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New amide and dioxopiperazine derivatives from leaves of *Breynia nivosa*

Blessing O. Umeokoli[a,b], Felix A. Onyegbule[b], Festus B.C. Okoye[b], Hao Wang[a], Rainer Kalscheuer[a], Werner E.G. Müller[c], Rudolf Hartmann[d], Zhen Liu[a,*], Peter Proksch[a,*]

[a] Institute of Pharmaceutical Biology and Biotechnology, Heinrich-Heine-Universität Düsseldorf, 40225 Düsseldorf, Germany
[b] Department of Pharmaceutical and Medicinal Chemistry, Nnamdi Azikiwe University, Awka, Nigeria
[c] Institute of Physiological Chemistry, Universitätsmedizin der Johannes Gutenberg-Universität Mainz, 55128 Mainz, Germany
[d] Institute of Complex Systems: Strukturbiochemie, Forschungszentrum Juelich, 52428 Juelich, Germany

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ABSTRACT

The first chemical investigation of leaves of *Breynia nivosa* from Nigeria resulted in the isolation of two new amide derivatives breynivosamides A and B (**1** and **2**) and two new dioxopiperazine derivatives breynivosines A and B (**4** and **5**) together with seven known compounds (**3**, **6**–**11**). The structures of the new compounds were elucidated by 1D, 2D NMR and HRESIMS data as well as by comparison with the literature. All isolated compounds were tested for the cytotoxic and antimicrobial activities. Only cristatin A (**6**) showed cytotoxicity against the L5178Y mouse lymphoma cell line with an IC_{50} value of 13.9 μM while breynivosamide A (**1**) exhibited moderate antimicrobial activity against *Mycobacterium tuberculosis* with an MIC value of 25 μM.

1. Introduction

Breynia nivosa (Phyllanthaceae), commonly known as snow bush, is a tropical shrub with variegated attractive foliage. It is widely grown in tropical Africa as a flower plant. In Nigeria, its medicinal applications include treatment of headaches, toothaches, tooth infections, fever and malaria. Leaves of *B. nivosa* have been reported to show antimicrobial, analgesic, anti-inflammatory, antioxidant and antimalarial activities [1–3]. A series of structurally diverse metabolites have been discovered from the genus *Breynia*, such as neolignans, triterpenes and sterols as well as sulfur-containing spiroketal, terpenic and phenolic glycosides [4–7]. Until now, no studies have been reported on the chemical constituents of *B. nivosa*. In the present study, eleven compounds (Fig. 1) were isolated from leaves of *B. nivosa* including two new amide derivatives breynivosamides A and B (**1** and **2**) and two new dioxopiperazine derivatives breynivosines A and B (**4** and **5**). The structure elucidation of the new compounds and their bioacitities are reported herein.

2. Results and discussion

The molecular formula of compound **1** was determined as $C_{32}H_{30}O_5N_2$ based on HRESIMS data, indicating 19 degrees of unsaturation. The ^{1}H NMR spectrum (Table 1) showed three sets of signals of monosubstituted benzene rings (δ_H 7.70, 7.39, 7.51; δ_H 7.22, 7.26, 7.18; δ_H 7.67, 7.36, 7.47) and one set of signals of *para*-disubstituted benzene ring (δ_H 7.06, H-5 and 9; δ_H 6.70, H-6 and 8) as confirmed by the COSY correlations. In addition, one oxygenated methylene at δ_C 66.4, δ_H 4.40 and 4.12 (CH_2-1′), two nitrogenated methines at δ_C 56.3, δ_H 4.73 (CH-2) and δ_C 51.7, δ_H 4.56 (CH-2′) as well as two aliphatic methylenes at δ_C 37.0, δ_H 3.18 and 3.04 (CH_2-3) and at δ_C 37.8, δ_H 2.94 and 2.88 (CH_2-3′) were observed. These data were similar to those of the co-isolated known compound anabellamide (**3**) [8] except for the replacement of a monosubstituted benzene ring by a *para*-disubstituted benzene ring in compound **1**. An additional hydroxy group was suggested to be located at this *para*-disubstituted benzene ring based on the difference of the molecular formula of **1** compared to that of **3**. The COSY correlation between H-2 and H_2-3 along with the HMBC correlations from H-5 and H-9 to C-3 and C-7 (δ_C 157.2) confirmed the attachment of the additional hydroxy group at the C-7 position (Fig. 2). The remaining structure of compound **1** was identical to that of **3** as elucidated by detailed analysis of the 2D NMR spectra of **1**. Compound **1** is suggested to share the same configuration as **3** due to their similar NMR data and close biogenetic relationship. Thus, the structure of compound **1** was elucidated as shown, representing a new amide derivative, for which the name breynivosamide A is proposed.

Breynivosamide B (**2**) has the molecular formula $C_{34}H_{31}O_4N_3$ as determined by HRESIMS. An indole ring was established by the COSY correlations between H-5 (δ_H 7.57, d)/H-6 (δ_H 6.99, t), H-6/H-7 (δ_H 7.08, t) and H-7/H-8 (δ_H 7.32, d) in addition to the HMBC correlations from H-5 to C-4 (δ_C 110.7), C-7 (δ_C 122.2) and C-8a (δ_C 137.8), from H-6 to C-8 (δ_C 112.1) and C-4a (δ_C 128.4), from H-7 to C-5 (δ_C 118.9) and

* Corresponding authors.
E-mail addresses: zhenfeizi0@sina.com (Z. Liu), proksch@uni-duesseldorf.de (P. Proksch).

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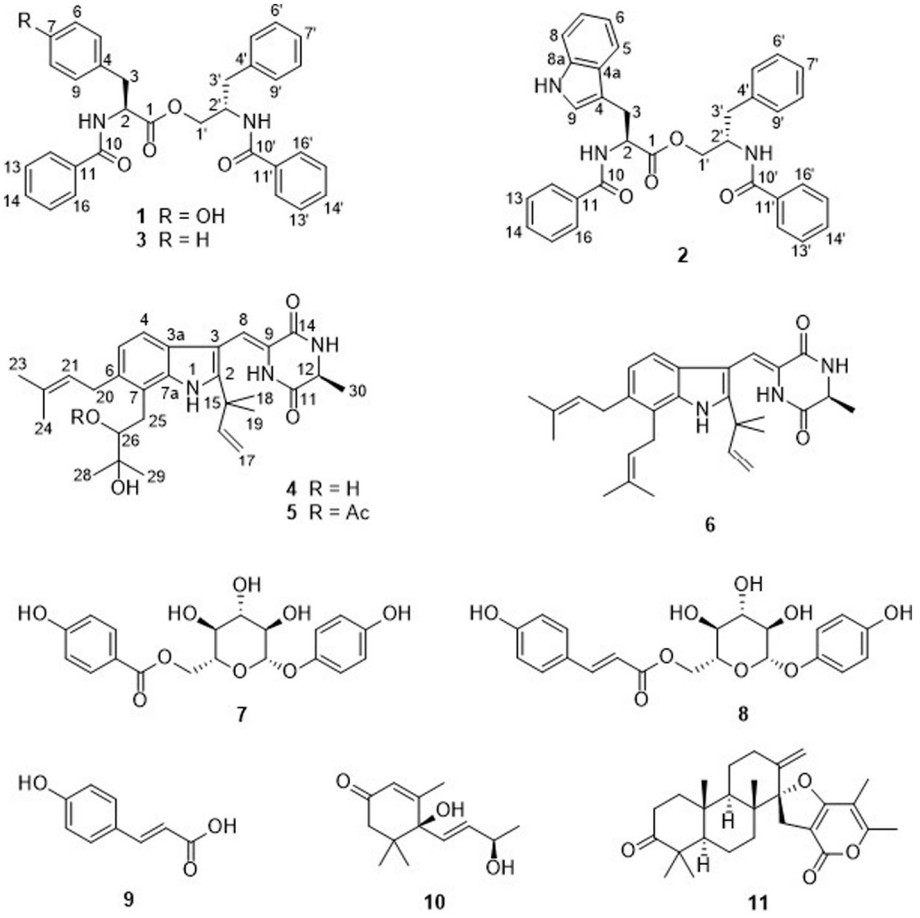

Fig. 1. Compounds isolated from leaves of *Breynia nivosa*.

C-8a, from H-8 to C-6 (δ_C 119.6) and C-4a, and from H-9 (δ_H 7.12, s) to C-4, C-4a and C-8a. This indole ring was attached to C-3 as deduced from the HMBC correlations from H-9 to C-3 (δ_C 27.9) and in turn from H_2-3 (δ_H 3.44 and 3.33) to C-4, C-4a and C-9 (δ_C 124.1). After detailed examination of the 2D NMR spectra of **2**, its remaining structure was elucidated to be identical to those of **1** and **3**.

The molecular formula of compound **4** was determined to be $C_{29}H_{39}O_4N_3$ on the basis of its HRESIMS data. The UV and ^{1}H NMR spectrum of **4** (Table 2) were similar to those of co-isolated known dioxopiperazine akloid cristatin A (**6**) [9–10] except for the replacement of one double bond by an oxygenated methine at δ_C 79.4 and δ_H 3.34 (CH-26), an oxygenated quaternary carbon at δ_C 72.5 (C-27) and two hydroxy groups at δ_H 5.09 (d, OH-26) and 5.00 (s, OH-27). Their locations were confirmed by the COSY correlations between OH-26/H-26 and H-26/H_2-25 (δ_H 3.27 and 2.70) together with the HMBC correlations from OH-27, Me-28 (δ_H 1.18) and Me-29 (δ_H 1.19) to C-26 and C-27 and from H_2-25 to C-6 (δ_C 131.9), C-7 (δ_C 122.8) and C-7a (δ_C 135.1) (Fig. 2). The remaining structure of **4** was the same as that of **6** as determined by the 2D NMR spectra of **4**. As shown in Fig. 1, the absolute configuration at C-12 was proposed to be the same as in **6** in consideration of the close biogenetic relationship of both compounds whereas the absolute configuration at C-26 remained unsolved due to the limited amount of **4**. Compound **4** is a new dioxopiperazine derivative, for which the name breynivosine A is proposed.

The HRESIMS of breynivosine B (**5**) gave the molecular formula $C_{31}H_{41}O_5N_3$. Compared to **4**, additional signals for an acetoxy group at δ_C 169.5, 20.6 and δ_H 1.75 were observed in the NMR spectra (Table 2) of **5**. The location of this acetoxy substituent at C-26 was cofirmed by the obvious downfield shift of H-26 (δ_H 4.87) and a key HMBC correlation from H-26 to the carboxy carbon of the acetoxy group in **5**. Detailed analysis of the 2D NMR spectra of **5** revealed the remaing structure of **5** is identical to that of **4**.

The seven known compounds were identified as asperphenamate (**3**) [8], cristatin A (**6**) [9–10], breynioside A (**7**) [7], robustaside A (**8**) [11], (*E*)-*p*-coumaric acid (**9**) [12], blumenol A (**10**) [13] and lygodinolide (**11**) [14] by comparison with literature data.

Lignanamides like compounds **1–3** are usually products of bacteria and fungi but are rarely found in plants [8,15]. Compounds **4–6** are tryptophan-containing 2,5-dioxopiperazine alkaloids, which have been isolated from fungi of *Aspergillus*, *Eurotium* [16] and from higher plants such as *Lepidagathis cristata* and *Acacia auriculiformis* [9–10]. To the best of our knowledge, this is the first report of lignanamides and tryptophan-containing 2,5-dioxopiperazine alkaloids from plants of the genus *Breynia*.

All isolated compounds (**1 − 11**) were evaluated for their cytotoxic activity against the L5178Y mouse lymphoma cell line using the MTT method. Only cristatin A (**6**) showed activity with an IC_{50} value of 13.9 μM. Oxidation at the C-26/C-27 (**4** and **5** vs. **6**) led to total loss of activity. In addition, an antimicrobial activity assay against *Mycobacterium tuberculosis* was carried out for all isolated compounds. Only the new compound breynivosamide A (**1**) exhibited moderate activity with an MIC value of 25 μM. The presence of tyrosine in **1** seems to be important for the activity compared to histidine in **2** and phenylalanine in **3**.

3. Experimental section

3.1. General experimental procedures

Optical rotations were measured with a P-1020 polarimeter. 1D and 2D NMR spectra were recorded on a Bruker ARX 600 or 700 NMR spectrometer. Mass spectra were obtained with a Finnegan MAT TSQ-