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Two New Alkaloids from a Marine-derived Fungus *Neosartorya fischeri*

Bin Wu[1], Gang Chen[2], Zhuo-gang Liu[1*] and Yuhu Pei[2]

[1] *Department of Hemotology, Shengjing Hospital of China Medical University, Shenyang 110016, China*

[2] *School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University, Shenyang 110016, China*

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Abstract: Investigation of EtOAc extract from the fermentation broth of the fungus *Neosartorya fischeri* led to the isolation of two novel alkaloids and one known compound with antitumor activity against HL-60 cell lines. Their structures were elucidated mainly by NMR and HR-TOF-MS, as well as on comparison with the reported data.

Keywords: *Neosartorya fischeri*; fungus; HL-60.

1. Introduction

Neosartorya is a teleomorphic (sexual) state of *Aspergillus* section. In recent years, many new bioactive compounds were found as secondary metabolites produced by *Neosartorya* species, such as sartorymensin, a new indole alkaloid, and new analogues of tryptoquivaline and fiscalins produced by *Neosartorya siamensis* [1] as well as fischerindoline, a pyrroloindole sesquiterpenoid isolated from *Neosartorya pseudofischeri*, with in vitro growth inhibitory activity in human cancer cell lines [2]. Therefore, the prospects are great to find new compounds for drugs candidates from *Neosartorya* species. We focused on the constituents of the *Neosartorya fischeri*. isolated from the marine mud sample collected in Hainan province of China in August 2009. The fermentation broth of the fungus *Neosartorya fischeri* showed strong cytotoxic activity against HL-60 cell lines with IC_{50} value of 6.25 μg. Herein, we report the isolation and structural elucidation of the new compounds **1** and **2** as well as their cytotoxic activities against HL-60 cell lines.

2. Materials and Methods

2.1. Microorganism Material

The Strain of *Neosartorya fischeri* was isolated from marine mud in the intertidal zone of Hainan Province of China in 2009 and identified by DNA extractions and PCR amplifications by researcher

* Corresponding author: E-Mail: liu_zhuogang@163.com; Phone: 086-24-2389-2617

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Xin Wu. The strain has been deposited in the School of Traditional Chinese Materia Medica, Shenyang Pharmaceutical University.

2.2 Fermentation and Isolation

The initial cultures were maintained on the potato dextrose agar isolation medium (extract of potato in 20 %, glucose 2 %, agar 2 %, fins salt company 3.3 %). Then, the mycelia were cut and aseptically transferred to a 250-mL Erlenmeyer flask containing 100 mL of culture media (barley sugar 2 %, ajinomoto 1 %, glucose 1 %, yeast extract 0.3 %, steep water 0.1%, mannitol 2 %, KH_2PO_4 0.05 %, $MgSO_4 \cdot 7H_2O$ 0.03 %, $CaCO_3$ 0.2 %, fins salt company 3.3%, pH 6.5). The flask was incubated at 28℃ on a rotary shaker, with 180 rpm, for 8 days.

The fermentation broth of the *Neosartorya fischeri* (80 L) was separated into the mycelial mass and the aqueous layer. The aqueous layer was concentrated to 1000 mL and extracted with ethyl acetate to give the crude extract (23 g). The mycelial mass was ultrasonically processed with acetone to get the crude extract (75 g). By the detection of thin-layer chromatography, the ethyl acetate soluble fraction was very similar with the acetone crude extract, so two fractions were combined to afford the crude extract (98 g). The crude extract was subjected to silica gel column, eluted with $CHCl_3$-CH_3OH (100:1→0:1), yielding eleven fractions. Fraction five (11 g) was subjected to column chromatography on silica gel and eluted with petroleum ether-acetone (100:0 - 1:1) to give seven subfractions. Subfraction six (1.5 g) was purified by Sephadex LH-20 column chromatography ($CHCl_3$:CH_3OH; 1:1) and preparative HPLC (CH_3CN-H_2O 37:63, flow rate 4 mL/min, wavelength 210 nm) to obtain compound **1** (6 mg, retention time 25 min), compound **2** (10 mg, retention time 35 min) and compound **3** (12 mg, retention time 40 min)

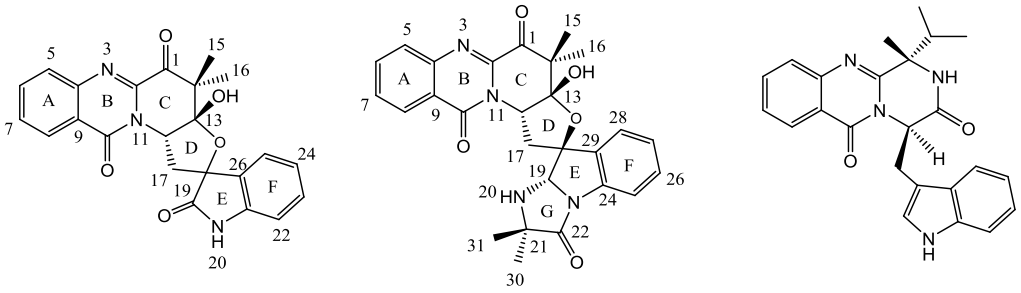


Figure 1. Structures of compounds **1-3**

3. Results and Discussion

3.1. Structure elucidation

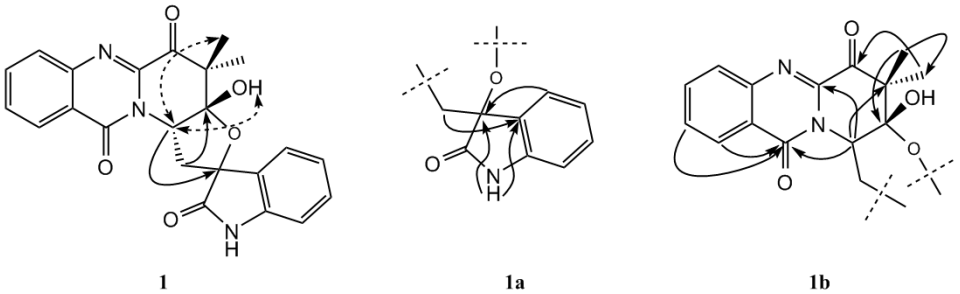
Investigation of EtOAc extract from the fermentation broth of the endophytic fungus *Neosartorya fischeri* led to the isolation of two new compounds, tryptoquivaline T (**1**), tryptoquivaline U (**2**) and one known compound fiscalin B (**3**) [3,4]. All of them were isolated for the first time from the strain. The bioactivity of compounds **1**-**3** on apoptosis of HL-60 cells were test with the same procedure as we described previously [5] and compounds **1**, **2** and **3** show the IC_{50} values at 82.3, 90.0, 8.88 μM respectively.

Compound **1** was obtained as a white amorphous powder, with $[\alpha]^{20}_{,D}$ -125.0 (*c* 5.5, MeOH). The molecular formula was determined to be $C_{23}H_{19}N_3O_5$ by HRESIMS at *m/z* 418.1397 $[M+H]^+$. The IR spectrum showed vibration peaks due to a hydroxyl group (3420 cm^{-1}), an aromatic ring (1470 and 1620 cm^{-1}), carbonyl groups (1734 cm^{-1}) and amide groups (1663 cm^{-1}).

^1H NMR spectrum of **1** gave two 1,2-disubstituted benzyl systems at δ_H 7.56 (dd, *J* = 0.9, 7.5 Hz), 7.81 (td, *J* = 7.8, 1.5 Hz), 8.00 (d, *J* = 8.1 Hz), 8.28 (dd, *J* = 7.8, 0.9 Hz) and δ_H 6.51 (d, *J* = 7.5 Hz), 6.94 (dd, *J* = 0.9, 7.8 Hz), 7.14 (t, *J* = 7.5 Hz), 7.33 (d, *J* = 7.5 Hz), respectively. And two methyl groups at δ 1.29 (3H, s), 1.39 (3H, s) were also observed in the ^1H NMR spectrum. The ^{13}C NMR

spectrum gave one ketal carbon signal at δ 107.4, three carboxyl groups at δ 161.5, 177.1, 191.2 and thirteen sp^2 carbon signals at δ 110.6, 122.3, 123.2, 124.8, 126.7, 128.7, 129.1, 129.5, 130.5, 134.8, 141.2, 144.2, 146.6, confirming the existence of two 1,2- disubstituted benzyl systems.

Among the NMR signals above, the NMR signals which were assigned to one 1,2-disubstitued benzyl system, determined by the HSQC spectrum at δc 141.2, 110.6 (δ_H 6.51), 130.5 (δ_H 7.14), 123.2 (δ_H 6.94), 124.8 (δ_H 7.33), 129.1 and one carboxyl group at δc 177.1 were almost identical with those of the corresponding moiety in the literature [1]. And the existence of fragment **1a** in compound **1** was confirmed by the correlations in the HMBC spectrum from H-25 at δ_H 7.33 to C-18 at δc 84.3; from NH at δ_H 8.31 to C-18 at δc 84.3, C-26 at δc 129.1; from H-17 at δ_H 2.80 to C-26 at δc 129.1. The other 1,2-disubstituted benzyl system determined by the HSQC spectrum at δc 141.2, 110.6 (δ_H 6.51), 130.5 (δ_H 7.14), 123.2 (δ_H 6.94), 124.8 (δ_H 7.33), 129.1 and one carboxyl group at δc 177.1 indicated the existence of a quinazolin-4(3*H*)-one moiety with a substitution at pyrone ring compared with the literature [1]. And the existence of fragment **1b**, a quinazolin-4(3*H*)-one moiety combined with a pyran ring, in compound **1** was elucidated by the correlations in the HMBC spectrum from H-15 at δ_H 1.29 to C-16 at δc 15.3, C-1 at δc 191.2 C-13 at δc 107.4; from H-12 at δ_H 5.21 to C-10 at δc 161.5, C-2 at δc 144.2; from H-6 at δ_H 7.81 and H-7 δ_H 7.56 at to C-10 at δc 161.5. Fragment **1a** and **1b** were connected to form the final structure by the correlations in HMBC sprectrum from H-17 δ_H 2.80 to C-13 at δc 107.4 and from H-12 at δ_H 5.21 to C-19 at δc 177.1.



1 **1a** **1b**

Figure 2. Fragments and key HMBC correlations of compound **1**

The relative configurations of C-2, 12 and C-13 were determined by analysis of the NOE spectrum, in which correlations between H-15 and H-11, -OH and H-11 were observed, suggesting the H-15, -OH and the H-11 were at the same plane of the structure. On the basis of the above evidence, compound **1** was elucidated as shown in Figure 1 and named tryptoquivaline T.

Compound **2** was obtained as a white amorphous powder, with $[\alpha]^{20}_{,D}$ -183.0 (*c* 5.5, MeOH). The molecular formula was determined to be $C_{27}H_{26}N_4O_5$ by HRESIMS at *m/z* 487.1976 $[M+H]^+$. The IR spectrum showed vibration peaks due to a hydroxyl group (3426 cm^{-1}), an aromatic ring (1468 and 1607 cm^{-1}) and amide groups (1682 cm^{-1}).

Compared with compound **1**, the NMR signals of compound **2** assignable to ring A to F were similar to those of compound **1** except the different chemical shift of the carbonyl groups located at ring E and two more methyl groups at δ_H 1.03, 1.06, two more carbon signals at δ_C 64.9, 82.0 were observed in the NMR spectrum of compound **2**. The HRESIMS of **2** revealed the existence of an extra nitrogen atom compared with those of **1**. Besides the evidence above, intensive analysis of the HMBC spectrum, in which correlations from H-30 at δ_H 1.03, H-31 at δ_H 1.06 to C-22 at δc 175.6 ; from H-28 at δ_H 7.64 to C-18 at δc 85.3; from H-19 at δ_H 4.99 to C-17 at δc 39.5 were observed, led to the elucidation of fragment **2b** and the structure of compound **2** was determined as shown in Figure 3.

The relative configuration of **2** was also elucidated by the analysis of the NOE spectrum. Correlations between H-15, hydroxyl signal of C-13 and H-11; H-11 and 17a; H-17b and H-28 revealed the relative configuration of **2** as shown in Figure 3 and compound **2** was named as tryptoquivaline U.

Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) data for compound **1** and **2** (in DMSO- d_6, δ in ppm, J in Hz).

compound **1**			compound **2**		
Position	δ_C	δ_H	Position	δ_C	δ_H
1	191.2	/	1	192.4	/
2	144.2	/	2	143.8	/
4	146.6	/	4	146.4	/
5	129.5	8.00 (d, 8.1)	5	129.0	7.91 (m)
6	134.8	7.81 (td, 7.8, 1.5)	6	134.8	7.91 (m)
7	128.7	7.56 (dd, 0.9, 7.5)	7	128.6	7.68 (m)
8	126.7	8.28 (dd, 7.8, 0.9)	8	126.3	8.23 (d, 7.8)
9	122.3	/	9	122.4	/
10	161.5	/	10	160.7	/
12	61.9	5.21 (d, 5.7）	12	61.3	4.93 (d, 8.4)
13	107.4	/	13	105.9	/
14	50.1	/	14	50.2	/
15	20.9	1.29 (s, 3H)	15	20.6	1.12 (s, 3H)
16	15.3	1.39 (s, 3H)	16	16.3	1.19 (s, 3H)
17a	40.7	2.80 (d, 15.0)	17a	39.5	2.64 (d, 15.3)
17b		3.15 (dd, 15.3, 6.0)	17b		3.55 (dd, 15.3, 6.6)
18	84.3	/	18	85.3	/
19	177.1	/	19	82.0	4.99 (d, 9.6)
20-NH	/	8.31 (s)	20-NH	/	2.36 (d, 9.6)
21	141.2	/	21	64.9	/
22	110.6	6.51 (d, 7.5)	22	175.6	/
23	130.5	7.14 (t, 7.5)	24	138.4	/
24	123.2	6.94 (dd, 0.9. 7.8)	25	114.9	7.30 (d, 7.2)
25	124.8.	7.33 (d, 7.5)	26	130.0	7.38 (t, 7.2)
26	129.1	/	27	125.2	7.24 (dd, 7.5, 7.2)
OH	/	3.80 (br. s)	28	126.6	7.64 (d, 7.5)
			29	136.6	/
			30	25.9	1.03 (s, 3H)
			31	25.2	1.06 (s, 3H）
			OH	/	6.91 (br s)

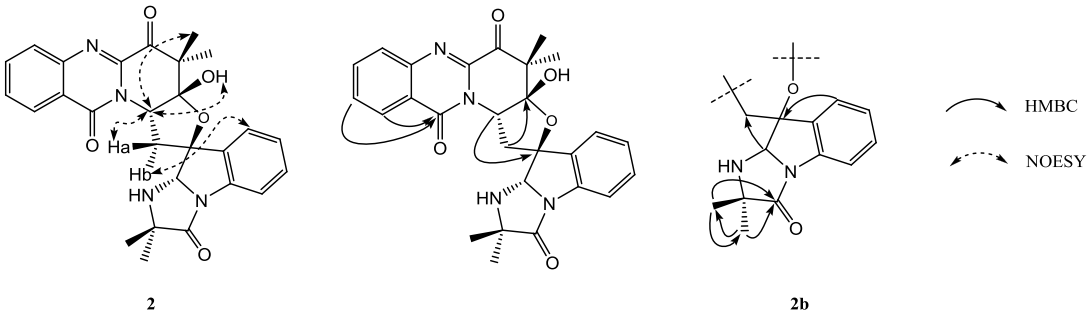


Figure 3. Fragments and key HMBC correlations of compound **2**

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/RNP

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