Chinese Journal of Oceanology and Limnology
Vol. 34 No. 5, P. 1072-1075, 2016
http://dx.doi.org/10.1007/s00343-016-5097-y

Phenylquinolinones with antitumor activity from the Indian Ocean-derived fungus *Aspergillus versicolor* Y31-2*

LI Peihai (李培海), FAN Yaqin (樊亚琴), CHEN Hao (陈颢)**,
CHAO Yaxi (晁雅熙), DU Ning (杜宁), CHEN Junhui (陈军辉)

Key Lab of Marine Bioactive Substances, *First Institute of Oceanography*, *State Oceanic Administration* (*SOA*), *Qingdao 266000*, *China*

Received Mar. 30, 2015; accepted in principle Jun. 8, 2015; accepted for publication Aug. 18, 2015

Abstract Two phenylquinolinones, including one new compound (**1**) and a previously isolated compound (**2**), were isolated from the ethyl acetate extracts of the fungus *Aspergillus versicolor* Y31-2, which was obtained from seawater samples collected from the Indian Ocean. The structures of these compounds were established by spectroscopic analyses. 4-(3-Hydroxyphenyl)-3-methoxyquinolin-2(1H)-one (**1**) exhibited moderate cytotoxicity against MCF-7 (human breast carcinoma cell line) and SMMC-7721 (human liver cancer cell line) cells with IC_{50} values of 16.6 and 18.2 μmol/L, respectively. To the best of our knowledge, this study represents the first reported account of the isolation of compounds **1** and **2** as the secondary metabolites of the seawater derived fungus *Aspergillus versicolor* from the Indian Ocean.

Keyword: phenylquinolinones; anti-tumor; *Aspergillus versicolor*

1 INTRODUCTION

Phenylquinolinones are a family of alkaloids that can be produced by *Penicillium* (Wei et al., 2011), *Aspergillus* (Fremlin et al., 2009) and the roots of several different plants (Abbadi et al., 1989). Compounds belonging to this family exhibit a wide range of interesting biological activities (Kobayashi and Harayama, 2009), as exemplified by the farnesyl transferase inhibitor developed by Pfizer as an anticancer agent, which is currently undergoing clinical trials (Andresen et al., 2004). Based on their interesting biological properties, phenylquinolinones have attracted considerable attention in recent years as potential drug candidates. As part of our research towards the isolation of bioactive compounds with novel structures from marine-derived fungi, we investigated the EtOAc extract of the fungal strain *Aspergillus versicolor*, which was isolated from seawater samples taken from the Indian Ocean. This study resulted in the isolation of one new phenylquinolinone derivative, 4-(3-hydroxyphenyl)-3-methoxyquinolin-2(1H)-one (**1**), as well as the analogue 3-O-methylviridicatin (**2**). Both of these compounds represent rare examples of naturally produced phenylquinolinones, which were characterized from the culture extract of the fungus. It is noteworthy that 3-O-methylviridicatin (**2**) has been reported to inhibit the replication of human immunodeficiency virus (HIV) with an IC_{50} of 2.5 μmol/L (Heguy et al., 1998). The structures of compounds **1** and **2** were established by spectroscopic analyses. The cytotoxic activities of these compounds were evaluated against five tumor cell lines, including MCF-7 (human breast carcinoma cell line), SMMC-7721 (human liver cancer cell line), HEK293 (human embryonic kidney cancer cell line), A549 (human pulmonary carcinoma) and T-47D (human ductal breast epithelial tumor cell line) cells. Compound **1** exhibited moderate cytotoxicity against MCF-7 and

* Supported by the National Basic Research Program of China (973 Program) (No. 2015CB755906), the National High Technology Research and Development Program of China (863 Program) (No. 2013AA092901-2), the China Ocean Mineral Resources Research and Development Association (No. DY125-15-T-07), the Public Welfare Project of SOA (No. 201205027), and the Basic Scientific Fund for National Public Research Institutes of China (No. GY0213G12)
** Corresponding author: hchen@fio.org.cn
LI Peihai and FAN Yaqin contributed equally to this work.

SMMC-7721 with IC_{50} values of 16.6 and 18.2 μmol/L, respectively. This paper describes the isolation, structural determination and cytotoxicity of the phenylquinolinone compounds isolated from this fungus.

2 EXPERIMENTAL

2.1 General experimental procedures

Ultraviolet (UV) spectra were recorded on an Eppendorf BioSpectrometer system (Eppendorf, Hamburg, Germany). ^{1}H nuclear magnetic resonance (NMR), ^{13}C NMR, distortionless enhancement by polarization transfer, heteronuclear multiple-quantum coherence, heteronuclear multiple-bond correlation (HMBC), correlation spectroscopy (COSY) and nuclear overhauser effect spectroscopy spectra were recorded using a JEOL JNM-ECP 600 spectrometer (JEOL, Tokyo, Japan). Mass spectra were determined on an Agilent Technologies G1969A mass spectrometer (Agilent, Santa Clara, USA). An analytical high performance liquid chromatography (HPLC) system (Hitachi, Tokyo, Japan), consisting of an organizer, UV detector (L-2400), pump (L-2130) and Hitachi software (D-2000) was used for the purification of the compounds with a C_{18} column (YMC-pack ODS-A, 150 mm×4.6 mm, I.D., S-5 μmol/L, 12 nm, 1 mL/min). Semipreparative HPLC purifications were conducted on the same system using a Prep RP-18 column (YMC-pack ODS-A, 250 mm×10 mm, I.D., S-5 μmol/L, 12 nm, 2.5 mL/min) with UV detection. Purifications by column chromatography were performed over Silica gel (200–300 mesh, Qingdao Marine Chemical Factory, Qingdao, China), Lobar LiChroprep RP-18 (40–63 mm, Merck, Darmstadt , Germany) and Sephadex LH-20 (Amersham Biosciences, Piscataway, USA).

2.2 Fungal material

The fungus *Aspergillus versicolor* Y31-2 was obtained from sea water samples collected from the Indian Ocean at a depth of about 30 m (88°0′6″E, 4°0′6″S), in 2013. The samples were cultured on Malt Extract Agar (MEA: Malt extract, 17 g; Peptone, 3 g; Agar, 20 g; sea water, 1 L) in a plate containing the antibiotic chloramphenicol (100 μg/mL). A single colony was transferred onto another MEA plate and was identified according to its morphological characteristics and 18S rRNA gene sequences (GenBank accession No. KP872504) (Fan et al., 2013). The pure reference culture was deposited in our laboratory at -80°C, whilst the producing strain was prepared on PDA slants and stored at 4°C.

2.3 Fermentation and extraction

The fungus *Aspergillus versicolor* Y31-2 was cultured under static conditions in 300 mL of fermentation medium in a 1-L conical flask containing 17 g of malt extract and 3 g of peptone per liter of sea water at 28°C for 30 days. The whole fermentation broth (15 L) of cultivated medium was extracted exhaustively with EtOAc, yielding 8.2 g of extract; the mycelia were extracted with 80% (v/v) aqueous acetone, yielding 4.2 g of extract (Fan et al., 2013). The extracts of the fermentation broth and the mycelia were combined because the analysis of these extracts by HPLC revealed that their chemical compositions were similar.

2.4 Purification

The combined extracts from *Aspergillus versicolor* Y31-2 were subjected to column chromatography over silica gel using a vacuum-liquid chromatography column, eluting with a stepwise gradient of petroleum ether (PE) and CH_2Cl_2 (2:1 and 0:1, v/v), followed by a stepwise gradient of CH_2Cl_2 and MeOH (100:1, 50:1, 30:1, 10:1, 1:1 and 0:1, v/v) to yield eight major primary fractions (Fr.1–8) (Fan et al., 2013). Fraction 6 was purified by reversed-phase C_{18} silica gel column chromatography eluting with a stepwise gradient of 10%–100% MeOH in H_2O to give 10 sub-fractions (Fr.6.1–6.10). Fraction 6.5 was further purified by Sephadex LH-20 eluting with MeOH to obtain compound **1** (4.4 mg). Fraction 6.6 was also resolved in a similar manner over Sephadex LH-20 eluting with MeOH, followed by HPLC purification to yield compound **2** (15 mg).

2.5 Cytotoxicity assay

The cytotoxic activities of compounds **1** and **2** were evaluated against five tumor cell lines: MCF-7 (human breast carcinoma cell line), SMMC-7721 (human liver cancer cell line), HEK293 (human embryonic kidney cancer cell line), A549 (human pulmonary carcinoma) and T-47D (human ductal breast epithelial tumor cell line) cells. The cytotoxic activities were determined according to a previously reported method (Bergeron et al., 1984).

Table 1 ^{1}H and ^{13}C NMR data of 1 in DMSO-d_6 and 2 in $CDCl_3$-*d* (at 600 and 150 MHz, respectively)

1			2		
Position	δ_C	δ_H (*J* in Hz)	Position	δ_C	δ_H (*J* in Hz)
1	-	-	1	-	12.63, brs
2	158.6, C	-	2	161.3, C	-
3	144.9, C	-	3	145.4, C	-
4	137.7, C	-	4	139.6, C	-
5	119.9, C	-	5	121.1, C	-
6	125.9, CH	7.05, d (8.0)	6	126.6, CH	7.22, d (8.0)
7	121.9, CH	7.09, t (7.5)	7	122.8, CH	7.12, t (7.5)
8	128.6, CH	7.42, t (7.1)	8	128.4, CH	7.44, t (7.1)
9	115.1, CH	7.37, d (8.1)	9	116.2, CH	7.49, d (8.1)
10	135.8, C	-	10	135.6, C	-
11	134.7, C	-	11	133.6, C	-
12	116.1, CH	6.69, s	12	128.4, CH	7.38, d (7.1)
13	157.7, C	-	13	129.6, CH	7.53, t (7.2)
14	115.1, CH	6.86, d (7.8)	14	129.0, CH	7.53, m
15	129.5, CH	7.31, t (8.0)	15	129.6, CH	7.53, t (7.2)
16	119.4, CH	6.70, d (7.0)	16	128.4, CH	7.38, d (7.1)
17	59.5, CH_3	3.70, s	17	60.5, CH_3	3.84, s

3 SPECTRAL DATA

4-(3-Hydroxyphenyl)-3-methoxyquinolin-2(1*H*)-one (1)

White amorphous powder; UV (MeOH) λ_{max} 203, 221, 279 and 322 nm; ^{1}H NMR (DMSO-d_6, 600 MHz): δ 7.05(1H, d, *J*=8.0 Hz, H-6), 7.09 (1H, t, *J*=7.5 Hz, H-7), 7.42(1H, t, *J* =7.1 Hz, H-8), 7.37 (1H, d, *J*=8.1 Hz, H-9), 6.69(1H, s, H-12), 6.86 (1H, d, *J*=7.8 Hz, H-14), 7.31 (1H, t, *J*=8.0 Hz, H-15), 6.70 (1H, d, *J*=7.0 Hz, H-16), 3.70 (3H, s, H-17). ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.6 (C, C-2), 144.9 (C, C-3), 137.7 (C, C-4), 119.9 (C, C-5), 125.9 (CH, C-6), 121.9 (CH, C-7), 128.6 (CH, C-8), 115.1 (CH, C-9), 135.8 (C, C-10), 134.7 (C, C-11), 116.1 (CH, C-12), 157.7 (C, C-13), 115.1 (CH, C-14), 129.4 (CH, C-15), 119.4 (CH, C-16), 59.5 (CH_3, C-17). High-resolution electrospray ionisation mass spectrometry *m*/*z* 268.0980 $[M+H]^+$ (calcd for $C_{16}H_{14}NO_3$, 268.097 4).

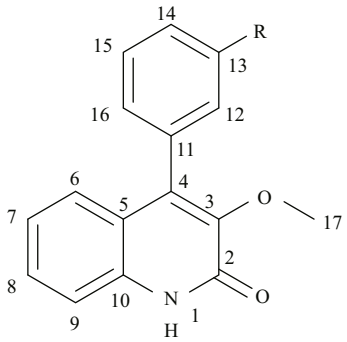
3-*O*-Methylviridicatin (2)

White amorphous powder; UV (MeOH) λ_{max} 203, 221, 279 and 322 nm; ^{1}H NMR ($CDCl_3$, 600 MHz): δ 7.22 (1H, d, *J*=8.0 Hz, H-6), 7.12 (1H, t, *J*=7.5 Hz, H-7), 7.44 (1H, t, *J*=7.1 Hz, H-8), 7.49 (1H, d, *J*=8.1 Hz, H-9), 7.38 (1H, d, *J*=7.1 Hz, H-12), 7.53 (1H, t, *J*=7.2 Hz, H-13), 7.53 (1H, m, H-14), 7.53 (1H, t, *J*=7.2 Hz, H-15), 7.38 (1H, d, *J*=7.1 Hz, H-16), 3.84 (3H, s, H-17). ^{13}C NMR (150 MHz, $CDCl_3$): δ 161.3 (C, C-2), 145.4 (C, C-3), 139.6 (C, C-4), 121.1 (C, C-5), 126.6 (CH, C-6), 122.8 (CH, C-7), 128.4 (CH, C-8), 116.2 (CH, C-9), 135.6 (C, C-10), 133.6 (C, C-11), 128.4 (CH, C-12), 129.6 (CH, C-13), 129.0 (CH, C-14), 129.6 (CH, C-15), 128.4 (CH, C-16), 60.5 (CH_3, C-17).

4 RESULT AND DISCUSSION

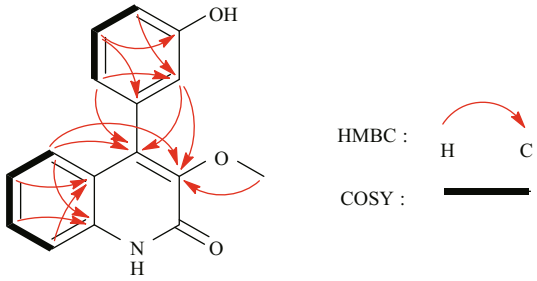
4.1 Structure identification of compound 1 and 2

Compound **1** was obtained as a white amorphous powder. The molecular formula of this compound was determined to be $C_{16}H_{13}NO_3$ by high-resolution electrospray ionisation mass spectrometry, which gave a peak with an *m*/*z* value of 268.098 0 $[M+H]^+$, indicating 11 degrees of unsaturation. The ^{1}H NMR, ^{13}C NMR and distortionless enhancement by polarization transfer spectra of compound **1** (Table 1) revealed the presence of twelve aromatic carbon atoms (eight methines and four quaternary carbons) with eight protons (three triplets, four doublets and one singlet). These signals were indicative of an *ortho*-disubstituted benzene ring system, as well as a 1,3-disubstituted benzene system. This deduction was further verified by the key COSY correlations between H-6 (δ_H 7.05, d, 8.0 Hz)/H-7 (δ_H 7.09, t, 7.5 Hz)/H-8 (δ_H 7.42, t, 7.1 Hz)/H-9 (δ_H 7.37, d, 8.1 Hz) and H-14 (δ_H 6.86, d, 7.8 Hz)/H-15 (δ_H 7.31, t, 8.0 Hz)/H-16 (δ_H 6.70, d, 7.0 Hz), together with the HMBC correlations from H-8 and H-6 to the quaternary carbon (C-10, δ_C 135.8); from H-9 and H-7 to the quaternary carbon (C-5, δ_C 119.9); from H-14 and H-16 to the methine C-12 (δ_C 116.1; δ_H 6.69, s); and from H-15 to the two quaternary carbons, one of which was oxygenated (C-11, δ_C 134.7; C-13, δ_C 157.7) (Fig.1). The ^{1}H and ^{13}C NMR spectra also revealed the presence of two aromatic carbons (one oxygenated) at δ_C 137.7 and 144.9, a methoxy group (δ_C 59.5; δ_H 3.70, s) and an amido bond at δ_C 158.6, which appeared to be conjugated to another double bond. Having accounted for 10 of 11 degrees of unsaturation in this molecule, we turned out attention to the final degree of unsaturated. With this in mind, we identified a planar structure, which was established by forming a hexatomic ring with the lactam, as evidenced by the HMBC correlations from H-6, H-12, H-16 to C-4 (δ_C



1 R=OH
2 R=H

Fig.1 Important HMBC and COSY correlations of 1



1

Fig.2 Structure of compounds 1 and 2

137.7), H-17 to C-3 (δ_C 144.9) and significant four-bond correlations from H-6, H-12 to C-3 (Fig.1). Compound **1** was therefore determined to be 4-(3-hydroxyphenyl)-3-methoxyquinolin-2(1H)-one (Fig.2).

The fungal metabolite 3-O-methylviridicatin (**2**) was identified by detailed spectroscopic analysis and, where possible, through a comparison of its spectroscopic data with literature data. Compound **2** was initially reported in 1963 following its isolation from a strain of *P. cyclopium* (Birkinshaw et al., 1963).

4.2 Anti-tumor activity of compounds 1 and 2

Compounds **1** and **2** were assayed for their cytotoxic activities against five tumor cell lines, including MCF-7, SMMC-7721, HEK293, A549 and T-47D cells. Compound **1** displayed cytotoxic activities against MCF-7 and SMMC-7721 cells with IC_{50} values of 16.6 and 18.2 μmol/L, respectively. Compared with adriamycin, which gave IC_{50} values of 1.2 and 1.5 μmol/L against MCF-7 and SMMC-7721 cells, compound **1** showed reasonable levels of cytotoxicity.

It is envisaged that compound **1** will also show anti-HIV activity because the related compound **2** has been reported to exhibit reasonable activity against HIV with an IC_{50} value of 2.5 μmol/L, although further work is required to determine the activity of this compound against HIV.

References

Abbadi N E, Weniger B, Lobstein A, Quirion J C, Anton R. 1989. New Alkaloids of *Chiococca alba*. *Planta Medica*, **55**(7): 603-604.

Andresen B M, Couturier M, Cronin B, D'Occhio M, Ewing M D, Guinn M, Hawkins J M, Jasys V J, LaGreca S D, Lyssikatos J P, Moraski G, Ng K, Raggon J W, Stewart A M, Tickner D L, Tucker J L, Urban F J, Vazquez E, Wei L L. 2004. Streamlined processes for the synthesis of a farnesyl transferase inhibitor drug candidate. *Org. Proc. Res. Dev.*, **8**(4): 643-650.

Bergeron R J, Cavanaugh P F, Kline S J, Hughes R G, Elliott G T, Porter C W. 1984. Antineoplastic and antiherpetic activity of spermidine catecholamide iron chelators. *Biochem. Biophys. Res. Commun.*, **121**(3): 848-854.

Birkinshaw J h, Luckner M, Mohammed Y S, Mothes K, Stickings C E. 1963. Studies in the biochemistry of micro-organisms. 114. viridicatol and cyclopenol, metabolites of *Penicillium Viridicatum* Westling and *Penicillium Cyclopium* Westling. *Biochemical Journal*, **89**(2): 196-202.

Fan Y Q, Wang Y, Liu P P, Fu P, Zhu T H, Wang W, Zhu W M. 2013. Indole-diterpenoids with Anti-H1N1 activity from the aciduric fungus *penicillium camemberti* OUCMDZ-1492. *Journal of Natural Products*, **76**(7): 1 328-1 336.

Fremlin L J, Piggott A M, Lacey E, Capon R J. 2009. Cottoquinazoline A and Cotteslosins A and B, metabolites from an Australian marine-derived strain of *Aspergillus Versicolor*. *Journal of Natural Products*, **72**(4): 666-670.

Heguy A, Cai P, Meyn P, Houck D, Russo S, Michitsch R, Pearce C, Katz B, Bringmann G, Feineis D, Taylor D L, Tyms A S. 1998. Isolation and characterization of the fungal metabolite 3-O-methylviridicatin as an inhibitor of tumour necrosis factor alpha-induced human immunodeficiency virus replication. *Antivir. Chem. Chemother.*, **9**(2): 149-155.

Kobayashi Y, Harayama T. 2009. A concise and versatile synthesis of viridicatin alkaloids from cyanoacetanilides. *Organic Letters*, **11**(7): 1 603-1 606.

Wei M Y, Yang R Y, Shao C L, Wang C Y, Deng D S, She Z G, Lin Y C. 2011. Isolation, structure elucidation, crystal structure, and biological activity of a marine natural alkaloid, viridicatol. *Chemistry of Natural Compounds*, **47**(2): 322-325.