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5-Hydroxyindole-type alkaloids, as *Candida albicans* isocitrate lyase inhibitors, from the tropical sponge *Hyrtios* sp.

Hyi-Seung Lee [a], Kyung-Mi Yoon [b], Yu-Ri Han [a], Kyung Jin Lee [c], Soon-Chun Chung [b], Tae-Im Kim [b], So-Hyoung Lee [b], Jongheon Shin [d,*], Ki-Bong Oh [b,e,*]

[a] *Marine Natural Products Laboratory, Korea Ocean Research and Development Institute, Ansan, PO Box 29, Seoul 425-600, Republic of Korea*
[b] *Department of Agricultural Biotechnology, Seoul National University, San 56-1, Sillim-dong, Gwanak-gu, Seoul 151-921, Republic of Korea*
[c] *Invertebrates Research Division, National Institute of Biological Resources, Incheon 404-708, Republic of Korea*
[d] *Natural Products Research Institute, College of Pharmacy, Seoul National University, Seoul 151-742, Republic of Korea*
[e] *Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Republic of Korea*

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ABSTRACT

Chemical investigations of the tropical marine sponge *Hyrtios* sp. have resulted in the isolation of a new alkaloid, 1-carboxy-6-hydroxy-3,4-dihydro-β-carboline (**1**) together with the known metabolites, 6-hydroxy-3,4-dihydro-1-oxo-β-carboline (**2**), 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**3**), serotonin (**4**), hyrtiosin A (**5**), 5-hydroxyindole-3-carbaldehyde (**6**), and hyrtiosin B (**7**). Their structures were elucidated on the basis of mass spectrometry and detailed 2D NMR spectroscopic data. Hyrtiosin B (**7**) displayed a potent inhibitory activity against isocitrate lyase (ICL) of *Candida albicans* with an IC_{50} value of 89.0 μM.

Candida albicans is a commensal flora and considered to be an opportunistic fungal pathogen. It is the leading cause of fungal infections especially in immunosuppressed patients.[1] In tissues, an invading *C. albicans* can be detected and phagocytosed by macrophages and neutrophils.[2] It has been reported that the genes of the glyoxylate cycle are highly induced when *C. albicans* is phagocytosed by macrophage.[3] The inside environment of phagolysosome, abundant in fatty acids or their breakdown products as carbon sources, makes *C. albicans* utilize the enzymes of the glyoxylate cycle to permit to use C_2 carbon sources in this environment. This is achieved through the activity of two unique enzymes, isocitrate lyase (ICL) and malate synthase (MLS). The *C. albicans* mutant strain lacking the glyoxylate cycle enzyme ICL is markedly less virulent in a mouse model of systemic candidiasis and less persistent in internal organs than is the wild-type strain.[3,4] ICL and the glyoxylate cycle, therefore, are envisaged as attractive drug targets for the development of antimicrobial agents effective against a wide range of pathogens, including both fungi and bacteria. Moreover, ICL inhibition drugs would be predicted to have less host toxicity because the enzymes of the glyoxylate cycle are not found in mammals.

Only several ICL inhibitors are reported to date; these include 3-nitropropionate,[5] 3-bromopyruvate,[6] 3-phosphoglycerate,[7] mycenon,[8] oxalate,[9] and itaconate.[9] However, these are not pharmacologically suitable for testing in vivo because of their toxicity and low activity. The development of selective ICL inhibitors with suitable pharmacological properties would definitively open the door to testing in animal models to establish both the importance of the glyoxylate cycle in disease and the proof of concept for the therapeutic potential of ICL inhibition. As part of our efforts to discover ICL inhibitors, we have recently reported the isolation and evaluation of ICL inhibitory activities of some bromophenols and sesterterpene sulfates from marine sponges.[10,11] Bromophenols compounds exhibited potent ICL inhibitory activity and protected rice plants from the rice blast fungus *Magnaporthe grisea* infection.[10] Sesterterpene sulfates isolated from the tropical sponge *Dysidea* sp. showed inhibitory activity against ICL of *C. albicans*.[11]

In the course of our research to discover novel ICL inhibitors, we encountered the marine sponge *Hyrtios* sp. collected in Chuuk State, Federated States of Micronesia whose crude extract exhibited moderate inhibitory activity (48% inhibition at 50 μg/ml) against ICL of *C. albicans*. Bioassay-guided separation of the crude extract using various chromatographic techniques yielded a new

* Corresponding authors. Tel.: +82 2 880 2484; fax: +82 2 762 8322 (J.S.); tel.: +82 2 880 4646; fax: +82 2 873 3112 (K.-B.O.).
E-mail addresses: shinj@snu.ac.kr (J. Shin), ohkibong@snu.ac.kr (K.-B. Oh).

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β-carboline alkaloid together with known related alkaloids. Herein, we describe the isolation and biological activities of these compounds.

The specimens of *Hyrtios* sp. (Thorectidae family)[12] were collected from Federated States of Micronesia in 2003 and 2007. The freeze-dried sponge (329 g) was sliced and repeatedly extracted with MeOH (1 L ×2) and CH_2Cl_2 (1 L ×1). The extract was filtered and concentrated under reduced pressure to afford 55.3 g of crude extract. The residue was partitioned between H_2O (41.4 g) and *n*-BuOH (13.4 g), then the organic layer was dried and re-partitioned between 15% aqueous MeOH (10.0 g) and *n*-hexane (3.41 g). An aliqout of aqueous MeOH layer (7.82 g) was subjected to C_{18} reversed-phase flash chromatography using gradient mixtures of MeOH and H_2O (elution order: 50%, 40%, 30%, 20%, 10% aq. MeOH, and 100% MeOH). The fraction eluted with 50% aqueous MeOH (2.31 g) was dried and separated by reversed-phase HPLC (YMC-ODS-A C_{18} column, 250 × 10 mm; 80% aqueous MeOH) to yield 16.1, 1.3, 72.2, and 2.2 mg of compounds **1**, **4**, **5**, and **6**, respectively. The fraction eluted with 40% aqueous MeOH (192.3 mg) was purified on a semipreparative HPLC (YMC-ODS-A C_{18} column, 250 × 10 mm; 65% aq. MeOH) to afford 39.5, 9.3, 3.7, and 1.0 mg of compounds **1**, **2**, **3**, and **7**, respectively (Fig. 1).

Compound **1** was obtained as reddish-yellow solid.[13] The molecular formula of compound **1** was deduced to be $C_{12}H_{10}O_3N_2$ by HRFABMS. The ^{1}H NMR spectrum (Table 1) of **1** measured in DMSO-d_6 exhibited three aromatic protones at δ 7.47 (d), 6.96 (br d) and 6.86 (br s) engaged in a 1,2,4-trisubstituted benzene ring and two vicinal methylenes at δ 3.83 (t) and 3.08 (t). The ^{13}C NMR spectrum (Table 1) of **1** displayed resonance for 12 carbons including seven quaternary carbons. A combination of the HSQC and HMBC data allowed assignment of the characteristic ^{13}C NMR signals, and the HMBC correlations from H-3 to C-1, C-4 and C-4a, from H-4 to C-3, C-4a and C-9a, and from H-5 to C-4a, C-6, C-7 and C-8a supported the presence of 6-hydroxy-dihydro-β-carboline moiety. Considering the molecular formular and unassigned NMR signals for δ_H11.65 and δ_C 158.69, it was suggested that the remaining part was COOH. Compound **1** is therefore 1-carboxy-6-hydroxy-3,4-dihydro-β-carboline, which is a new compound.

Compound **2** was determined to be 6-hydroxy-3,4-dihydro-1-oxo-β-carboline by combined spectroscopic analyses and comparison of NMR data with those reported previously.[14,15] The ^{1}H and ^{13}C NMR spectra of compounds **3**–**7** were closely related to each other and all of them possess a 5-hydroxyindole moiety. Structures of these compounds were readily identified by the comparison of the reported[14,16] spectral data as being 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**3**), serotonin (**4**), hyrtiosin A (**5**), 5-hydroxyindole-3-carbaldehyde (**6**), and hyrtiosin B (**7**).

Table 1
NMR data for compound **1** in DMSO-d_6 (δ in ppm)

Position	δ_H	δ_C		HMBC (δ_H to δ_C)
1		158.64[a]	s	
3	3.83 (t, *J* = 8.1 Hz)	41.6	t	1, 4, 4a
4	3.08 (t, *J* = 8.1 Hz)	18.8	t	3, 4a, 9a
4a		122.8	s	
4b		124.5	s	
5	6.86 (br s)	102.5	d	4a, 6, 7, 8a
6		151.9	s	
7	6.96 (br d, *J* = 8.8 Hz)	120.7	d	5, 8a
8	7.47 (d, *J* = 8.8 Hz)	114.9	d	4b, 6
8a		136.6	s	
9a		125.0	s	
COOH	11.65 (br s)	158.69[a]	s	

[a] Interchangeable signals.

The cloning, expression, and purification of ICL from the genomic DNA of *C. albicans* (ATCC 10231) were carried out as described previously.[9] The compounds **1**–**7** were evaluated for their inhibitory activities against ICL of *C. albicans* according to a previously documented procedure.[9,17,18] The inhibitory potencies, expressed as IC_{50} values, of the tested compounds are shown in Table 1 and are compared to that of a known ICL inhibitor, 3-nitropropinate (IC_{50}: 50.7 μM).[19] Among the compounds tested, compound **7** was found to be a strong ICL inhibitor (IC_{50}: 89.0 μM). However, compound **2** was inactive and the other alkaloid compounds **1** and **3**–**6** revealed only moderate to weak activity against *C. albicans* ICL (Table 2). By comparing chemical structures of compounds **3**–**6**, it was found that the ICL inhibitory activities of these 5-hydroxyindole-type alkaloids are markedly affected by a substitution of functional group at the C-3 position. A substitution by a hydrophilic

Table 2
Inhibitory effect of compounds **1**–**7** on the activity of ICL enzyme and fungal growth of *Candida albicans* ATCC 10231[a]

Compound	ICL IC_{50} (μM) (μg/ml)	MIC (μM) (μg/ml)
1	379.6 (87.4)	>868.7 (200)
2	>989.1 (200)	>989.1 (200)
3	799.77 (152.9)	>1046.1 (200)
4	301.3 (53.1)	>1134.9 (200)
5	318.0 (60.8)	>1046.1 (200)
6	247.0 (39.8)	>1024.0 (200)
7	89.0 (28.5)	>624.4 (200)
3-Nitropropinate	50.7 (6.0)	>1690.0 (200)
Amphotericin B	ND[b]	1.7 (1.6)

[a] 3-Nitropropinate and amphotericin B inhibitors of ICL and fungus, respectively, were used as positive controls.
[b] ND, not determined.

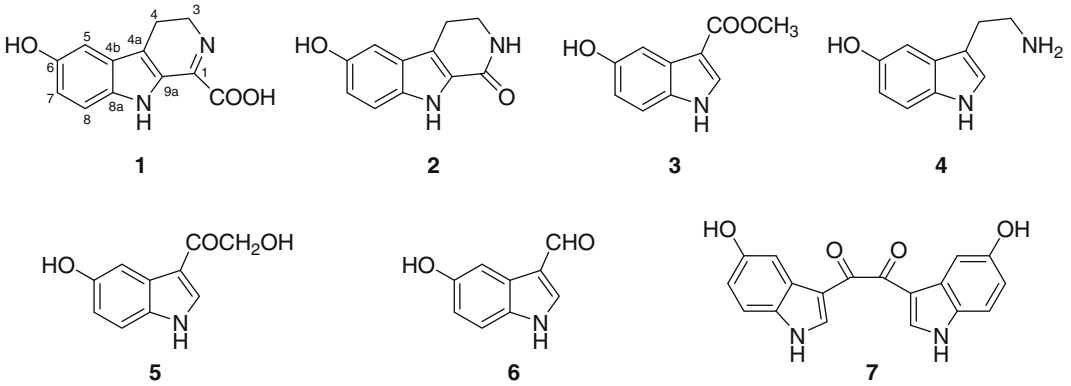

Figure 1. Structure of the isolated compounds from the tropical marine sponge *Hyrtios* sp.

group at the C-3 position results in an increase of the ICL inhibitory activity. The order of ICL inhibitory activity was 5-hydroxy-1*H*-indole-3-carboxylic acid methyl ester (**3**) < hyrtiosin A (**5**) ⩽ serotonin (**4**) < 5-hydroxyindole-3-carbaldehyde (**6**). The in vitro antimicrobial activities of compounds **1**–**7** were also assessed against medically important pathogenic fungi including *C. albicans* (Table 1) and bacteria.[20] All of these compounds were inactive against tested microorganisms at 200 μg/ml (data not shown).

Marine sponges of the genus *Hyrtios* (Thorectidae family) have proven to be a rich source of structurally diverse metabolites including alkaloids, mainly sesterterpenes, sesquiterpene quinones, and macrolides.[21] Many of them possess important biological activities as illustrated with the promising anticancer altohyrtins.[22] In this study, we have shown that specimen of the marine sponge *Hyrtios* sp. collected from Federated States of Micronesia contain 5-hydroxyindole-type alkaloids, as ICL inhibitors. The alkaloidal content of the Micronesian *Hyrtios* sp. we have studied is similar to that of the Indonesian *Hyrtios erectus* studied by Salmoun et al.,[14] as both samples contained 6-hydroxy-3,4-dihydro-1-oxo-β-carboline, serotonin, 5-hydroxyindole-3-carbaldehyde, and hyrtiosin B. Interestingly, several tryptamine derivatives and trisindoline were found to be produced by a fungus *Aspergillus niger* (separated from *Hyrtios proteus*)[23,24] and a bacterium of the genus *Vibrio* (separated from *Hyrtios altum*),[25] respectively. This suggests that the 5-hydroxyindole-type alkaloids found in *Hyrtios* sp. could be of symbiotic origin.

In conclusion, biological and chemical investigations of the crude extract of the Micronesian marine sponge *Hyrtios* sp. led to the isolation of a new 5-hydroxyindole-type alkaloid, 1-carboxy-6-hydroxy-3,4-dihydro-β-carboline, together with six known related alkaloids. Their structures were elucidated on the basis of mass spectrometry and detailed 2D NMR spectroscopic data. Among the isolated alkaloids, hyrtiosin B displayed a potent inhibitory activity against isocitrate lyase (ICL) of *C. albicans*. It was also found that the ICL inhibitory activities of 5-hydroxyindole-type alkaloids are markedly affected by a substitution of functional group at the C-3 position. Since the enzymes of the glyoxylate cycle are not found in mammals, the isolated alkaloid compounds are good starting candidates for ICL inhibitor design.

Acknowledgments

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12. *Sponge material*: This sponge was collected by SCUBA diving at 20–30 m depth from Weno island, Chuuk Atoll, Federated States of Micronesia in 2003. This specimen was identified *Hyrtios* sp. by Dr. K.J. Lee. The shape of sponges is elongate form with many branches, 1.5–3 cm thick and 3–7.5 cm high. Some branches were divided into two at the tip. Oscules are not visible. The color is dark blue or black in life and same color in alcohol. The texture is firm and hard but easily broken and brittle. The surface is covered with low and round conules, under 1 mm high and unarmored. All fibers are heavily cored with small sands. The primary fibers, 100–150 μm in diameter, are almost full with large amount of sands and spicules. The secondary fibers, 70–130 μm in diameter, are heavily cored with detritus also. A voucher specimen of this horny sponge (registry No. L-Spo. 1) was deposited at the Natural History Museum, Hannam University, Korea.
13. *1-Carboxy-6-hydroxy-3,4-dihydro-β-carboline* (***1***): Mp 221–224 °C (dec.); UV (MeOH) λ_{max} (log ε) 390 (3.92) nm; IR (KBr) ν_{max} 3352, 2949, 1660, 1454, 1033 cm^{-1}; ^{1}H and ^{13}C NMR see Table 1; HRFABMS(+) *m/z* 253.0585 $[M+Na]^+$, (calcd for $C_{12}H_{10}O_3N_2Na$, *m/z* 253.0589).
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