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Chaetominedione, a new tyrosine kinase inhibitor isolated from the algicolous marine fungus *Chaetomium* sp.

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A marine fungal isolate, identified as *Chaetomium* sp., was cultivated and found to produce a novel benzonaphthyridinedione derivative, chaetominedione (**1**). In addition to the known fungal metabolites, 2-furancarboxylic acid (**2**) and 5-(hydroxymethyl)-2-furancarboxylic acid (**3**) were obtained. The structures of all the compounds were determined based on extensive spectroscopic measurements (1D and 2D NMR, MS, UV, and IR). The total extract and compound **1** had significant inhibitory activity toward p56^{lck} tyrosine kinase (18.7% and 93.6% enzyme inhibition at 200 μg/mL, respectively).

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Marine micro-organisms have historically provided a rich source of structurally diverse, biologically active secondary metabolites.[1] Bioactive compounds produced by marine fungi are more or less similar to the metabolites produced by terrestrial fungi.[2] The unusual habitats of these fungi and their tendency to produce biologically active substances has led to much interest in this group of micro-organisms.[3] There are reports of unique biologically active compounds isolated from marine fungi, for example, those concerning anomalin A and B, ascosalipyrrolidinone, trichodermanone A, trimeric terrestrol A, and acremonin A.[4–8]

Fungi of the *Chaetomium* genus (Ascomycota of the family Chaetomiaceae) are well known as soil and contaminant fungi, and also are noted for their secondary metabolite content with significant biological activities. To date, more than 120 compounds have been reported from fungi belonging to this genus, including chaetochromins B, C, and D, chaetocins B and C, chetracin A, isocochliodinol, neocochliodinol, musanahol, and globosuxanthone.[9–14]

Protein tyrosine kinases play an important role in the signal transduction pathways which regulate essential cellular processes (e.g., growth). Thus, targeting of protein tyrosine kinases would be a good approach for the therapeutic intervention against pathological processes, including proliferative disorders, inflammatory responses, and cancer.[15]

The crude extract of the marine fungus, identified as *Chaetomium* sp. (Chaetomiaceae, Ascomycetes), isolated from the alga *Valomia utricularis*, collected from the waters around the Azores (Atlantic Ocean), showed antimicrobial effects toward *Microbotryum violaceum* (250 μg/disk, 3 mm inhibition zone), inhibition of reverse transcriptase of the human immunodeficiency virus type 1 (HIV-1-RT) (30.5% at 66 μg/mL, positive control Foscarnet, 10 μM, rest activity of HIV-1-RT 13%), and inhibition of p56^{lck} tyrosine kinase (TK) (18.7% at 200 μg/mL, positive control was piceatannol, 3 mM, rest activity of 2%).

As a continuation of previous projects,[4,8,16] *Chaetomium* sp. was cultivated on a liquid biomalt medium with added artificial sea water (the same concentration of salt as present in natural sea water, see Supplementary data).[17] Successive fractionation of the EtOAc extract by vacuum liquid chromatography (VLC) over reversed phase (RP-18) silica followed by reversed phase (RP-18) HPLC yielded novel benzonaphthyridine derivative **1**, and two known furan derivatives **2** and **3**.

The structure elucidation commenced when the molecular formula of $C_{17}H_{12}N_2O_4$ was established by LCESIMS, *m/z* 309 $[M+H]^+$ in positive-ion mode, and *m/z* 307 $[M-H]^+$ in negative-ion mode. This result was validated by HRFABMS, *m/z* 308.0818 $[M]^+$ and 309.0889 $[M+H]^+$. The ^{13}C NMR spectra (^{1}H decoupled and DEPT) of **1** showed 16 resonances attributable to $1 \times CH_2$, $7 \times CH$, and $9 \times C$ groups (Table 1). It was clear that the difference between the molecular formula and the number of ^{13}C NMR resonances indicated two carbonyl groups (C-1 and C-3), which overlapped in the ^{13}C spectrum. Extensive interpretation of the HMBC spectrum indicated the presence of two carbonyl groups. Nine of the 13 elements of unsaturation, as indicated by the molecular formula

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Table 1
^{1}H [$(CD_3)_2CO$, 300 MHz] and ^{13}C NMR [$(CD_3)_2CO$, 75.5 MHz] spectral data for compound **1**[a]

Position	δ ^{1}H[c]	*J*/Hz	δ ^{13}C[b]	HMBC
1			165.9 (s)	
3			165.9 (s)	
4			122.2 (s)	
4a			133.6 (s)	
5a			142.2 (s)	
6	7.76 d	8.0	113.6 (d)	C-8, C-10a
7	7.63 dd	8.0, 7.7	130.0 (d)	C-5a, C-9
8	7.38 dd	8.0, 7.7	121.8 (d)	C-6, C-10a, C-7
9	8.43 d	8.0	122.8 (d)	C-5a, C-7, C-9a, C-10a
9a			122.1 (s)	
10	8.85 s		115.3 (d)	C-1, C-2′, C-4, C-4a, C-5a, C-9a, C-10a
10a			131.9 (s)	
2′			153.0 (s)	
3′	7.61 d	3.3	112.2 (d)	C-2′, C-4, C-4′, C-5′
4′	6.61 d	3.3	110.4 (d)	C-2′, C-3′, C-5′, C-1″
5′			158.5 (s)	
1″	4.71 s		57.4 (t)	C-4′, C-5′
NH[d]	11.44 br s			

[a] All assignments are based on 1D and 2D measurements (HMBC, HMQC, COSY).
[b] Implied multiplicities were determined by DEPT (C = s, CH = d, CH_2 = t).
[c] *J* in Hz.
[d] Tentative assignment. The 1″-OH was not observed.

of **1**, could be attributed to seven carbon–carbon double bonds and two carbonyl groups (see Table 1); the molecule thus, has four rings. As the ^{1}H and ^{13}C NMR data enabled all but three of the hydrogen atoms within **1** to be accounted for, it was evident that the remaining three protons were present as part of a hydroxyl function or attached to nitrogen, a deduction supported by IR data (ν_{max} 3280). After association of all the protons with directly bonded carbons via 2D NMR (HMQC) spectral measurements, it was possible to deduce the structure of **1** by interpretation of the ^{1}H–^{1}H COSY and ^{1}H–^{13}C HMBC spectra. From the ^{1}H–^{1}H COSY spectrum of **1**, a ^{1}H–^{1}H spin system between H-9 and H-8, H-8 and H-7, H-7 and H-6 was observed, indicating the four olefinic protons residing on the same aromatic ring. Long-range C-H correlations observed between the resonances of H-9 and those of C-7, C-5a, and C-9a, between H-7 and C-5a and C-9, between H-8 and C-6 and C-9, and between H-6 and C-8 established ring A. These correlations were in good agreement with the coupling constants δ 8.43 (d, *J* = 8.0, H-9); 7.38 (dd, *J* = 8.0, 7.7, H-8); 7.63 (dd, *J* = 8.0, 7.7, H-7); 7.76 (d, *J* = 8.0, H-6) and with NOESY correlations observed between H-9 and H-8, H-8 and H-7, and H-7 and H-6. Long-range correlations, this time observed from H-10 to C-4a, C-9a and C-10a, and between H-9 and C-10a together with the NOESY correlation between H-9 and H-10 and the downfield chemical shifts of C-5a (142.2, s) and C-4a (133.6, s) indicating that both carbons are attached to nitrogen, led to the deduction of ring B. The ^{1}H and ^{13}C NMR resonances for CH-3′ (δ 7.61, d, *J* = 3.3, δ 112.2, d) and CH-4′ (δ 6.61, d, *J* = 3.3, δ 110.4, d) indicated an *ortho*-coupled furan ring. This deduction was supported by HMBC correlations observed between H-3′ and C-2′, C-4′ and C-5′, and between H-4′ and C-2′, C-3′, and C-5′. A CH long-range coupling between H_2-1″ and C-4′ and C-5′ showed methylene group CH_2-1″ to be connected to C-5′. HMBC correlations, this time observed between H-10 and C-4 and also correlations between H-3′ and C-4 indicated that the ring D was attached to ring C through the C–C bond between C-2′ and C-4. The C-1 carbonyl group has to be attached to C-10a because of the HMBC coupling observed between H-10 and C-1. Ring C was established by deduction through subtraction of the remaining part of compound **1** from the molecular formula. This deduction was supported by UV maxima 270 and 378 nm, and the IR data (ν_{max} 1713, 1613 cm^{-1}).[18] It was clear from the ^{13}C NMR spectrum that the structure was not present in the enol-form, due to the absence of a ^{13}C chemical shift around 150 ppm. The attachment of the furan moiety at C-3

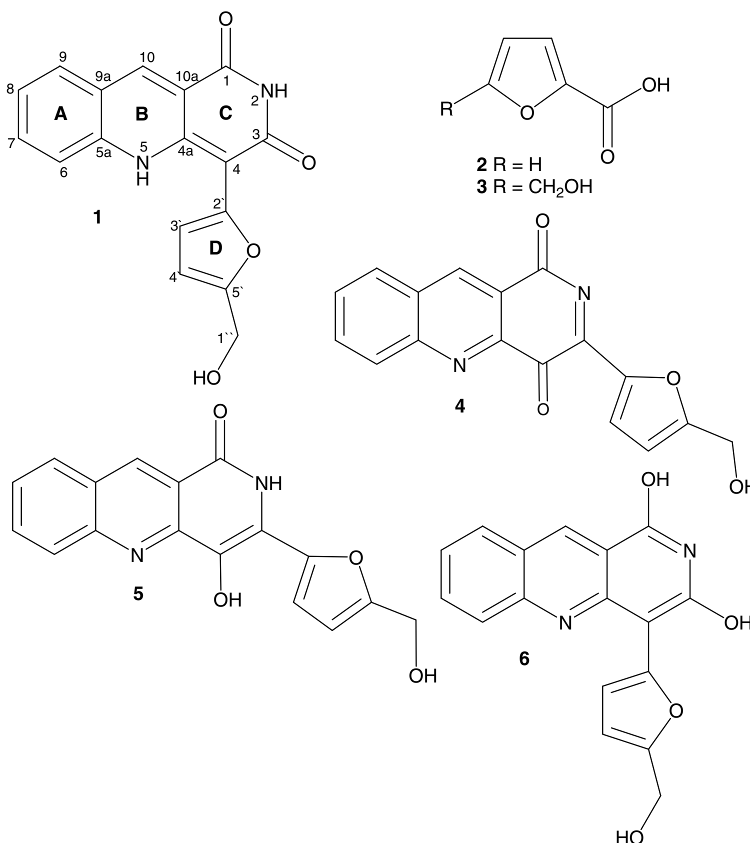

Table 2
Inhibitory activity of the total extract and compounds **1** and **3** toward HIV-1 reverse transcriptase, tyrosine kinase p56^{lck}, and their antimicrobial activity

Assay	Extract	1	3
Tyrosine kinase[a,c,d]	81.3	6.6	0
HIV-1-RT[a,b,d]	69.5	98.4	98.3
Bacteria[e]	Not active	Not active	Not active
Fungi[f]	3 mm[g] for *M.v.*	Not active	Not active
Chlorella fusca	Not active	Not active	Not active

[a] Percentage of enzyme activity observed relative to negative control (100% HIV-1-RT or TK p56^{lck}).
[b] Reduction of enzyme activity to 80% or less was regarded as a significant inhibition. Foscarnet (10 μM, rest activity of HIV-1-RT 13%) was used as a positive control.
[c] Reduction of enzyme activity to 40% or less was regarded as a significant inhibition. Piceatannol (3 mM, rest activity of 2%, Boehringer Mannheim, Germany, purity 99.9%) was used as a positive control.
[d] Sample concentration for HIV-1-RT test 66 μg/mL, and for TK p56^{lck} test 200 μg/mL.
[e] Tested against *Escherichia coli* (*E.c.*) and *Bacillus megaterium* (*B.m.*).[h]
[f] Tested against *Microbotryum violaceum* (*M.v.*), *Eurotium repens* (*E.r.*), *Mycotypha microspora* (*M.m.*).[h]
[g] The radius of the resultant zone of inhibition was measured from the edge of the filter disk.
[h] 250 μg/disk for the extract and 50 μg/disk for pure compound.

instead of C-4 (structures **4** and **5**) was discounted based on the fact that the ^{13}C chemical shift of the carbonyl group at C-4 in **4** should appear at 178 ppm and that the *m/z* value would be 306. The other possibility is the enol-form **6**, which does not fit with the ^{13}C and UV data. A literature survey indicated that there are no similar compounds published except for one paper describing the synthesis of riboflavin derivatives.[19] Thus, compound **1** is a new natural product based on a benzonaphthyridinedione skeleton. The trivial name chaetominedione is proposed for **1**.

2-Furancarboxylic acid (**2**)[20] and 5-(hydroxymethyl)-2-furancarboxylic acid (**3**)[21] were identified by comparison of the spectroscopic data with published values.

ELISA-based bioassays with HIV-1-RT and TK p56^{lck} allowed us to determine enzyme inhibitory activities. The total extract and compounds **1** and **3** had significant TK p56^{lck} enzyme inhibitor activity (18.7%, 93.6%, 100% enzyme inhibition at 200 μg/mL, respectively), and no inhibition of HIV-1-RT reverse transcriptase, except the moderate activity observed for the total extract (Table 2). Antimicrobial activities were measured using agar diffusion assays, and no significant activity was found except for the rather moderate antimicrobial effects of the total extract. Finally, the total extract was tested for its cytotoxicity using the brine shrimp lethality assay, and no activity was found.

4-[5-Hydroxymethyl)-2-furyl]benzo[b][1,6]naphthyridine-1,3(2H,5H)-dione (**1**): (4 mg, 98% purity); yellowish amorphous powder ($(CH_3)_2CO$). R_f 0.7, silica gel 60 F254 $CHCl_3/MeOH/H_2O$ (80:20:0.1). $[\alpha]_D^{22}$ (0.2, MeOH); UV λ_{max} (MeOH) (log ε) 218 (4.7), 270 (4.7), 378 (4.3) nm;. IR (film): 3280, 2924, 2853, 1713, 1613, 1514, 1457, 1375, 1247 cm^{-1}. Positive ion *m/z* 309 $[M+H]^+$; Negative ion *m/z* 307 $[M-H]^+$. EIMS *m/z* (% rel. int.) 222 $[M-C_2H_2N_2O_2]^+$ (100), 205 (32), 177 (28), 165 (10), 151 (8), positive FABMS *m/z* 308.0818 $[M]^+$ and HRFABMS *m/z* 308.0818 $[M]^+$ and *m/z* 309.0889 $[M+H]^+$ (calculated for $C_{17}H_{12}N_2O_4$, 308.0797).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.08.064.

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