Taylor & Francis
Taylor & Francis Group

Natural Product Research
Formerly Natural Product Letters

ISSN: 1478-6419 (Print) 1478-6427 (Online) Journal homepage: https://www.tandfonline.com/loi/gnpl20

Iridoids and cycloartane saponins from *mussaenda pilosissima* valeton and their inhibitory NO production in BV2 cells

Vu Kim Thu, Nguyen Xuan Bach, Cong Tien Dung, Nguyen The Cuong, Tran Hong Quang, Youn-Chul Kim, Hyuncheol Oh & Phan Van Kiem

To cite this article: Vu Kim Thu, Nguyen Xuan Bach, Cong Tien Dung, Nguyen The Cuong, Tran Hong Quang, Youn-Chul Kim, Hyuncheol Oh & Phan Van Kiem (2020): Iridoids and cycloartane saponins from *mussaenda pilosissima* valeton and their inhibitory NO production in BV2 cells, Natural Product Research, DOI: 10.1080/14786419.2020.1737059

To link to this article: https://doi.org/10.1080/14786419.2020.1737059

NATURAL PRODUCT RESEARCH
https://doi.org/10.1080/14786419.2020.1737059

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Iridoids and cycloartane saponins from *mussaenda pilosissima* valeton and their inhibitory NO production in BV2 cells

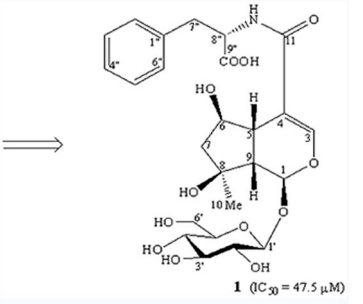
Vu Kim Thu[a], Nguyen Xuan Bach[d], Cong Tien Dung[a], Nguyen The Cuong[e], Tran Hong Quang[b], Youn-Chul Kim[c], Hyuncheol Oh[c] and Phan Van Kiem[b,d]

[a]Hanoi University of Mining and Geology, Bac Tu Liem, Hanoi, Viet Nam; [b]Institute of Marine Biochemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Viet Nam; [c]College of Pharmacy, Wonkwang University, Iksan, Republic of Korea; [d]Graduate University of Sciences and Technology, VAST, Hanoi, Viet Nam; [e]Institute of Ecology and Biological Resources, VAST, Hanoi, Viet Nam

ABSTRACT

One new iridoid glycoside (**1**), shanzhiside *N*-L-phenylalanyl ester along with seven known compounds, mussaenoside (**2**), shanzhiside methyl ester (**3**), barlerin (**4**), mussaendodise G (**5**), mussaendodise U (**6**), mussaendodise P (**7**), and mussaglaoside B (**8**) have been isolated from *Mussaenda pilosissima* Valeton. Their chemical structures were elucidated by spectroscopic methods, 1 D-, 2 D-NMR, and mass spectra. Compounds **1-7** showed significant inhibitory activity on LPS-stimulated NO production in BV2 cells with the IC_{50} values ranging from 43.5 to 65.2 μM.

Mussaenda pilosissima



ARTICLE HISTORY
Received 15 November 2019
Accepted 22 February 2020

KEYWORDS
Mussaenda pilosissima; terpenoid; shanzhiside N-L-phenylalanyl ester; NO inhibitory activity

1. Introduction

Mussaenda genus belongs to Rubiaceae family with more than 240 species. In Vietnam, some species of *Mussaenda* genus have been used in traditional medicine for

CONTACT Phan Van Kiem phankiem@yahoo.com

Supplemental data for this article can be accessed at https://doi.org/10.1080/14786419.2020.1737059.

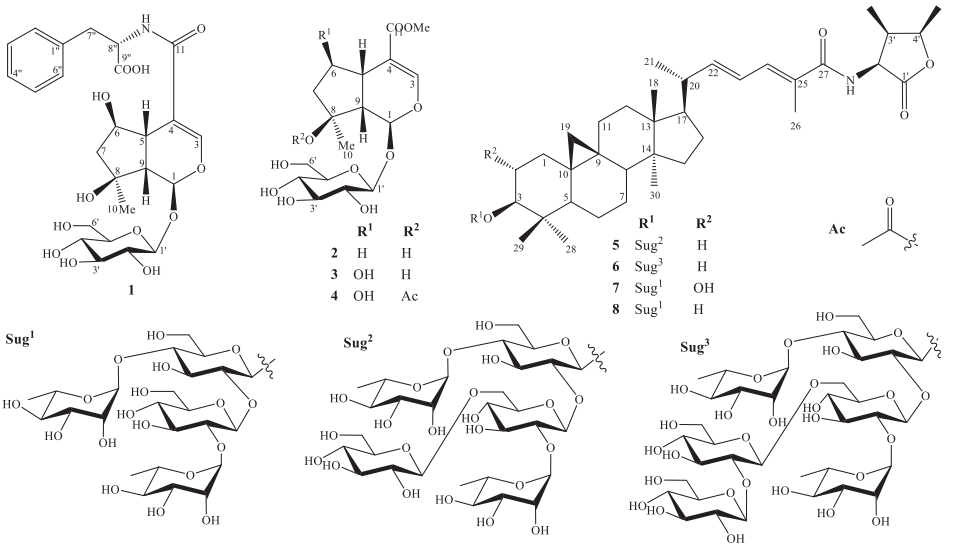


Figure 1. Chemical structures of compounds **1**–**8**.

the treatment of different ailments such as sore throat, and stomach troubles (Chi 1999). The whole plants of *M. pilosissima* have been used in oriental medicine for the treatment of kidney disease (Hien and Xuyen 2011). However, chemical and biological investigation of *M. pilosissima* have not been reported yet. Chemical investigation on *Mussaenda* genus indicated the presence of triterpenoids, iridoids, and flavonoids (Zhao et al. 1996; Thu et al. 2019). In addition, these compounds have been proposed to have biological effects, such as anti-inflammatory and anticancer activities (Astalakshmi and Sundara Ganapathy 2017). Our previous study reported the isolation and structural determination of cycloartane-*type* triterpene saponins from *Mussaenda glabra* (Thu et al. 2019). We reported herein the isolation and structural elucidation, and inhibitory NO production of one new iridoid glycoside and seven known compounds from *M. pilosissima*.

2. Results and discussion

Compound **1** was obtained as a white amorphous powder and its molecular formula was determined to be $C_{25}H_{33}NO_{12}$ by HR-ESI-MS at *m/z* 562.1895 $[M+Na]^+$ (Calcd. for $[C_{25}H_{33}NO_{12}Na]^+$, 562.1895). The ^{1}H-NMR spectrum of **1** (CD_3OD) showed the signals of one olefinic proton at δ_H 7.07 (1H, s), four methine protons at δ_H 5.43 (1H, d, $J = 3.5$ Hz), 3.79 (1H, m), 3.06 (1H, dd, $J = 3.5$, 10.0 Hz), and 2.53 (1H, dd, $J = 3.5$, 10.0 Hz), and one methyl group at δ_H 1.26 (3H, s) suggested the presence of an iridoid (Takamura et al. 2007); five aromatic protons at δ_H 7.17 (1H, t, $J = 8.0$ Hz), 7.22 (2H, t, $J = 8.0$ Hz), and 7.26 (2H, t, $J = 8.0$ Hz) suggested the existent of a phenyl group; and one anomeric proton of a monosaccharide at δ_H 4.63 (1H, d, $J = 8.0$ Hz). The ^{13}C-NMR and DEPT spectra of **1** indicated the signals of 25 carbons, including 2 carbonyl carbons at δ_C 168.75 and 178.5, 3 non-protonateds at δ_C 79.0, 114.4, and 139.9, 16

methines at δ_C 41.4, 52.0, 57.6, 71.7, 74.8, 77.1, 77.9, 78.3, 94.6, 99.7, 127.2, 129.1 × 2, 130.6 × 2, and 148.0, 3 methylenes at δ_C 39.6, 49.8, and 62.9, and 1 methyl carbon at δ_C 24.7. Analysis of ^{1}H- and ^{13}C-NMR data indicated the structure of **1** was similar to those of eucomoside B (Takamura et al. 2007). The structural difference between **1** and eucomoside B was the iridoid moiety. In addition, the iridoid moiety of **1** was found to be similar to shanzhiside methyl ester (**3**) (Kobayashi et al. 1986) which also reported from *Mussaenda pilosissima*. The HMBC correlations (Figure S1) from H-5 (δ_H 3.06) to C-3 (δ_C 148.0)/C-4 (δ_C 114.4); from H-3 (δ_H 7.07) to C-4 (δ_C 114.4)/C-5 (δ_C 41.4)/C-11 (δ_C 168.7) suggested the position of the double bond at C-3/C-4 and the carbonyl group at C-11. The HMBC correlations from H-5 (δ_H 3.06)/H-7 (δ_H 1.81 and 2.06) to C-6 (δ_C 77.1); from H-10 (δ_H 1.26) to C-7 (δ_C 49.8)/C-8 (δ_C 79.0)/C-9 (δ_C 52.0) indicated the position of the hydroxyl groups at C-6 and C-8. The D-glucose moiety of **1** was identified by acid hydrolysis (identified as TMS derivative by gas chromatography method). In addition, the location of the β-D-glucopyranosyl at C-1 was confirmed by HMBC correlation between glc H-1′ (δ_H 4.63) and C-1 (δ_C 94.6). The HMBC correlations between H-7″ (δ_H 3.00) and C-2″/C-6″ (δ_C 130.6)/C-1″ (δ_C 139.9)/C-8″ (δ_C 57.6)/C-9″ (δ_C 178.5) suggested the presence of the phenylalanine moiety. The *N*-amide bond between the phenylalanine and C-11 of the iridoid was confirmed by HMBC correlation from H-8″ (δ_H 4.66) to C-11 (δ_C 168.7). In addition, the L-configuration of phenylalanine moiety was supported by comparing by the chemical shifts at C-8″ (δ_C 57.6) and C-9″ (δ_C 178.5) of **1** with those of geniposidic acid *N*-L-phenylalanyl ester [C-8″ (δ_C 57.2) and C-9″ (δ_C 178.5)] and geniposidic acid *N*-D-phenylalanyl ester [C-8″ (δ_C 56.8) and C-9″ (δ_C 175.0)] (Takamura et al. 2007). The configurations of oxygenated group at C-1 and hydroxyl groups at C-6 and C-8 were proved as β by the NOESY correlations between H-10 (δ_H 1.26) and H-1 (δ_H 5.43)/H-6 (δ_H 3.79) (Figure S1). Therefore, the structure of new compound **1** was determined to be shanzhiside *N*-L-phenylalanyl ester.

The structures of known compounds were identified as musaenoside (**2**) (Sticher et al. 1981), shanzhiside methyl ester (**3**) (Kobayashi et al. 1986), barlerin (**4**) (Kobayashi et al. 1986), mussaendodise G (**5**) (Weimin et al. 1996), mussaendodise U (**6**) (Zhao et al. 1997), mussaendodise P (**7**) (Zhao et al. 1994), and mussaglaoside B (**8**) (Thu et al. 2019) (Figure 1) by analyzing the NMR and MS methods in comparison with the reported values in the literature.

All compounds were evaluated for their inhibitory NO production in BV2 cells, LPS-stimulated. Compounds were firstly examined screen cytotoxicity and inhibitory activity on NO production at the concentration of 80 μM. All tested compounds did not show any cytotoxicity (cell viability $>$ 95%). Compounds **1-7** inhihbited NO production in BV2 cells LPS-stimulated with the inhibitory percentages $>$ 50%. Thus, compounds **1-7** were evaluated inhibitory NO production at the various concentrations (80, 40, 20, 10, and 1.0 μM) to get IC_{50} values. As the results, compounds **1-7** showed significant inhibitory activity on LPS-stimulated NO production in BV2 cells with the IC_{50} values ranging from 43.5 to 65.2 μM, compared to those of positive control, L-NMMA (IC_{50} value of 22.1 μM) (Table 1).

Table 1. Inhibitory NO effect of compounds in BV2 cells, LPS-stimulated.

Compound	IC_{50} (μM)
1	47.5 ± 2.4
2	43.5 ± 2.2
3	45.6 ± 2.3
4	63.0 ± 3.1
5	45.8 ± 2.3
6	45.2 ± 2.3
7	65.2 ± 3.3
L-NMMA[a]	22.1 ± 1.2

[a]L- NMMA as a positive control. Compound **8** showed inhibitory NO production 38.5% at 80 μM. Results are presented as the MEAN values ± SD obtained from three independent experiments, carried out in triplicate.

3. Experimental

3.1. General

All NMR spectra were recorded on a Bruker 500 MHz AVANCE. HR-ESI-MS was obtained from Agilent 6550 iFunnel Q-TOF LC/MS system. Optical rotation was determined on a Jasco DIP-370 digital polarimeter. Column chromatography was performed on RP-18 resins (30 − 50 μm, Fuji Silysia Chemical Ltd.) or silica-gel (Kieselgel 60, 230-400 mesh, Merck). Thin layer chromatography (TLC), RP-18 $F_{254}S$ (0.25 mm, Merck) and silica-gel 60 F_{254} (0.25 mm, Merck) plates were used.

3.2. Plant material

The leaves of *Mussaenda pilosissima* Valeton. were collected at Vinhphuc, Viet Nam in February 2017 and identified by one of the authors. A voucher specimen (NCCT-P69) was deposited at Hanoi University of Mining and Geology.

3.3. Extraction and isolation

The dried powder of leaves of *M. pilosissima* (4.2 kg) was sonicated with hot methanol (3 times). The extract was removed solvent under low pressure to yield of a solid extract (MP, 360 g). MP was suspended in water and then partitioned with *n*-hexane, dichloromethane, ethyl acetate giving *n*-hexane (MPA1, 56.8 g), dichloromethane (MPA2, 97.8 g), ethyl acetate extracts (MPA3, 23 g) and water layer (MPA4). MPA4 was chromatographed on a Diaion HP-20 column eluting with water to remove salty and sugar components, then increase concentration of methanol in water (25, 50, 75 and 100%) to obtain four fractions, MPA4A-MPA4D. The MPA4B was subjected on a silica gel column eluting with a gradient solvent of dichloromethane/methanol (20/1, 10/1, 5/1, v/v) to give three sub-fractions MPA4B1-MPA4B3. MPA4B1 was chromatographed on a RP-18 column using methanol/water (1/2, v/v) to give MPA4B1A-MPA4B1C fractions. MPA4B1A was chromatographed on a RP-18 column eluting with acetone/water (1/4, v/v) to obtain compound **3** (100.0 mg). MPA4B1C was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/3/0.2, v/v/v) to yield compounds **1** (10.0 mg) and **2** (24.0 mg). MPA4B3 was chromatographed on a RP-18

column using acetone/water (1/5, v/v) to give two fractions, MPA4B3A and MPA4B3B. MPA4B3B was then purified on a RP-18 column using methanol/water (1/3, v/v) to yield compound **4** (6.5 mg). The MPA4D fraction was chromatographed on a silica gel column eluting with dichloromethane/methanol (10/1, v/v) to give four sub-fractions MPA4D1-MPA4D4. MPA4D3 was chromatographed on a RP-18 column using acetone/water (1/1.2, v/v) to give three fractions, MPA4D3A-MPA4D3C. MPA4D3A was chromatographed on a silica gel column eluting with dichloromethane/acetone/water (1/4/0.3, v/v/v) to yield compounds **6** (12.7 mg) and **8** (8.0 mg). Finally, compounds **5** (40.0 mg) and **7** (8.6 mg) was yielded from MPA4D3C fraction using a silica gel column eluting with dichloromethane/acetone/water (1/4/0.3, v/v/v).

3.3.1. Shanzhiside N-L-phenylalanyl ester (1)

White amorphous powder; $[\alpha]_D^{25} = -16.5$ (*c* 0.1 MeOH); HR-ESI-MS *m/z*: 562.1895 $[M+Na]^+$ (Calcd. for $[C_{25}H_{33}NO_{12}Na]^+$, 562.1895); ^{1}H-NMR ($CD_3OD$, 500 MHz) δ_H: iridoid: 5.43 (d, $J = 3.5$ Hz, H-1), 7.07 (s, H-3), 3.06 (dd, $J = 3.5$, 10.0 Hz, H-5), 3.79 (m, H-6), 2.06 (dd, $J = 7.0$, 13.5 Hz, H_a-7), 1.81 (dd, $J = 6.0$, 13.5 Hz, H_b-7), 2.53 (dd, $J = 3.5$, 10.0 Hz, H-9), 1.26 (s, H-10); glucose: 4.63 (d, $J = 8.0$ Hz, H-1′), 3.20 (dd, $J = 8.0$, 9.0 Hz, H-2′), 3.37 (dd, $J = 9.0$, 9.0 Hz, H-3′), 3.26 (dd, *J* 9.0, 9.0 Hz, H-4′), 3.30 (m, H-5′), 3.65 (dd, $J = 6.0$, 12.0 Hz, H_a-6′), 3.91 (dd, $J = 2.5$, 12.0 Hz, H_b-6′), 7.22 (d, $J = 8.0$ Hz, H-2″, 6″), 7.26 (t, $J = 8.0$ Hz, H-3″, 5″), 7.17 (t, $J = 8.0$ Hz, H-4″), 3.00 (dd, $J = 7.0$, 14.0 Hz, H_a-7″), 3.28 (m, H_b-7″), 4.66 (dd, $J = 5.0$, 8.0 Hz, H-8″); ^{13}C-NMR (CD_3OD, 125 MHz) δ_C: iridoid: 94.6 (C-1), 148.0 (C-3), 114.4 (C-4), 41.4 (C-5), 77.1 (C-6), 49.8 (C-7), 79.0 (C-8), 52.0 (C-9), 24.7 (C-10), 168.7 (C-11), glucose: 99.7 (C-1′), 74.8 (C-2′), 77.9 (C-3′), 71.7 (C-4′), 78.3 (C-5′), 62.9 (C-6′), phenylalanine: 139.9 (C-1″), 130.6 (C-2″, 6″), 129.1 (C-3″, 5″), 127.2 (C-4″), 39.6 (C-7″), (C-), 57.6 (C-8″), 178.5 (C-9″).

3.4. Acid hydrolysis

Compound (**1**, 2.0 mg) was dissolved in 1.0 N HCl (dioxane–H_2O, 1:1, v/v, 1.0 mL) and heated to 80 °C in a water bath for 3 h. Acidic solution was dried under a N_2 gas stream, suspended with H_2O then partitioned with $CHCl_3$. The aqueous layer was concentrated to dryness using N_2. The residue was dissolved in dry pyridine (0.1 mL), followed by addition of L-cysteine methyl ester hydrochloride in pyridine (0.06 M, 0.1 mL). The reaction mixture was heated at 60 °C for 2 h. Trimethylsilylimidazole solution (0.1 mL) was then added, followed by heating at 60 °C for 1.5 h. The dried product was partitioned with *n*-hexane and H_2O (0.1 mL each), and the organic layer was analyzed by gas chromatography (GC): column DB-5 (0.32 mm ID × 30 m length), detector FID, column temp 210 °C, injector temp 270 °C, detector temp 300 °C, carrier gas He (2 mL/min). With same above conditions, the standard sugars gave peaks at t_R (min) 14.11 and 14.26 for D- and L-glucose. Peaks at t_R (min) 14.11 of D-glucose for **1** was observed.

3.5. Inhibitory NO production in BV-2 cells assay

BV-2 cells were maintained in DMEM supplemented with 5% FBS and 1% penicillin-streptomycin. To measure NO production, BV-2 cells were dispensed into wells of a

96-well-plate (4×10^4 cells/well). After 24 h, BV2 cells were treated different concentration (80, 40, 20, 10, and 1.0 μM) of compound 30 min prior to 100 ng/mL of LPS treatment. NG-Monomethyl-L-arginine (L-NMMA), a well-known NO synthase inhibitor, was tested as a positive control. After 24 h of treatment, nitrite, a soluble oxidation product of NO, was measured in the culture media using the Griess reaction. The supernatant (50 μL) was harvested and mixed with an equal volume of Griess reagent (1% sulphanilamide, 0.1% N-1-naphthylethylenediamine dihydrochloride in 5% phosphoric acid). After 10 min, the absorbance at 540 nm was measured using an Emax microplate reader (molecular devices). After sample aliquot was collected for Griess assay, MTT (0.2 mg/mL) was directly added to cultures followed by incubation at 37 °C for 2 h to estimate cell viability. The supernatant was aspirated and 200 μL of DMSO was added to dissolve the insoluble formazan. Absorbance at 540 nm was then measured using a microplate reader. Data were expressed as percent cell viability relative to control cultures. The biological assays were performed as three experimental experiment. Data are expressed as the mean ± standard deviation (SD) of three independent experiments. Statistical analysis was performed using GraphPad Prism software, version 6.0 (GraphPad Software Inc, San Diego, CA, USA).

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This research is funded by Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 104.01-2016.27.

ORCID

Cong Tien Dung http://orcid.org/0000-0002-7703-3092

References

Astalakshmi N, Sundara Ganapathy R. 2017. A comprehensive review on the genus: *Mussaenda*. Int J Pharm Sci Rev Res. 8:534–541.

Chi VV. 1999. The dictionary of Vietnamese medicinal plants. Hanoi: Hanoi Medicine.

Hien DS, Xuyen DT. 2011. Muong ethnic groups's medicinal plants for therapeutic kidney in Hang Kia- Pa Co Nature reserve, Hoabinh province, Vietnam. Vietnam National Conference for Ecology and Biological Resources, Hanoi. 4: 1121–1126.

Kobayashi S, Mima A, Kihara M, Imakura Y. 1986. Iridoid glucosides from *Lamium amplexicaule*. Chem Pharm Bull. 34(2):876–880.

Sticher O, Uesato S, Nagajima K, Inouye H. 1981. Ladroside (= 6'-Caffeoyl-mussaenoside), a new Iridoid glucoside from *Veronica officinalis L.* (*Scrophulariaceae*) and the elucidation of the absolute configuration at C(8) of mussaenoside. Helv Chim Acta. 64:16–24.

Takamura C, Hirata T, Ueda T, Ono M, Miyashita H, Ikeda T, Nohara T. 2007. Iridoids from the green leaves of *Eucommia ulmoides*. J Nat Prod. 70(8):1312–1316.

Thu VK, Bach NX, Anh LT, Trang DT, Nhiem NX, Tai BH, Van Kiem P, Van Minh C, Park S, Seo Y, et al. 2019. Discovery of cycloartane-type triterpene saponins from *Mussaenda glabra*. Phytochem Lett. 33:39–45.

Weimin Z, Rensheng X, Guowei Q, Vaisar T, Lee MS. 1996. Saponins from *Mussaenda pubescens*. Phytochemistry. 42(4):1131–1134.

Zhao W-M, Yang G-J, Xu R-S, Qin G-W. 1996. New Saponins from *Mussaenda pubescens*. Nat Prod Lett. 8(2):119–126.

Zhao W, Wolfender J-L, Hostettmann K, Cheng K, Xu R, Qin G. 1997. Triterpenes and triterpenoid saponins from *Mussaenda pubescens*. Phytochemistry. 45(5):1073–1078.

Zhao W, Xu J, Qin G, Xu R, Wu H, Weng G. 1994. New triterpenoid Saponins from *Mussaenda pubescens*. J Nat Prod. 57(12):1613–1618.