Chinese Herbal Medicines 16 (2024) 235–238

Contents lists available at ScienceDirect

Chinese Herbal Medicines

journal homepage: www.elsevier.com/locate/chmed

Original Article

New indole derivatives from endophytic fungus *Colletotruchum* sp. HK-08 originated from leaves of *Nerium indicum*

Huiqin Chen [a,b,1], Hao Zheng [a,b,1], Caihong Cai [a,b], Hao Wang [a,b], Cuijuan Gai [a,b], Zhiqiong Tan [c], Haofu Dai [a,b,*], Wenli Mei [a,b,*]

[a] *Hainan Key Laboratory of Research and Development of Natural Product from Li Folk Medicine, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China*
[b] *Hainan Institute for Tropical Agricultural Resources, Haikou 571101, China*
[c] *School of Life and Pharmaceutical Sciences, Hainan University, Haikou 570228, China*

ARTICLE INFO

Article history:
Received 16 April 2023
Revised 24 May 2023
Accepted 18 July 2023
Available online 18 January 2024

Keywords:
Colletotruchum sp.
endophytic fungus
indole derivatives
Nerium indicum Mill.
secondary metabolites

ABSTRACT

Objective: To study secondary metabolites from endophytic fungus *Colletotruchum* sp. HK-08 originated from the leaves of *Nerium indicum*.
Methods: The compounds were isolated by various column chromatographic techniques, and their structures were elucidated by spectroscopic techniques [high resolution electrospray ionization mass spectroscopy (HRESIMS), one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance spectroscopy (NMR)], as well as comparison with literature data. The Ellman method was used to determine the acetylcholinesterase (AChE) inhibitory activity.
Results: Four indole derivatives were identified from *Colletotruchum* sp. HK-08, including 6′-hydroxymonaspiloindole (**1**), 2-(2-oxoindolin-3-yl)ethyl 2-(4-hydroxyphenyl) acetate (**2**), 2-(2-oxoindolin-3-yl) ethyl 2-(2-hydroxyphenyl)acetate (**3**), and monaspiloindole (**4**). Compound **4** presented weak AChE inhibitory activity with IC_{50} value of (69.30 ± 6.27) μmol/L [tacrine as the positive control, with IC_{50} value of (0.61 ± 0.07) μmol/L].
Conclusion: Compounds **1–3** were new compounds, and compound **4** had weak AChE inhibitory activity.

1. Introduction

Endophytic fungi are a group of fungi living in plants tissues and keeping company with part or whole plants life cycle without causing any noticeable symptoms of infection (Bacon & White, 2000; Porras-Alfaro & Bayman, 2011). Due to the long period of co-evolution, an interdependent relationship between endophytes and their hosts has been established, which also shape peculiar natural product patterns of the endophytic fungi, and some of them play significant roles in increasing host resistance to diseases, improving defense mechanisms, as well as in ecosystem processes (Aly, Debbab, & Proksch, 2013; Faeth & William, 2002; Soliman & Raizada, 2013; Sun et al., 2011). Therefore, diversity endophytic fungi exited in plants have become one of the most important sources of natural products. Herein, the ongoing phytochemical study on the endophytic fungus *Colletotruchum* sp. HK-08 from the leaves of *Nerium indicum* Mill. has led to identify three new indole derivatives, together with one known analogue (Fig. 1).

2. Materials and methods

2.1. General experimental procedures

1D and 2D NMR spectra were recorded on Bruker AV III spectrometer (Bruker, Bremen, Germany) at 500 MHz (^{1}H) or 125 MHz (^{13}C), using tetramethylsilane (TMS) as an internal standard. High resolution electrospray ionization mass spectroscopy (HRESIMS) spectra were obtained by an API QSTAR Pulsar mass spectrometer (Bruker, Bremen, Germany). Circular dichroism (CD) data were determined by a JASCO J-715 spectrophotometer (Biologic, Paris, France). Optical rotations were collected using a Rudolph Autopol III polarimeter (Anton Paar, Graz, Austria). Semi-preparative high performance liquid chromatography (HPLC) was carried out using a Agilent Technologies 1260 Infinity equipped with a Agilent DAD G1315D detector (Agilent, Palo Alto, USA) by a reverse-phased column (YMC-packed C_{18},

* Corresponding authors.
E-mail addresses: daihaofu@itbb.org.cn (H. Dai), meiwenli@itbb.org.cn (W. Mei).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.chmed.2023.07.004
1674-6384/

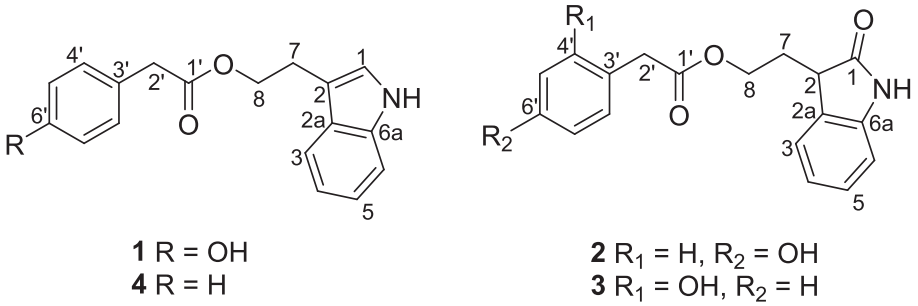

Fig. 1. Structures of compounds **1**–**4**.

250 mm × 10 mm, 5 μm). Silica gel (60–80, 200–300 mesh, Qingdao Marine Chemical Co., Ltd., Qingdao, China), octadecylsilyl (ODS) gel (20–45 μm, Fuji Silysia Chemical Co., Ltd., Nagoya, Japan) and Sephadex LH-20 (Merck, Darmstadt, Germany) were used for column chromatography. Thin-layer chromatography (TLC) was conducted on precoated silica gel G plates (Qingdao Marine Chemical Co., Ltd., Qingdao, China).

2.2. Fungal materials

Colletotruchum sp. HK-08 was isolated from the leaves of *N. indicum*, collected from Haikou, China, in June 2018. The identification of the fungus was performed according to a molecular biological protocol by DNA amplification and sequencing of the internally transcribed spacer (ITS) region. The voucher strain was deposited in the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, where a voucher specimen (No. 20180625) was deposited.

2.3. Extract and isolation

The fermentation was performed on solid rice for 30 d. The culture was diced and extracted with ethyl acetate (EtOAc). The crude extract (198.0 g) was suspended in 95% methanol (MeOH), and partitioned with petroleum ether (PE) to remove fatty acid. The MeOH part was concentrated, and subjected to vacuum liquid chromatography (VLC) using a step gradient of PE-EtOAc (100:1–0:1, volumn percent), to yield 15 fractions (Frs. 1–15). Fr. 8 (7.0 g) was applied to Sephadex LH-20 eluted with MeOH to give 10 fractions (Frs. 8.1–10). Fractionation of Fr. 8.7 (912.0 mg) on silica gel (PE: EtOAc 40:1–1:1, volumn percent) afforded Frs. 8.7.1–8.7.8. Fr. 8.7.3 (120.2 mg) was separated with silica gel (PE: EtOAc 20:1–1:1, volumn percent) to get compound **4** (20.0 mg). Fr. 8.7.4 (120.2 mg) was separated with silica gel (PE: acetone 30:1–1:1, volumn percent) to get compound **1** (0.9 mg). Fr. 10 (6.2 g) was submitted to Sephadex LH-20 (MeOH as eluent) to obtain 14 fractions. Fr. 10.5 (357.5 mg) was separated by Sephadex LH-20 ($CHCl_3$: MeOH 1:1, volumn percent), and then purified by semi-preparative HPLC [C_{18} column, acetonitrile (MeCN): H_2O 58:42, volumn percent as eluent, flow rate = 4 mL/min, wavelength 220, 265 nm] to give compound **3** (2.0 mg, t_R = 8.2 min). Fr. 10.12 (501.2 mg) was separated by repeated silica gel, and PE: EtOAc 30:1–1:1 (volumn percent) and PE: EtOAc 15:1–1:1 (volumn percent) as eluent respectively, to obtain compound **2** (5.0 mg).

2.4. Acetylcholinesterease (AChE) inhibitory activity

The Ellman method was used to determine the AChE inhibitory activity (Ellman et al., 1961). AChE from Electrophorus electricus was purchased from Sigma-Aldrich Co., Ltd., (Saint Louis, USA). Briefly, the samples were dissolved in dimethyl sulfoxide (DMSO) at the highest final concentration of 200 μmol/L, and then 2-fold diluted to get a serial of concentrations. 10 μL sample and 150 μL phosphate buffer (pH 8.0), together with 20 μL AChE solution (0.1 U/mL) were mixed in the 96-well plate, and incubated in 30 °C for 15 min. Subsequently, 10 μL 5,5′-dithiobis (2-nitrobenzoic acid) (2 mmol/L) and 10 μL *S*-acetylthiocholine iodide (10 mmol/L) were added, and incubated for another 30 min. The absorbance (A) values were measured at 412 nm using a microplate reader. DMSO was used as a negative control. Tacrine was used as reference compound. All assays were performed in triplicate. The percentage inhibition was calculated using the following equation: Inhibition (%) = $(A_{control} - A_{compound})/(A_{control} - A_{blank}) \times 100\%$.

3. Results and discussion

3.1. Compounds isolation and identification

Compound **1** was obtained as brown solid. The molecular formula was established as $C_{18}H_{17}NO_3$ based on the HRESIMS pseudo-molecular ion peak at *m*/*z* 318.102 6 [M + Na]$^+$ (calcd for $C_{18}H_{17}NO_3Na$, 318.100 1). By analysis of the 1D and 2D NMR, the structure presented highly similarity to that of monaspiloindole (Cheng et al., 2008), except for the substituent on the aromatic ring. Accordingly, 2-(1*H*-indol-3-yl)ethoxy fragment was determined by the signals in ^{1}H and ^{13}C-NMR (Tables 1 and 2), displaying one aromatic ring [δ_C 127.6 (C-2a); δ_C 118.9/δ_H 7.62 (d, *J* = 7.9 Hz, H-3); δ_C 119.6/δ_H 7.13 (t, *J* = 7.8 Hz, H-4); δ_C 122.2/δ_H 7.20 (t, *J* = 7.5 Hz, H-5); δ_C 111.3/δ_H 7.35 (d, *J* = 8.1 Hz, H-6); δ_C 136.3 (C-6a)], one double bond [δ_C 122.2/δ_H 6.91 (d, *J* = 2.0 Hz, H-1); δ_C 112.0 (C-2)], one NH group [δ_H 8.0 (br s, NH)], and two ethyl groups [δ_C 24.8/δ_H 3.09 (t, *J* = 7.0 Hz, H_2-7); δ_C 65.2/δ_H 4.38 (t, *J* = 7.0 Hz, H_2-8)]. Furthermore, heteronuclear multiple-bond (HMBC) correlations (Fig. 2) from H-1 to C-2, C-2a and C-6a; from H-3 to C-2, C-5 and C-6a; from H-6 to C-2a and C-4; from H_2-7 to C-1, C-2, C-2a and C-8; from H_2-8 to C-2 and C-7; as well as ^{1}H–^{1}H correlated spectrometry (^{1}H–^{1}H COSY) correlations of H-3/H-4/H-5/H-6, and H_2-7/H_2-8 confirmed the 2-(1*H*-indol-3-yl)ethoxy fragment. The remaining NMR signals appearing in the spectra consisted the 4-hydroxyphenylacetyl fragment, which included one typical AA'BB' aromatic system [δ_C 130.6/δ_H 7.09 (d, *J* = 8.5 Hz, H-4′, 8′); δ_C 115.6/δ_H 6.73 (d, *J* = 8.5 Hz, H-5′, 7′); δ_C 126.2 (C-3′); δ_C 154.9 (C-6′)], one ethyl group [δ_C 40.7/δ_H 3.57 (s, H-2′)], and one carbonyl group [δ_C 172.4 (C-1′)], and supported by the HMBC correlations from H-2′ to C-1′, C-3′, C-4′ and C-8′. Finally, the two fragments were connected by the HMBC correlation of H-8/C-1′. Thus, the structure of compound **1** was elucidated, and named as 6′-hydroxymonaspiloindole.

Compound **2** was isolated as yellow oil. Its molecular formula was deduced to be $C_{18}H_{17}NO_4$ based on the HRESIMS

Table 1
^{1}H-NMR spectroscopic data for compounds **1–3** (500 MHz, δ in ppm, J in Hz).

Positions	δ and J		
	1[a]	**2**[b]	**3**[b]
1	6.91, d (2.0)		
2		3.54, m	3.55, m
3	7.62, d (7.9)	7.23, d (7.7)	7.24, d (7.5)
4	7.13, t (7.8)	7.02, t (7.2)	7.01, t (7.5)
5	7.20, t (7.5)	7.22, t (8.1)	7.21, t (7.7)
6	7.35, d (8.1)	6.90, d (7.7)	6.90, d (7.7)
7	3.09, t (7.0)	2.28, m	2.30, dt (12.8, 6.4)
	3.09, t (7.0)	2.28, m	2.19, dq (13.6, 6.5)
8	4.38, t (7.0)	4.22, dt (11.6, 6.0)	4.27, dt (12.2, 6.2)
	4.38, t (7.0)	4.08, m	4.15, dd (12.2, 5.4)
2′	3.57, s	3.40, d (3.7)	3.52, d (2.2)
4′	7.09, d (8.5)	7.02, d (8.4)	
5′	6.73, d (8.5)	6.70, d (8.4)	6.74, d (7.5)
6′			7.06, t (7.5)
7′	6.73, d (8.5)	6.70, d (8.4)	6.75, t (7.4)
8′	7.09, d (8.5)	7.02, d (8.4)	7.07, d (7.4)
NH	8.0, br s		

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

Table 2
^{13}C-NMR spectroscopic data for compounds **1–3** (125 MHz, δ in ppm).

Positions	δ		
	1[a]	**2**[b]	**3**[b]
1	122.2	181.7	182.1
2	112.0	44.5	44.4
2a	127.6	130.1	130.3
3	118.9	125.3	125.4
4	119.6	123.4	123.3
5	122.2	129.2	129.1
6	111.3	110.9	110.9
6a	136.3	143.6	143.6
7	24.8	29.7	30.2
8	65.2	62.6	62.5
1′	172.4	173.9	173.9
2′	40.7	40.9	36.6
3′	126.2	126.2	122.5
4′	130.6	131.4	156.7
5′	115.6	116.3	115.8
6′	154.9	157.7	129.4
7′	115.6	116.3	120.4
8′	130.6	131.4	132.1

[a] Measured in $CDCl_3$.
[b] Measured in CD_3OD.

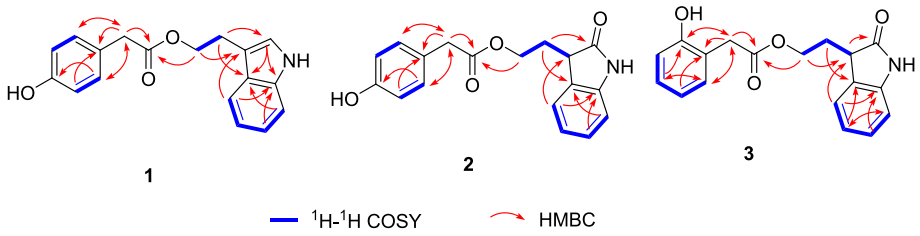

Fig. 2. Key ^{1}H–^{1}H COSY and HMBC correlations of compounds **1**–**3**.

pseudomolecular ion peak at m/z 334.104 4 $[M + Na]^+$ (calcd for $C_{18}H_{17}NO_4Na$, 334.105 0). The ^{1}H and ^{13}C-NMR (Tables 1 and 2) presented the structural similarity with that of **1**, with the only difference on the indole ring. Further analysis found that the double bond on the indole ring was disappear, and appeared one methine [δ_C 44.5/δ_H 3.54 (m, H-2)] and one carbonyl group [δ_C 181.7 (C-1)]. The ^{1}H–^{1}H COSY correlations (Fig. 2) of H-2/H_2-7 (δ_H 2.28)/H_2-8 (δ_H 4.22, 4.08), together with the HMBC correlations from H-2 to C-1; from H-3 (δ_H 7.23) to C-2 (δ_C 44.5) and C-6a (δ_C 143.6); as well as from H_2-7 to C-1, C-2 and C-2a (δ_C 130.1) confirmed the indolin-2-one instead of indole ring in the structure. Therefore, the structure was identified as 2-(2-oxoindolin-3-yl) ethyl 2-(4-hydroxyphenyl)acetate.

Compound **3** was afforded as yellow oil, possessing the molecular formula of $C_{18}H_{17}NO_4$ determined from HRESIMS data which showed a pseudomolecular ion peak at m/z 334.105 3 $[M + Na]^+$

($C_{18}H_{17}NO_4Na$, 334.105 0). The structure of **3** was showed highly similarity with that of **2**, except for the position of hydroxy group on the aromatic ring. The typical 1,2-disubstituted aromatic ring presented in ^{1}H-NMR spectrum [δ_H 6.74 (d, J = 7.5 Hz, H-5′); δ_H 7.06 (t, J = 7.5 Hz, H-6′); δ_H 6.75 (t, J = 7.4 Hz, H-7′); δ_H 7.07 (d, J = 7.4 Hz, H-8′)], and HMBC correlations from H-2′ (δ_H 3.52) to C-3′ (δ_C 122.5), C-4′ (δ_C 156.7) and C-8′ (δ_C 132.1) confirmed the 2-hydroxyphenylacetyl fragment. Thus, the compound was identified as 2-(2-oxoindolin-3-yl)ethyl 2-(2-hydroxyphenyl)acetate.

3.2. AChE inhibitory activity

All the isolated compounds were tested their AChE inhibitory activity, and the results showed that only compound **4** presented weak activity with IC_{50} value of (69.30 ± 6.27) μmol/L [tacrine as the positive control, with IC_{50} value of (0.61 ± 0.07) μmol/L].

4. Conclusion

Four indole derivatives including three new compounds were isolated from the endophytic fungus *Colletotruchum* sp. HK-08, which originated from the leaves of *N. indicum*. Compound **4** presented weak AChE inhibitory activity.

CRediT authorship contribution statement

Huiqin Chen: Data curation, Formal analysis, Visualization, Writing – original draft. **Hao Zheng:** Data curation, Formal analysis, Visualization. **Caihong Cai:** Data curation, Visualization. **Hao Wang:** Methodology, Data curation. **Cuijuan Gai:** Methodology, Data curation. **Zhiqiong Tan:** Project administration. **Haofu Dai:** Project administration. **Wenli Mei:** Supervision.

Declaration of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared in influence the work reported in this paper.

Acknowledgements

This study was supported by the Hainan Natural Science Foundation (No. 220RC702).

Appendix A. Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.chmed.2023.07.004.

References

Aly, A. H., Debbab, A., & Proksch, P. (2013). Fungal endophytes-secret producers of bioactive plant metabolites. *Pharmazie, 68*(7), 499–505.
Bacon, C. W., & White, J. (2000). *Microbial endophytes*. CRC Press.
Cheng, M. J., Wu, M. D., Chen, I. S., & Yuan, G. F. (2008). Secondary metabolites from the mycelia of the fungus *Monascus pilosus* BCRC38072. *Chemical Pharmaceutical Bulletin, 56*(3), 394–397.
Ellman, G. L., Courtney, K. D., Andres, V. J. R., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology, 7*(2), 88–95.
Faeth, S. H., & William, F. F. (2002). Fungal endophytes: common host plant symbionts but uncommon mutualists. *Integrative and Comparative Biology, 42*(2), 360–368.
Porras-Alfaro, A., & Bayman, P. (2011). Hidden fungi, emergent properties: Endophytes and microbiomes. *Phytopathology, 49*, 291.
Soliman, S. S. M., & Raizada, M. N. (2013). Interactions between co-habitating fungi elicit synthesis of taxol from an endophytic fungus in host *Taxus* plants. *Frontiers in Microbiology, 4*(3), 1–3.
Sun, X., Guo, L. D., & Hyde, K. (2011). Community composition of endophytic fungi in *Acer truncatum* and their role in decomposition. *Fungal Diversity, 47*(1), 85–95.