Tetrahedron Letters 55 (2014) 2789–2792

Contents lists available at ScienceDirect

Tetrahedron Letters

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

Marine bacterial inhibitors from the sponge-derived fungus *Aspergillus* sp.

Yaming Zhou [a], Abdessamad Debbab [a], Victor Wray [b], WenHan Lin [c], Barbara Schulz [d], Rozenn Trepos [e], Claire Pile [e], Claire Hellio [e], Peter Proksch [a], Amal H. Aly [a,*]

[a] Institut für Pharmazeutische Biologie und Biotechnologie, Heinrich-Heine-Universität, Universitätsstrasse 1, D-40225 Düsseldorf, Germany
[b] Helmholtz Centre for Infection Research, Inhoffenstraße 7, D-38124 Braunschweig, Germany
[c] National Research Laboratories of Natural and Biomimetic Drugs, Peking University, Health Science Center, 100083 Beijing, People's Republic of China
[d] Institut für Mikrobiologie, Technische Universität Braunschweig, Spielmannstraße 7, D-31806 Braunschweig, Germany
[e] School of Biological Sciences, King Henry 1st Street, University of Portsmouth, Portsmouth PO1 2DY, UK

ARTICLE INFO

Article history:
Received 29 November 2013
Revised 15 February 2014
Accepted 18 February 2014
Available online 13 March 2014

Keywords:
Aspergillus
Marine natural products
Sponge-derived fungi
Tethya aurantium

ABSTRACT

Chromatographic separation of a crude extract obtained from the fungus *Aspergillus* sp., isolated from the Mediterranean sponge *Tethya aurantium*, yielded a new tryptophan derived alkaloid, 3-((1-hydroxy-3-(2-methylbut-3-en-2-yl)-2-oxoindolin-3-yl)methyl)-1-methyl-3,4-dihydrobenzo[*e*][1,4]diazepine-2,5-dione (**1**), and a new meroterpenoid, austalide R (**2**), together with three known compounds (**3**–**5**). The structures of the new compounds were unambiguously elucidated on the basis of extensive one and two-dimensional NMR (^{1}H, ^{13}C, COSY, HMBC, and ROESY) and mass spectral analysis. Interestingly, the compounds exhibited antibacterial activity when tested against a panel of marine bacteria, with **1** selectively inhibiting *Vibrio* species and **2** showing a broad spectrum of activity. In contrast, no significant activity was observed against terrestrial bacterial strains and the murine cancer cell line L5178Y.

Introduction

Prevention or treatment of bacterial disease outbreaks in aquacultures is a major challenge facing this industry.[1] For instance, destructive infections caused by bacteria of the genus *Vibrio* and those causing necrotizing hepatopancreatitis (NHP) are the main diseases commonly affecting shrimp farms.[2] Hence, there is a great need for new antibiotics to combat such diseases and the resulting stock loss, especially with the development of bacterial resistance to traditionally used antibiotics.[1]

A potential source of novel antibacterial compounds is marine-derived fungi, which have attracted considerable attention in recent years.[3–5] They have been isolated from virtually every possible marine habitat, including inorganic matter, microbial communities, plants, invertebrates and vertebrates. In particular, sponges have yielded numerous fungal strains, which have been reported to produce a variety of pharmacologically active and structurally diverse metabolites.[3,6–11] The need of these organisms to adapt and survive in an environment that is significantly different from that of terrestrial organisms may have shaped their natural product patterns resulting in many cases in the production of unique secondary metabolites.[12–15]

The chemical profiles of both terrestrial and marine *Aspergillus* species have been studied by several research groups, and a vast diversity of secondary metabolites with novel structures and interesting biological activities was already elucidated.[16–25] In continuation of our previous studies on the sponge-derived *Aspergillus* sp. strain, isolated from the Adriatic Sea sponge *Tethya aurantium*,[6,25] two new compounds, 3-((1-hydroxy-3-(2-methylbut-3-en-2-yl)-2-oxoindolin-3-yl)methyl)-1-methyl-3,4-dihydrobenzo[*e*][1,4]diazepine-2,5-dione (**1**) and austalide R (**2**), as well as the known compounds 8-*O*-4-dehydrodiferulic acid (**3**), cytochalasin Z17 (**4**), and dihydroisoflavipucine (**5**) (Fig. 1), were now isolated and identified. All compounds exhibited antibacterial activity against marine-derived strains, with **1** selectively inhibiting *Vibrio* species and **2** showing a broad spectrum of activity, which may raise the prospect of using such compounds as antifouling agents or to combat epizootics in aquaculture in the future.

Results and discussion

The crude ethyl acetate extract of the fungus *Aspergillus* sp. was subjected to repeated column chromatography, followed by semi

* Corresponding author. Tel.: +49 211 81 14173; fax: +49 211 81 11923.
E-mail address: amal.hassan@uni-duesseldorf.de (A.H. Aly).

http://dx.doi.org/10.1016/j.tetlet.2014.02.062
0040-4039/

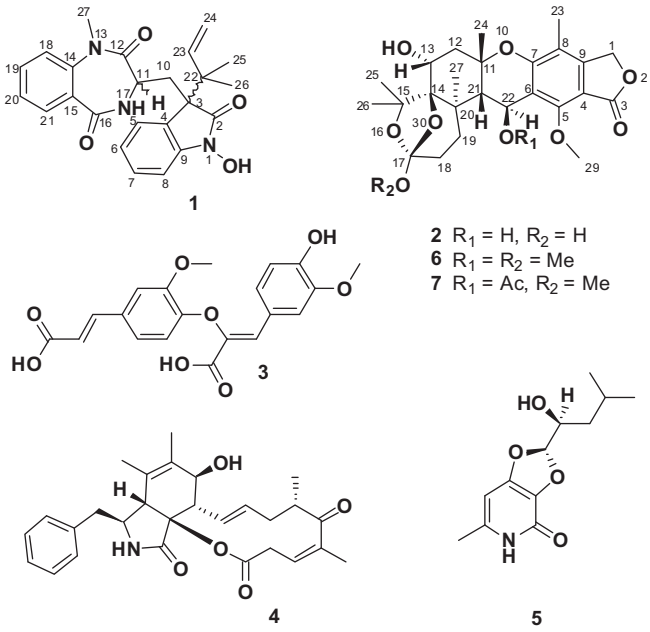

Figure 1. Structures of **1**–**7**.

preparative HPLC separation, to afford two new compounds (**1** and **2**), along with three known compounds (**3**–**5**) (Fig. 1).

The molecular formula of **1** was established as $C_{24}H_{25}N_3O_4$ on the basis of the $[M+H]^+$ signal at *m*/*z* 420.1917 in the HRESIMS. The UV absorbance bands observed at λ_{max} (MeOH) 214.1, 250.1, and 290.0 nm suggested the presence of an indoline chromophore.[26] The ^{1}H NMR and COSY spectra of **1** (Table 1) revealed the presence of eight aromatic protons corresponding to two ABCD spin systems resonating at δ_H 7.30, 6.90, 7.21, and 6.73 ppm (H-5 to H-8, respectively) and at δ_H 7.24, 7.50, 7.18, and 7.27 ppm (H-18 to H-21, respectively), an olefinic ABX spin system at δ_H 4.94/5.02 (H_2-24) and 6.07 (H-23) ppm, an aliphatic ABX spin system at δ_H 2.30/2.70 (H_2-10) and 2.90 (H-11) ppm, two geminal methyl groups at δ_H 0.98 ppm (H_3-25 and H_3-26), a nitrogen bearing methyl group at δ_H 3.15 ppm (H_3-27), and a NH group at δ_H 8.18 ppm (H-17).

Table 1
^{1}H, ^{13}C NMR, COSY, and HMBC data of **1** at 300 (^{1}H) and 100 (^{13}C) MHz (DMSO-d_6, δ in ppm, *J* in Hz)

Position	δ_H	δ_C	COSY	HMBC
1				
2		172.3		
3		54.7		
4		124.8		
5	7.30 d (7.8)	126.6	6	3, 7, 9
6	6.90 dt (0.1, 7.6)	120.8	5, 7	4, 7, 8
7	7.21 t (7.7)	128.0	6, 8	5, 8, 9
8	6.73 d (7.6)	106.3	7	4, 6, 9
9		142.7		
10	2.30 dd (7.7, 14.9) 2.70 dd (3.2, 14.9)	28.9	11	2, 3, 4, 11, 12, 22
11	2.90 br m	49.6	10, 17	
12		169.9		
13				
14		140.1		
15		128.2		
16		166.8		
17	8.18 d (5.9)		11	10, 11, 15
18	7.24 d (8.3)	121.6	19	15, 20
19	7.50 dt (1.7, 8.5)	132.0	18, 20	14, 18, 21
20	7.18 t (8.4)	125.1	19, 21	15, 18
21	7.27 d (8.3)	128.8	20	14, 16, 19
22		41.8		
23	6.07 dd (10.8, 17.4)	142.9	24	22, 25, 26
24	4.94 dd (0.1, 17.4) 5.02 dd (0.1, 10.9)	113.4	23	22, 23
25	0.98 s	21.3		3, 22, 23, 26
26	0.98 s	22.5		3, 22, 23, 25
27	3.15 s	34.9		12, 14

The ^{13}C NMR (Table 1) and DEPT spectra confirmed the presence of 24 carbon atoms in the structure of **1**, including one aliphatic and nine olefinic methine groups, one aliphatic and one olefinic methylene group, three methyl groups, as well as two aliphatic and seven olefinic quaternary carbon atoms, the latter including three amide carbonyl carbons resonating at δ_C 172.3, 169.9, and 166.8 ppm (C-2, C-12, and C-16, respectively). Furthermore, analysis of the HMQC spectrum allowed the assignment of proton signals to the corresponding proton bearing carbon atoms.

The identified spin systems of **1** were connected based on inspection of the HMBC spectrum (Table 1, Fig. 2). Correlations of the tertiary methyl group protons H_3-27 (δ_C 34.9 ppm) to the amide carbonyl C-12 and to C-14 (δ_C 140.1 ppm), of H-18 to C-15 (δ_C 128.2 ppm) and C-20, of H-21 to C-14, C-16, and C-19, of the amide proton H-17 to C-10 (δ_C 28.9 ppm), C-11 (δ_C 49.6 ppm), and C-15, and of H_2-10 to C-11 and C-12, established the 1-methyl-1,4-benzodiazepine-2,5-dione moiety of **1**. Further correlations of H_2-10 to C-2 (δ_C 172.3 ppm), C-3 (δ_C 54.7 ppm), C-4 (δ_C 124.8 ppm), and C-22 (δ_C 41.8 ppm), of H-5 to C-3, C-7, and C-9 (δ_C 142.7 ppm), and of H-8 to C-4, C-6 and C-9, corroborated the presence of an indolin-2-one moiety and revealed its connection with the 1,4-diazepine-2,5-dione ring through CH_2-10. The 2-methylbut-3-en-2-yl side chain was verified by correlations of both methyl groups CH_3-25 (δ_C 21.3 ppm) and CH_3-26 (δ_C 22.5 ppm) to each other and to C-3, C-22, and C-23 (δ_C 142.9 ppm), of the olefinic proton H-23 to C-22, C-25, and C-26, and of H_2-24 (δ_C 113.4 ppm) to C-22 and C-23. The hydroxyl group was located at N-1 as the assignment of all other atoms was completed, and based on comparison of observed chemical shift values with those reported for similar 1-hydroxyindolin-2-one substructures.[27] Hence, **1** was determined as a novel metabolite with an unusual structural framework and named 3-((1-hydroxy-3-(2-methylbut-3-en-2-yl)-2-oxoindolin-3-yl)methyl)-1-methyl-3,4-dihydrobenzo[*e*][1,4]diazepine-2,5-dione. Attempts to determine the relative configuration of **1** by analysis of the ROESY spectrum failed due to free rotation around the methylene bridge CH_2-10.

HRESIMS indicated the molecular formula $C_{25}H_{32}O_9$ for **2** in accordance with the $[M+H]^+$ signal at *m*/*z* 477.2119. Its UV spectrum showed characteristic maxima of an austalide at λ_{max} (MeOH) 222.6 and 268.5 nm.[28] The ^{1}H and ^{13}C NMR spectra (Table 2) revealed five methyl groups at δ_H (δ_C) 0.79 (18.2), 1.29 (28.8), 1.32 (29.1), 1.43 (25.6), and 1.99 (10.5) ppm (CH_3-27, -24, -26, -25, and -23, respectively), and one methoxy group at δ_H 4.01 (δ_C 62.5) ppm (OCH_3-29). Additionally, four methylene groups, including the oxygenated benzylic methylene group at C-1 (δ_H 5.25, δ_C 68.1 ppm), and three methine groups, two of which were situated

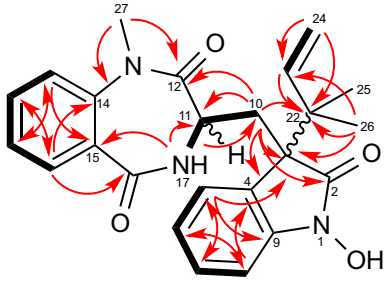

Figure 2. Key COSY (▬) and HMBC (⟶) correlations observed for **1**.