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Natural Products and Bioprospecting

SHORT COMMUNICATION

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Three new amide derivatives from the fungus *Alternaria brassicicola*

Fengli Li[1†], Saisai Gu[2†], Sitian Zhang[1], Shuyuan Mo[1], Jieru Guo[3*], Zhengxi Hu[1*] and Yonghui Zhang[1*]

Abstract

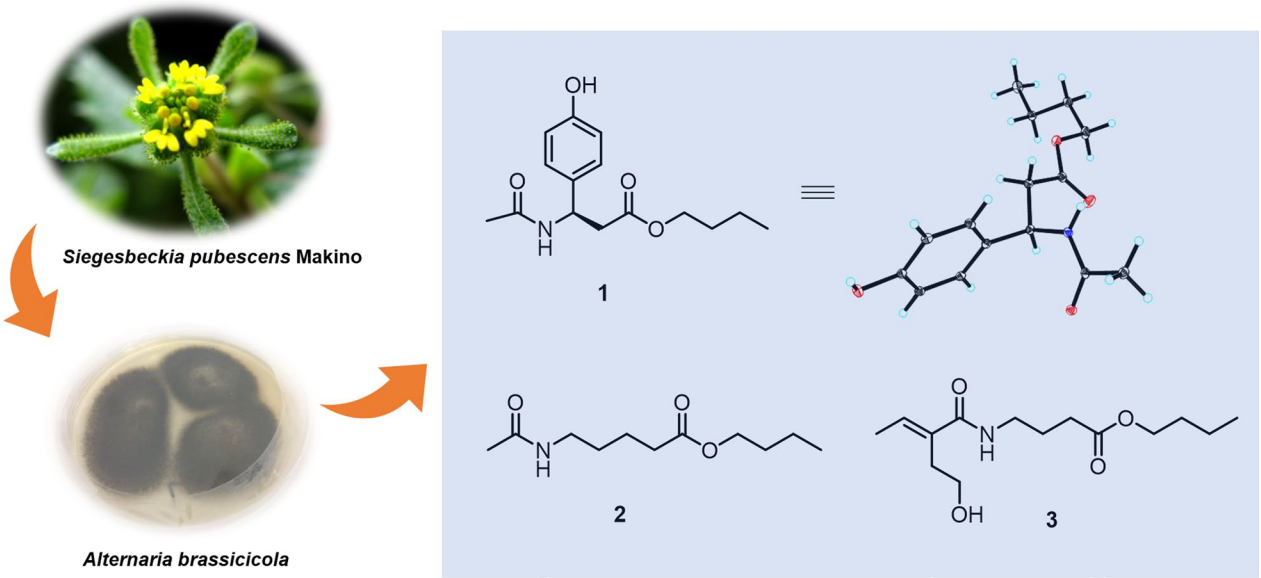
Three new amide derivatives (alteralkaloids A–C, **1**–**3**) and three known alkaloids (**4**–**6**) were afforded after phytochemical investigation of fungus *Alternaria brassicicola*. The structures of these compounds were confirmed by NMR spectroscopic and HRESIMS data. Furthermore, the absolute configuration of **1** was determined using the single-crystal X-ray diffraction analysis. Compounds **1**–**3** belong to a class of amide derivatives that have not been found in nature before, sharing the same characteristic signals of the butyl moiety and amide group. These isolated compounds mentioned above were tested for the cytotoxic activity.

Keywords *Alternaria brassicicola*, Secondary metabolites, Amide derivatives, Structural elucidation

†Fengli Li and Saisai Gu contributed equally to this work.

*Correspondence:
Jieru Guo
guojieru314@163.com
Zhengxi Hu
hzx616@126.com
Yonghui Zhang
zhangyh@mails.tjmu.edu.cn
Full list of author information is available at the end of the article

Graphical Abstract



1 Introduction

Alternaria fungi are one of the important biological resources with great potentials, from which a rich source of novel structures with a variety of bioactivities were discovered successively [1–3]. They mainly included nitrogen-containing metabolites [4], diterpenoids [5], meroterpenoids [6], and polyketides [7, 8]. And these metabolites exhibited a broad range of biological activities, such as anti-inflammatory [9], phytotoxic [10], cytotoxic [11], acetylcholinesterase inhibitory [12], and antimicrobial activities [13], which attracted, and still attract increasing attentions from the chemists and pharmacologists.

During the chemical investigation of structurally interesting and biologically active constituents from fungus *A. brassicicola*, our group have identified a variety of novel structures. Notably, brassicicene N represented the first fusicoccane-derived diterpenoid with a tetracyclic skeleton bearing an oxabicyclo[4.3.1]nonane unit [14]; alterbrassicene A possessed an unprecedented 5/9/4-fused carbocyclic skeleton, exhibiting potent IKKβ inhibitory effect [9]; alterbrassicicene A, featuring a newly transformed monocyclic carbon skeleton, showed strong agonistic action upon PPAR-γ [15]; alterbrassinoids A–D represented the first class of fusicoccane-derived diterpenoid dimers [16]. Based on our previous work, we amplified and fermented this potential fungus again. As a result, three new compounds (**1**–**3**) along with three known alkaloids (**4**–**6**) were afforded in the present phytochemical study on the fungus *A. brassicicola*. Herein, this paper aims to report the isolation, structural elucidation, and the cytotoxic activity of these isolated compounds (Fig. 1).

2 Results and discussion

Compound **1** was isolated as a colorless needle crystal, and its molecular formula was confirmed as $C_{15}H_{21}NO_4$ based on the HRESIMS data at *m/z* 302.1378 (Additional file 1). According to the characteristic signals of the 1D NMR data (Table 1) showing H-2′/H-6′ (δ_H 7.15, d J=8.5 Hz) and H-3′/H-5′ (δ_H 6.74, d, J=8.5 Hz); and C-1′ (δ_C 133.2), C-2′/C-6′ (δ_C 128.9), C-3′/C-5′ (δ_C 116.3), and C-4′ (δ_C 158.0), the *para*-substituted benzene ring can be deduced. The spin coupling system (Fig. 2) of H_3-7 (δ_H 0.9)/H_2-6 (δ_H 1.31)/H_2-5 (δ_H 1.53)/H_2-4 (δ_H 4.02) suggested the existence of a butyl fragment. The spin coupling system (Fig. 2) of H-1 (δ_H 5.25)/H_2-2 (δ_H 2.79), along with the HMBC correlations (Fig. 2) of H_2-4 and H-1 with C-3 (δ_C 172.4) and of H-2′ with C-1 (δ_C 51.2), suggested that the butyl moiety was connected to the 3-(4-hydroxyphenyl)propanoic acid group. In addition, the HMBC correlations from H_3-2″ (δ_H 1.92) and H-1 to C-1″ (δ_C 172.2) were observed, which confirmed an acetamido motif attached to the C-1. Thus, the planar structure of **1** was corroborated (Fig. 1). The absolute configuration of **1** was confirmed as 1*R* using the single-crystal X-ray diffraction analysis [Flack parameter = 0.01(5), CCDC 2049878] (Fig. 3). Therefore, the

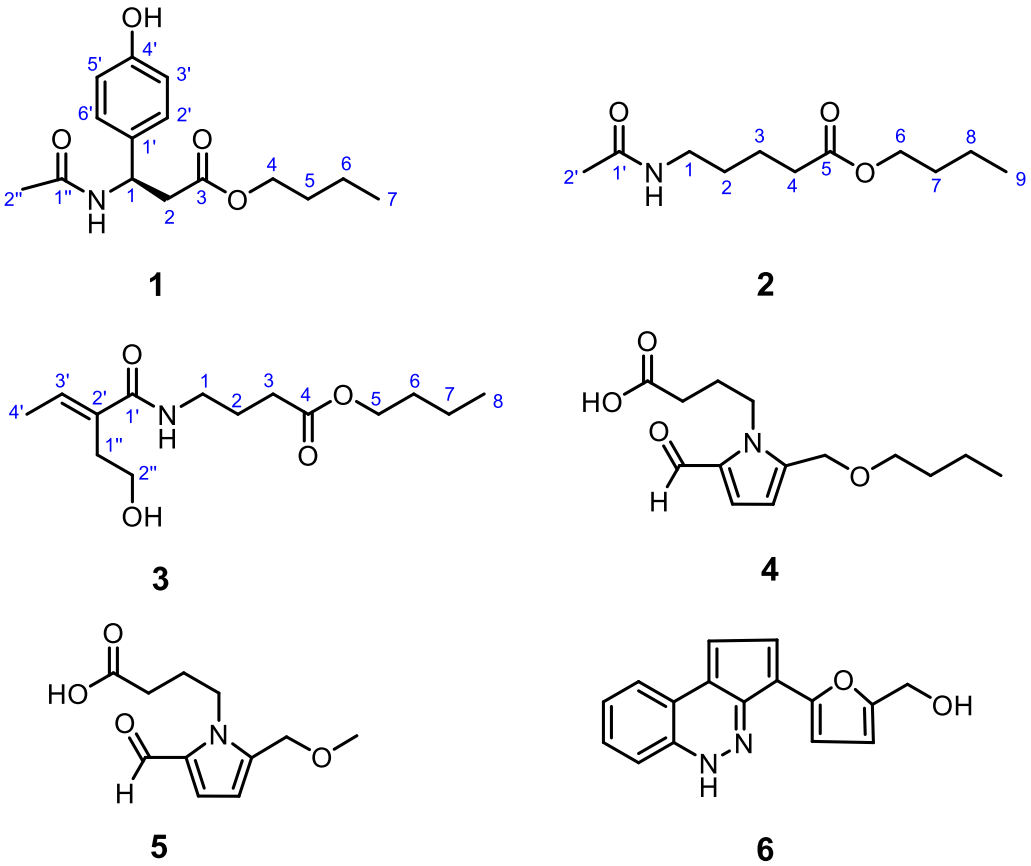


Fig. 1 Chemical structures of compounds **1**–**6**

structure of **1** was determined and named as alteralkaloid A.

Compound **2**, obtained as a colorless oil, showed an $[M + Na]^+$ peak at *m/z* 238.1473 in the HRESIMS analysis, matching a molecular formula of $C_{11}H_{21}NO_3$, which was indicative of two degrees of unsaturation. Based on its 1D NMR data (Table 1), a total of 11 carbons were observed, from which two methyl groups (δ_C 14.0 and 22.5), seven methylene groups (δ_C 20.2, 23.4, 29.8, 31.8, 34.6, 40.0, and 65.3), and two carbonyls (δ_C 173.2 and 175.3) were included. The characteristic signals [spin coupling system: H_2-6 (δ_H 4.07)/H_2-7 (δ_H 1.61)/H_2-8 (δ_H 1.40)/H_3-9 (δ_H 0.95)] of the butyl moiety were also observed in the ^1H–^1H COSY spectrum of **2** (Fig. 2). In addition, the spin coupling system of H_2-1 (δ_H 3.17)/H_2-2 (δ_H 1.52)/H_2-3 (δ_H 1.69, 1.55)/H_2-4 (δ_H 2.34) along with the HMBC correlations (Fig. 2) from H_2-3 and H_2-6 to C-5 (δ_C 175.3) and from H_3-2′ (δ_H 1.92) and H_2-1 to C-1′ (δ_C 173.2) were clearly observed, which defined the structure of **2** as butyl 5-acetamidopentanoate and this compound was named as alteralkaloid B (Additional file 1).

Compound **3** was obtained as a colorless oil. Its HRESIMS data at *m/z* 294.1689 indicated that the molecular formula was $C_{14}H_{25}NO_4$, suggesting three degrees of unsaturation. The 1D NMR (Table 1) and HSQC data showed 14 carbon signals, which was similar to that of **2**, including two methyl groups (δ_C 14.0 and 18.5), eight methylene groups (δ_C 20.2, 25.9, 31.8, 32.4, 39.3, 44.5, 60.8, and 65.4), two carbonyls (δ_C 175.0 and 169.7), and two olefinic carbons (δ_C 120.9 and 151.6). The structure contained one double bond and two carbonyl groups, suggesting that **3** was a chain compound. The ^1H–^1H COSY correlations (Fig. 2) of H_2-1 (δ_H 3.23)/H_2-2 (δ_H 1.80)/H_2-3 (δ_H 2.36) and H_2-5 (δ_H 4.08)/H_2-6 (δ_H 1.62)/H_2-7 (δ_H 1.40)/H_3-8 (δ_H 0.95), together with the HMBC correlations (Fig. 2) from H_2-2 and H_2-5 to C-4 (δ_C 175.0), suggested the existence of a butyl butyrate fragment (A unit). In addition, two spin coupling systems of H_2-1″ (δ_H 2.32)/H_2-2″ (δ_H 3.70) and H_3-4′ (δ_H 2.12)/H-3′ (δ_H 5.71) in the ^1H–^1H COSY spectrum of **3** (Fig. 2) and the key HMBC correlations (Fig. 2) of H_3-4′ and H_2-1″ with C-3′ (δ_C 120.9) and C-2′ (δ_C 151.6), indicated the existence of a 2-(2-hydroxyethyl)but-2-enamide fragment (B unit). The deduction that C-1 of A unit was connected to the nitrogen atom of B unit was supported by the HMBC correlation from H_2-1 to C-1′ (δ_C 169.7).

Table 1 ^{1}H NMR and ^{13}C NMR data (δ in ppm, J in Hz) for **1**–**3**

No.	1 (in methanol-d_4)		2 (in methanol-d_4)		3 (in methanol-d_4)	
	δ_C [a]	δ_H [b]	δ_C [a]	δ_H [b]	δ_C [a]	δ_H [b]
1	51.2 CH	5.25 t (7.6)	40.0 CH_2	3.17 t (6.6)	39.3 CH_2	3.23 t (6.9)
2	41.9 CH_2	2.79 m	29.8 CH_2	1.52 m	25.9 CH_2	1.80 m
3	172.4 C		23.4 CH_2	1.69 m, 1.55 m	32.4 CH_2	2.36 m
4	65.5 CH_2	4.02 t (6.6)	34.6 CH_2	2.34 t (7.3)	175.0 C	
5	31.8 CH_2	1.53 m	175.3 C		65.4 CH_2	4.08 t (6.6)
6	20.1 CH_2	1.31 m	65.3 CH_2	4.07 t (6.6)	31.8 CH_2	1.62 m
7	14.0 CH_3	1.90 t (7.4)	31.8 CH_2	1.61 m	20.2 CH_2	1.40 m
8			20.2 CH_2	1.40 m	14.0 CH_3	0.95 t (7.4)
9			14.0 CH_3	0.95 t (7.4)		
1′	133.2 C		173.2 C		169.7 C	
2′	128.9 CH	7.15 d (8.5)	22.5 CH_3	1.92 s	151.6 C	
3′	116.3 CH	6.74 d (8.5)			120.9 CH	5.71 q (1.4)
4′	158.0 C				18.5 CH_3	2.12 d (1.4)
5′	116.3 CH	6.74 d (8.5)				
6′	128.9 CH	7.15 d (8.5)				
1″	172.2 C				44.5 CH_2	2.32 m
2″	22.6 CH_3	1.92 s			60.8 CH_2	3.70 t (6.6)

"m" mean overlapped or multiplet with other signals

[a] Recorded at 100 MHz

[b] Recorded at 400 MHz

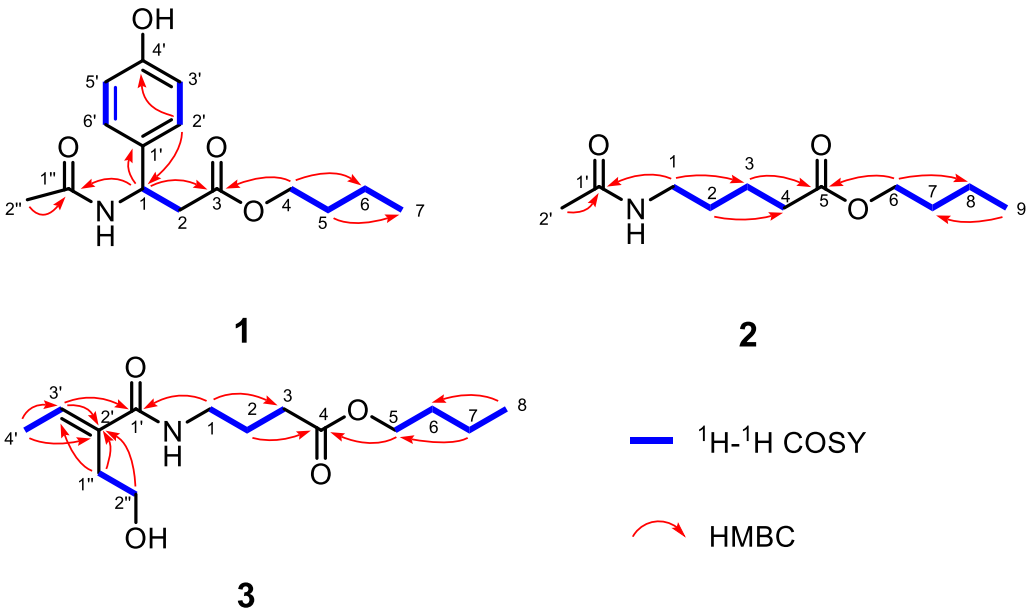


Fig. 2 Key ^{1}H–^{1}H COSY and HMBC correlations of compounds **1**–**3**

To sum up, the structure of **3** was confirmed and named as alteralkaloid C.

Three known compounds were confirmed as lobechine (**4**) [17], 4-[2-formyl-5-(methoxymethyl)-1*H*-pyrrol-1-yl]butanoic acid (**5**) [18], and 2-furanmethanol-(5′→11)-1,3-cyclopentadiene-[5,4-*c*]-1*H*-cinnoline (**6**) [19] by comparing their NMR data with the literatures.

Compounds **1**–**6** were tested for the cytotoxicity against six cell lines, including five human cancer cell

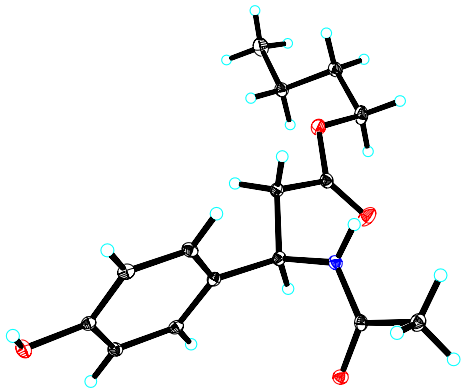


Fig. 3 X-Ray crystallographic structure of **1**

lines (HepG2, Hep3B, HT-29, HeLa, and OCVAR) and the normal liver cell LO2. Unfortunately, no compound showed obvious cytotoxicity against the above cell lines (IC_{50} > 40 μM) (Additional file 1).

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s13659-023-00391-2.

Additional file 1. Includes 1D and 2D NMR, HRESIMS, UV, and IR of compounds **1**–**3**.

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Declarations

Competing interests
The authors declare no competing interests.

Author details
[1]Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, School of Pharmacy, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China. [2]Department of Pharmacy, Union Hospital, Tongji Medical college, Huazhong University of Science and Technology, Wuhan 430022, China. [3]Department of Pharmacy, Tongji Hospital, Tongji Medical college, Huazhong University of Science and Technology, Wuhan 430033, China.

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