.9, C     |           | 177.9, C      |           |
| NMe | 2.69, s       | 52.3, CH3 | 2.84, s      | 52.5, CH3 | 2.80, s       | 52.7, CH3 |

aMeasured at 800 MHz
bMeasured at 100 MHz
cMeasured at 125 MHz
dMeasured at 500 MHz

The inhibitory effect of all compounds on aggregation of rabbit platelet induced by Thrombin (IIa) (1 U/mL) is shown in Table 6.
24 MeOH/H2O-eluted portion was purified by Sephadex LH-11. Positive control (IC50=12.2 µg/mL). The IC50 values of each compound (Table 7) were calculated by nonlinear regression using the formula: Inhibition (%) = 100 × (1 - (Compound Concentration / IC50)^4). The final structure of each compound has been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 1529565). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

### 3.4 Spectroscopic Data of Compounds

#### 3.4.1 Pipermullesine A (1)

Pale yellow powder; UV (MeOH) λmax (logε) 334 (4.22), 243 (4.11), 224 (3.94) nm; IR (KBr) νmax 1643, 1595, 1513, 1461, 1439, 1415, 1376, 1345, 1315, 1287, 1269, 1228, 1160, 1141, 1047, 1023, 907, 803 cm⁻¹; 1H NMR and 13C NMR data, see Table 1; ESIMS m/z 296 [M+Na]⁺, 569 [2 M+Na]⁺; HREIMS m/z 273.0997 [M⁺] (calcd for C15H15NO4, 273.1001).

Crystal data for pipermullesine A (1): C15H15NO4·H2O, M=291.30, monoclinic, a=4.9028(8) Å, b=21.1593(3) Å, c=13.4552(2) Å, α=90.00°, β=92.120(2)°, γ=90.00°, V=1394.9(4) Å³, T=100(2) K, space group P21/n, Z=4, μ(MoKα)=0.105 mm⁻¹, 14679 reflections measured, and 3883 independent reflections (Rint=0.0331). The final R1 value was 0.0434 (I>2σ(I)). The final wR2 value was 0.1212 (I>2σ(I)). The final R1 value was 0.0575 (all data). The final wR2 value was 0.1322 (all data). The goodness of fit on F² was 1.030. The crystallographic data for the structure of 1 have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 1529565). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

#### 3.4.2 Pipermullesine B (2)

Pale yellow powder; UV (MeOH) λmax (logε) 402 (3.62), 310 (3.07), 268 (3.43) nm; IR (KBr) νmax 3424, 1622, 1511, 1466, 1441, 1368, 1354, 1235, 1208, 1180, 1039 cm⁻¹; 1H and 13C NMR data, see Table 2; ESIMS m/z 291 [M+H]⁺, 313 [M+Na]⁺; HREIMS m/z 290.1620 [M⁺] (calcd for C16H22N2O3, 290.1630).

#### 3.4.3 Pipermullesine C (3)

Pale yellow powder; UV (MeOH) λmax (logε) 400 (3.62), 310 (3.10), 268 (3.42) nm; IR (KBr) νmax 3376, 1721, 1630, 1607, 1590, 1562, 1511, 1462, 1438, 1384, 1337, 1271, 1237, 1211, 1101, 1076, 1037 cm⁻¹; 1H and 13C NMR data, see Table 3; ESIMS m/z 415 [M+H]⁺, 437 [M+Na]⁺; HREIMS m/z 414.2223 [M⁺] (calcd for C22H30N4O4, 414.2223).
3.4.4 Pipermullamide A (4)

White solid; [α]_D^25 = −16.2 (c 0.08, MeOH); UV (MeOH) λ_max (log e) 204 (3.35) nm; ECD Δε (c 0.08, MeOH) +1.35 (217); IR (KBr) ν max 3429, 1713, 1626, 1460, 1415, 1384, 1299, 1274, 1126, 1079, 1046 cm⁻¹; Δε (log ε) 1299, 1274, 1126, 1079, 1046 cm⁻¹; H and 13C NMR data, see Table 4; ESIMS m/z 321 [M + H]⁺, 343 [M + Na]⁺; HREIMS m/z 320.2102 [M]⁺ (calcd for C₁₈H₂₈N₂O₃, 320.2100).

3.4.5 Pipermullamide B (5)

White solid; [α]_D^20 = −7.6 (c 0.18, MeOH); UV (MeOH) λ_max (log e) 203 (4.00) nm; ECD Δε (c 0.012, MeOH) +1.48 (214); IR (KBr) ν max 3442, 1666, 1609, 1494, 1456, 1385, 1312, 1256, 1226, 1091, 1031 cm⁻¹; Δε (log ε) 1255, 1229, 1098, 1032 cm⁻¹; H and 13C NMR data, see Table 4; ESIMS m/z 321 [M + H]⁺, 343 [M + Na]⁺; HREIMS m/z 320.2102 [M]⁺ (calcd for C₁₈H₂₈N₂O₃, 320.2100).

3.4.6 Pipermullamide C (6)

White solid; [α]_D^20 = −33.1 (c 0.09, MeOH); UV (MeOH) λ_max (log e) 206 (4.07) nm; ECD Δε (c 0.014, MeOH) + 2.50 (214); IR (KBr) ν max 3426, 1673, 1608, 1494, 1456, 1383, 1315, 1255, 1225, 1096, 1032 cm⁻¹; Δε (log ε) 1255, 1229, 1098, 1032 cm⁻¹; H and 13C NMR data, see Table 4; ESIMS m/z 329 [M + Na]⁺; HREIMS m/z 307.2017 [M + H]⁺ (calcd for C₁₇H₂₇N₂O₃, 307.2022).

3.4.7 Pipermullamide D (7)

White solid; [α]_D^21 = −13.7 (c 0.08, MeOH); UV (MeOH) λ_max (log e) 281 (3.47), 222 (4.28), 206 (4.16) nm; ECD Δε (c 0.037, MeOH) +1.40 (233), -1.15 (222), -0.94 (214); IR (KBr) ν max 3426, 1674, 1611, 1488, 1459, 1384, 1259, 1228, 1127, 1101 cm⁻¹; Δε (log ε) 1255, 1229, 1098, 1032 cm⁻¹; H and 13C NMR data, see Table 4; ESIMS m/z 360 [M + H]⁺, 382 [M + Na]⁺; HREIMS m/z 360.2288 [M + H]⁺ (calcd for C₂₀H₃₀N₃O₃, 360.2287).

3.4.8 Pipermullamide E (8)

White solid; [α]_D^21 = −32.2 (c 0.06, MeOH); UV (MeOH) λ_max (log e) 398 (1.74), 282 (3.09), 220 (3.90), 205 (3.89) nm; ECD Δε (c 0.028, MeOH) +1.21 (233), +1.87 (225), -0.51 (220), -3.34 (200); IR (KBr) ν max 3428, 1681, 1619, 1452, 1422, 1384, 1209, 1139, 1046 cm⁻¹; Δε (log ε) 1255, 1229, 1098, 1032 cm⁻¹; H and 13C NMR data, see Table 5; ESIMS m/z 360 [M + H]⁺, 382 [M + Na]⁺; HREIMS m/z 360.2293 [M + H]⁺ (calcd for C₂₀H₃₀N₃O₃, 360.2287).

3.4.9 Pipermullamide F (9)

White solid; [α]_D^20 = −12.2 (c 0.21, MeOH); UV (MeOH) λ_max (log e) 281 (3.53), 221 (4.31), 206 (4.15) nm; ECD Δε (c 0.013, MeOH) +1.30 (228), -1.80 (212), -4.48 (200); IR (KBr) ν max 3415, 3266, 1672, 1603, 1491, 1459, 1384, 1255, 1229, 1098, 961, 747 cm⁻¹; Δε (log ε) 1255, 1229, 1098, 961, 747 cm⁻¹; H and 13C NMR data, see Table 5; ESIMS m/z 346 [M + H]⁺, 368 [M + Na]⁺; HREIMS m/z 346.2128 [M + H]⁺ (calcd for C₁₉H₂₈N₃O₃, 346.2131).

3.5 Preparation of Pipermulesine B Trifluoroacetate (2a)

Compound 2 (1.6 mg, 0.00551 mmol) was performed on semipreparative HPLC [Welch Ultimate AQ-C₁₈ column, 50.0 μm, φ 4.6 × 300 mm, MeCN/H₂O (containing 0.05% TFA), 20:80, 1.0 mL/min] to obtain 2a (2.0 mg, t_R = 6.344 min; 0.00516 mmol, 94% yield): pale yellow needles (MeOH); mp 142–145 °C; 1H and 13C NMR data, see Table 2.

Crystal data for pipermulesine B trifluoroacetate (2a): C₁₆H₂₃N₃O₃·C₂F₃O₂, M = 404.38, a = 7.6163(3), b = 8.8383(3), c = 14.8434(5), α = α = 82.5370(10), β = 89.4710(10), γ = 134.470(10), V = 954.71(6) Å³, T = 100(2) K, space group P-1, Z = 2, μ(CuKα) = 1.046 mm⁻¹, 1311 reflections measured, 3381 independent reflections (R_int = 0.0623). The final R1 value was 0.1027 (I > 2σ(I)). The final wR(F²) value was 0.2945 (I > 2σ(I)). The final R1 value was 0.1048 (all data). The final wR(F²) value was 0.2990 (all data). The goodness of fit on F² was 1.412. The crystallographic data for the structure of 2a have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 1529558). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

3.6 Preparation of Pipermulesine C Trifluoroacetate (3a)

Compound 3 (4.0 mg, 0.00965 mmol) was performed on semipreparative HPLC [Welch Ultimate AQ-C₁₈ column, 5.0 μm, φ 4.6 × 300 mm, MeCN/H₂O (containing 0.05% TFA), 20:80, 1.0 mL/min] to obtain 3a (4.5 mg, t_R = 7.460 min; 0.00880 mmol, 91% yield): pale yellow needles (MeOH); mp 157–159 °C; 1H and 13C NMR data, see Table 3.

Crystal data for pipermulesine C trifluoroacetate (3a): C₁₂H₁₁N₃O₄·C₂F₃O₂, M = 528.53, a = 8.5097(10) Å, b = 12.3631(15) Å, c = 12.8583(15) Å, α = 86.3542(2º), β = 87.8048(2º), γ = 88.814(2º), V = 1225.1(3) Å³, T = 100(2) K, space group P-1, Z = 2, μ(MoKα) = 0.118 mm⁻¹, 13522.
reflections measured, 6727 independent reflections ($R_{int}=0.0383$). The final $R_f$ values were 0.0528 ($I>2\sigma(I)$). The final $wR(F^2)$ value was 0.1229 ($I>2\sigma(I)$). The final $R_f$ value was 0.0879 (all data). The final $wR(F^2)$ value was 0.1414 (all data). The goodness of fit on $F^2$ was 1.020. The crystallographic data for the structure of 3a have been deposited in the Cambridge Crystallographic Data Centre (deposition number CCDC 1589949). Copies of the data can be obtained free of charge from the CCDC via www.ccdc.cam.ac.uk.

3.7 In vitro Platelet Aggregation Assay

The inhibitory effects of compounds against rabbit platelet aggregation induced by PAF or Thrombin (IIa) were evaluated according to the published methods [50–53]. The details were presented in Supplementary Material.

4 Conclusion

Thirty-seven compounds were isolated from the folk Chinese medicine *Piper mullesua* with the “Huayu” function associated with the antiplatelet therapies. The antiplatelet compounds, especially (-)-mangochinine, pellitorine, and (2E,4E)-N-isobutyl-2,4-dodecadienamide, might be scientific evidence to support the traditional use of the plant as folk medicine. In order to make better use of the folk medicine to serve for human health, further research needs to be conducted on bioguided isolation of compounds from the plant, based on both in vitro and in vivo bioassay testing.

Acknowledgements This work was funded by the Southeast Asia Biodiversity Research Institute, Chinese Academy of Sciences (Y4ZK111B01), the Natural Science Foundation of Yunnan Province, China (2011FZ205), the International Partnership Program of Chinese Academy of Sciences (153631KYSB20160004), the Key Laboratory of Ethnomedicine (Minzu University of China) of Ministry of Education of China (KLEM-ZZ201806), and the National Natural Science Foundation of China (31761143001 & 31161140345).

Compliance with Ethical Standards

Conflict of interest Authors declare that there is no conflict of interest associated with this work.

Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

References

1. C. Chen, F.Q. Wang, X. Wang, Z.N. Xia, H. Guang, J. Tradit. Chin. Med. 37, 64–75 (2017)
2. X.L. Su, W. Su, Y. Wang, X. Ming, Y. Kong, Acta Pharm. Sinica 37, 1208–1217 (2016)
3. J.X. Zhao, Y.H. Wang, X.L. Su, R.Q. Mei, J. Yang, Y. Kong, C. L. Long, Nat. Prod. Bioprospect. 6, 161–166 (2016)
4. Y.H. Wang, S.L. Morris Natschke, J. Yang, H.M. Niu, C.L. Long, K.H. Lee, J. Tradit. Complement. Med. 4, 8–16 (2014)
5. Editorial Board of “Zhonghua Bencao”, *Zhonghua Bencao*, vol. 3 (Shanghai Scientific and Technological Press, Shanghai, 1999), pp. 424–449
6. K. Zhang, W. Ni, C.C. Chen, Y.T. Liu, Plant Divers. 20, 374–376 (1998)
7. Z.Q. Shen, Z.H. Chen, D.C. Wang, J. Kunming Med. Univ. 29, 23–25 (1997)
8. S. Srivastava, M.M. Gupta, V. Prajapati, A.K. Tripathi, S. Kumar, Phytother. Res. 15, 70–72 (2001)
9. S. Srivastava, M. Gupta, V. Prajapati, A. Tripathi, S. Kumar, Pharm. Biol. 39, 226–229 (2001)
10. S. Srivastava, M.M. Gupta, A.K. Tripathi, S. Kumar, Indian J. Chem. 39, 946–949 (2001)
11. S. Srivastava, R.K. Verma, M.M. Gupta, S. Kumar, J. Indian Chem. Soc. 77, 305–306 (2000)
12. D.D. Ding, Y.H. Wang, Y.H. Chen, R.Q. Mei, J. Yang, J.F. Luo, Y. Li, C.L. Long, Y. Kong, Phytochemistry 129, 36–44 (2016)
13. D.D. Zhang, J. Yang, J.F. Luo, X.N. Li, C.L. Long, Y.H. Wang, J. Asian Nat. Prod. Res. (2017). https://doi.org/10.1080/10286020.10286020.11346630
14. E. Claveau, I. Gillaizeau, J. Blu, A. Bruel, G. Coudert, J. Org. Chem. 72, 4832–4836 (2007)
15. S.V. Eswaran, D. Kaur, K. Khamaru, S. Prabhakar, T. Sony, P. Raghunathan, B. Ganguly, Tetrahedron Lett. 57, 1899–1902 (2016)
16. I.I. Rodrı´guez, A.D. Rodrı´guez, J. Nat. Prod. 66, 855–857 (2003)
17. C.J. Li, D. Brownson, T.J. Mabry, C. Perera, E.A. Bell, Phytochemistry 42, 443–445 (1996)
18. Y.H. Wang, C.L. Long, F.M. Yang, X. Wang, Q.Y. Sun, H.S. Wang, Y.N. Shi, G.H. Tang, J. Nat. Prod. 72, 1151–1154 (2009)
19. M. Gacek, K. Undheim, Tetrahedron 29, 863–866 (1973)
20. S.X. Qiu, C. Liu, S.X. Zhao, Z.C. Xia, N.R. Farnsworth, H.H.S. Fong, Tetrahedron Lett. 29, 4167–4170 (1998)
21. Y. Nishiyama, M. Moriyasu, M. Ichimaru, K. Iwasa, A. Kato, S.G. Mathenge, P.B. Chalo Mutiso, F.D. Juma, Phytochemistry 65, 939–944 (2004)
22. A. Kato, M. Moriyasu, M. Ichimaru, Y. Nishiyama, F.D. Juma, J. Nat. Prod. 65, 89–95 (1995)
23. C. Chen, F.Q. Wang, X. Wang, Z.N. Xia, H. Guang, J. Tradit. Chin. Med. 37, 64–75 (2017)
24. J.R. Stöhr, P.G. Xiao, R. Bauer, Planta Med. 65, 175–177 (1999)
25. A. Banerji, C. Das, Phytochemistry 28, 3039–3042 (1989)
26. A. Banerji, D. Bandyopadhyay, M. Sarkar, A.K. Siddhanta, S.C. Pal, S. Ghosh, K. Abraham, J.N. Shoolery, Phytochemistry 24, 279–284 (1985)
27. K. Likhitwitayawud, N. Ruangrungsi, G.L. Lange, C.P. Decicco, Tetrahedron 43, 3689–3694 (1987)
28. V.S. Parmar, S.C. Jain, S. Gupta, S. Talwar, V.K. Rajwanshi, R. Kumar, A. Azim, S. Malhotra, N. Kumar, R. Jain, Phytochemistry 49, 1069–1078 (1998)
29. E. Nomura, A. Kashiwada, A. Hosoda, K. Nakamura, H. Morishita, T. Tsuno, H. Taniguchi, Bioorgan. Med. Chem. 11, 3807–3813 (2003)
30. D. Takaoka, K. Watanabe, M. Hiroi, Bull. Chem. Soc. Jpn 49, 3564–3566 (1976)
31. T. Zhuang, B. Xu, L. Huang, X. Chen, J. Liang, W. Qu, J. China. Pharm. Univ. 45, 410–412 (2014)
32. H. Shimomura, Y. Sashida, M. Oohara, Phytochemistry 27, 634–636 (1988)
33. D.D. de L. Moreira, E.F. Guimaraes, M.A.C. Kaplan, Phytochemistry 55, 783-786 (2000)
34. S.H. Lee, I.K. Dong, J.A. Kim, Y. Jahng, Heterocycl. Commun. 11, 407–410 (2005)
35. K. Obst, B. Lieder, K.V. Reichelt, M. Backes, S. Paetz, K. Geißler, G. Krammer, V. Somoza, J.P. Ley, K.H. Engel, Phytochemistry 135, 181–190 (2017)
36. G. Erosa-Rejón, L.M. Peña-Rodríguez, O. Sterner, J. Mex. Chem. Soc. 53, 44–47 (2009)
37. T.V. Sung, B. Steffan, W. Steglich, G. Klebe, G. Adam, Phytochemistry 31, 1659–1661 (1992)
38. J.J. Sims, J.A. Pettus Jr., Phytochemistry 15, 1076–1077 (1976)
39. J.D.P. Teresa, J.G. Urones, I.S. Marcos, J.F. Ferreras, A.L. Bertelloni, P.B. Barcala, Phytochemistry 26, 1481–1485 (1987)
40. M. Matsuo, S. Urano, Tetrahedron 32, 229–231 (1976)
41. W.H. Chen, G.Y. Chen, J. Wang, Y. Hui, L. Liu, J.J. Han, X.P. Song, Chem. Nat. Compd. 51, 797–799 (2015)
42. J.G. Cui, L.M. Zeng, J.Y. Su, C.W. Lin, Chem. Res. Chin. Univ. 18, 400–404 (2002)
43. E.S. Elkhayat, S.R. Ibrahim, G.A. Mohamed, S.A. Ross, Nat. Prod. Res. 30, 814–820 (2016)
44. M.Y. Xia, L. Wang, Y.H. Wang, Nat. Prod. Res. Dev. 28, 1676–1685 (2016)
45. C.Y. Li, W.J. Tsai, A.G. Damu, E.J. Lee, T.S. Wu, N.X. Dung, T. D. Thang, L. Thanh, J. Agric. Food Chem. 55, 9436–9442 (2007)
46. T. Rukachaisirikul, P. Siriwattanakit, K. Sukcharoenphol, C. Wongvein, P. Ruttanaweang, P. Wongwattanavuch, A. Suksamrarn, J. Ethnopharmacol. 93, 173–176 (2004)
47. Y.N. Shi, F.F. Liu, M.R. Jacob, X.C. Li, H.T. Zhu, D. Wang, R.R. Cheng, C.R. Yang, M. Xu, Y.J. Zhang, Planta Med. 83, 143–150 (2016)
48. S.V. Pullela, A.K. Tiwari, U.S. Vanka, A. Vummenthula, H.B. Tatipaka, K.R. Dasari, I.A. Khan, M.R. Janaswamy, J. Ethnopharmacol. 108, 445–449 (2006)
49. S.K. Ku, I.C. Lee, J.A. Kim, J.S. Bae, Fitoterapia 91, 1–8 (2013)
50. G.V. Born, Nature 194, 927–929 (1962)
51. L.J. Küster, J. Filep, J.C. Frölich, Thromb. Res. 43, 425 (1986)
52. M. Wu, D. Wen, N. Gao, C. Xiao, L. Yang, L. Xu, W. Lian, W. Peng, J. Jiang, J. Zhao, Eur. J. Med. Chem. 92, 257–269 (2015)
53. X.Y. Zhu, H.C. Liu, S.Y. Guo, B. Xia, R.S. Song, Q.C. Lao, Y.X. Xuan, C.Q. Li, Zebrafish 13, 335–344 (2016)