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Studies on the antimicrobial activity of cadiolides and synoilides isolated from the ascidian *Synoicum* sp.

Synocium sp. 유래의 cadiolides와 synoilides의 항균활성 연구

2013 년 8 월

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Abstract

Studies on the antimicrobial activity of cadiolides and synoilides isolated from the ascidian *Synoicum* sp.

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Infectious diseases are currently the second major cause of death worldwide and third leading cause of death in economically advanced countries. Natural products have been the rich source of medicinal agents for the treatment of a variety of diseases. In particular, marine organisms are very prolific sources of structurally unique and biologically active metabolites. In this study, tris-aromatic furanones (cadiolide E,

G, H and I) and related bis-aromatic diesters (synoilide A and B) isolated from the dark red ascidian *Synoicum* sp., were evaluated for their inhibitory activities toward microbial growth and several target enzymes related to antimicrobial activities (isocitrate lyase, sortase A and Na^+/K^+-ATPase). In antimicrobial assay, cadiolides E-I displayed significant antibacterial activities against several Gram-positive and Gram-negative strains, while synoilides A and B exhibited either much weaker or no activity. These results suggest that the antibacterial activity of these compounds may be attributable to the presence of the furanone moiety. The isolated compounds were also evaluated for their inhibitory activity toward several target enzymes related to antimicrobial activities. These studies led to the identification of cadiolide E, H and I as potent inhibitors of isocitrate lyase (ICL) from *Candida albicans*, with IC_{50} values of 7.62, 17.16, and 10.36 μM, respectively. Growth phenotype of ICL deletion mutants and RT-PCR analysis data indicated that cadiolide E inhibits the ICL expression in *C. albicans* under C_2 carbon utilizing condition. Since the enzymes of the glyoxylate cycle are not found in mammals, cadiolides may be worth of investigation as antifungal agents to suppress virulence in the *C. albicans*.

Keywords : **Antibacterial activity, Isocitrate Lyase(ICL), Glyoxylate cycle, Ascidian *Synoicum* sp., ICL inhibition**

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1. Introduction

Antibiotics of a ‘golden age’ of discovery, lasting from 1945 to 1965, which sought naturally produced antibiotics from soil streptomyces and fungi. Many antibiotics groups, included aminoglycosides, tetracyclins, macrolides, glycopeptides, were reported in that time(1). The fifties and sixties that main problems of chemotherapy has been solved up to now(2). However, newly discovered antibiotics were decreased but the number of antibiotic. resistants such as MRSA(methicillin-resistant *Staphylococcus aureus*), VRSA(vancomycin-resistant *Staphylococcus aureus*) and MRAB(multidrug-resistant *Acinetobacter baumannii*) were increased(3-5). In this respect, requirements of discovering new antibiotics were critical to drug effect. For research drug candidate, many scientist were founded a numerous quantity of microbes lived in nature resource such as air, water, soil. In comparison these nature source, marine organisms were not yet explored well. Until now, marine natural products were reported that have diverse bioactive compound against cancer human pathogen. Furthermore, numerous untapped marine organisms live in oceans which cover approximately 75 percents of the planet earth. Especially, the deep sea is unique environment with various oxygen concentration, no light and high pressure.

Marine microorganisms, particularly marine actionomyces, have attracted increasing attention as one of the most important resources for a wide variety of biological active metabolites(6, 7). Diverse natural products from marine organisms have great potential in clinical trials(8). Thus, marine organism, such as sponge, microorganism lived in deep ocean sediment, is worthwhile to research bioactive compound which is novel structure.

Glyoxylate cycle is well known for anaplerotic variant of the tricarboxylic acid (TCA) cycle the net effect of which is the conversion of two molecules of acetyl-CoA to succinate (Fig. 1). It is well known that the glyoxylated cycle operates in bacteria, fungi, some protein and plants(9-12). In glyoxylate cycle, the net assimilation of carbon from C_2 compounds allow microorganisms to supplement the pool of TCA cycle intermediates necessary for many biosynthetic processes including gluconeogenesis. Most of all, pathogenic microorganisms utilize the glyoxylate cycle during infection(13). But, some of animal cell have no system(14). Genetic regulation of the glyoxylate cycle during microbial growth on acetate has been reviewed and in the last several years it has become evident that this pathway is important in microbial pathogenesis(15, 16). Three of the five involved enzymes with TCA cycle is shared in glyoxylate cycle. Except for three enzyme, isocitrate lyase (ICL) and malate synthase (MS) is key

enzyme in glyoxylate cycle.

Several research is supporting that ICL play role as essential enzyme in pathogenesis. Such as pathogenic fungi, *Candida albicans* and *Aspergillus fumigatus*, a clear role for ICL has been identified up to now(17-19). In case for *C. albicans*, an important pathogenic fungus of humans, the *ICL* of glyoxylate cycle is strongly upregulated. When *C. albicans* is exposed to macrophages and human neutrophils, the fact that ICL is induced is confirmed by previous experiment data(19, 20). A large variety of C_2 carbon sources including acetate, ethanol or lactate can be used for substrate as glyoxylate cycle. But all case do not adapt for virulence. *ICL* expression in virulence do not derive from certain carbon source including intermediate of lipid metabolism(21-23). Unlikely, ICL in *C. albicans* is essential enzyme of the utilization of diverse carbon source.

Because ICL is critical in pathogenesis of *C. albicans*, searching a effective inhibitor of ICL is attractive. With this necessity, antibiotic candidates against a wide range of pathogens, including both bacteria and fungi, are considered to be interesting in drug field. To date, several ICL inhibitors including 3-nitropropionate and 3-bromopyruvate have been identified(24, 25). However, these inhibitors are not pharmacologically suitable for testing in vivo because of their toxicity and low activity.

Ascidians (phylum Chordata, class Ascidiacea) produce a wide

variety of bioactive secondary metabolites(26). In a previous work,(27) asicidian *Synoicum* sp. which is dark red was collected off the coast of Chuja-do, southern Korea. Chemical investigation of this animal led to the isolation of new compounds, tris-aromatic furanones and related bis-aromatic diesters. Seperation of the crude extracts from *Synoicum* sp. using chromatic technic yielded six compounds (Fig. 2). The structures of these compounds, designated as cadiolides E and G-I (**1**-**4**) and synoilides A and B (**5** and **6**), were determined by extensive spectroscopic analyses (Fig. 3). The potential of these compounds as antibacterial and inhibitor of *C. albicans* ICL was described in this paper.

2. Materials and Methods

2.1 Isolation of compound 1-6 from *Synoicum* sp.

The purification and characterization of compounds 1–6 from the ascidian *Synoicum* sp. were carried out as described previously(27). Lyophilized specimens were repeatedly extracted with MeOH and CH_2Cl_2. The combined extracts (160.95 g) were successively partitioned between H_2O and n-BuOH; the latter fraction was repartitioned between H_2O-MeOH (15:85) (12.27 g) and n-hexane (3.56 g). An aliquot of the former layer (5.70 g) was separated by C_{18} reversed-phase flash chromatography using sequential mixtures of MeOH and H_2O as eluents (six fractions in gradient, H_2O-MeOH, from 50:50 to 0:100), acetone, and finally EtOAc.

On the basis of the results of ^1H NMR, the fractions eluted with H_2O-MeOH (50:50; 1.10 g) and H_2O-MeOH (10:90; 0.59 g) were chosen for separation. The fraction eluted with H_2O-MeOH (50:50) was separated by silica normal-phase flash chromatography using sequential mixtures of CH_2Cl_2 and MeOH as eluents (six fractions in gradient, CH_2Cl_2-MeOH, from 100:0 to 50:50) and finally MeOH. The fraction that eluted with CH_2Cl_2-MeOH (80:20; 0.33 g) was chosen for separation. It was separated by reversed-phase HPLC (YMC ODS-A column, 10 mm × 250 mm; H_2O-MeOH, 20:80, with 0.01% TFA),

yielding four peaks rich with secondary metabolites. Further purification of the first and second peak by reversed-phase HPLC (H_2O–MeCN, 45:55, with 0.01% TFA) provided compounds **5** and **6**, respectively. The other two peaks provided, in order of elution, compounds **1** and **4**, having high purity without further purification.

The H_2O–MeOH (10:90) fraction (0.59 g) was separated by reversed-phase HPLC (H_2O–MeOH, 20:80) to yield, in order of elution, compounds **2** and **3**. Final purifications of these metabolites were then accomplished by reversed-phase HPLC (H_2O–MeOH, 20:80, with 0.01% TFA). The purified metabolites were isolated in the following amounts: 21.5, 7.1, 34.2, 9.9, 15.6, and 19.7 mg of **1**–**6**, respectively.

2.2 Antibacterial activity assay

The following 6 microorganisms, obtained from the stock culture collection at American Type Culture Collection (ATCC) (Rockville, MD, U.S.A.) and NITE Biological Resource Center (NRBC) (Tokyo, Japan), were used in this study: *Staphylococcus aureus* ATCC 6538p, *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* NBRC 12708, *Salmonella enterica* ATCC 14028, *Proteus hauseri* NBRC 3851, *Escherichia coli* ATCC 35270. Bacteria were grown overnight in Luria Bertani (LB) broth at 37 ℃, harvested by centrifugation, and then washed twice with sterile distilled water. The antibacterial activity was determined by twofold microliter broth dilution method. Dilution of test compound dissolved in dimethyl sulfoxide (DMSO) were added to each well of 96-well microtiter plate containing fixed volume of m plate count broth (Difco). The concentration of compounds ranges 100-0.1 μg/ml. Each well was inoculated with an overnight culture of bacteria (10^5 CFU/ml), and inoculated at 37 ℃ for 16 hours. The minimum inhibitory concentration (MIC) were determined as the lowest concentration of test compounds that inhibited bacterial growth (28).

2.3 Antifungal activity assay

C. albicans SC5314 was grown in YNB (6.7% yeast nitrogen base) broth containing 2% glucose at 28 ℃ for 24 h, harvested by centrifugation, and washed twice with sterile distilled water. Each test compound was dissolved in DMSO and diluted with YNB containing 2% glucose or 2% potassium acetate to prepare serial twofold dilutions in the range of 100–0.1 μg/ml. Twenty microliters of the broth containing approximately 10^4 cells/ml of test fungus was added to each well of a 96-well microtiter plate. Culture plates were incubated for 48 h at 28 ℃. The MIC values were determined as the lowest concentration of test compounds that inhibited fungal growth. Amphotericin B and 3-nitropropionate were used as a reference compound.

2.4 Expression and purification of ICL

In a previous work, the cloning and purification of ICL from the genomic DNA *Candida albicans* (ATCC 10231) were carried out as described previously(29). Briefly, *C. albicans* strain were grown in YPD medium (1% Yeast extract, 2% Bacto peptone, and 2% Dextrose). Genomic DNA of *C. albicans* was prepared with Wizard genomic DNA purification Kit (Promega, U.S.A). Based on the nucleotide sequence for *C. albicans ICL1* (Genbank accession number AF222905), two synthetic primers (5'-a**gaattc**ctaccatgcttacactcc-3', forward primer; 5'-cttc**gtcgac**tcaaaattaagccttg-3', reverse primer) were designed to carry the *EcoR I* and *Sal I* recognition sites, respectively (bold). PCR reaction consisted of 4 min at 94 ℃ followed by 30 cycles of 1 min at 94 ℃, 1 min at 48 ℃ and 1 min at 72 ℃ followed by a single cycle at 1 min at 72 ℃. The resulting PCR product was cloned in the pBAD/Thio-TOPO vector according to the instructions of manufacturer. The *E. coli* TOP10 was chosen for transformation. The positive transformants were grown in LB medium containing ampicillin (50 μg/ml) at 37 ℃ for 16 hours ($O.D._{600}$ = 1~2). The pre-incubated cells (2 ml) were inoculated into 400ml of LB broth containing ampicillin and incubated with shaking at 37 ℃ until $O.D._{600}$ reached 0.5 approximately. At this point L-arabinose was added to the medium to a final concentration of over 0.02% and an additional

incubation was performed at 25 ℃ for 8 hours to induce the expression of ICL proteins. Growth at the low resulted in improved solubility of the recombinant system are fused to His-Patch(HP)-thioredoxin at the N-terminal for the simplified purification(30). Since HP-Thioredoxin has a Ni-binding property at pH 7.2, the expressed ICL could easily be purified by Ni-NTA affinity column chromatography (Qiagen, Hilden, Germany).

2.5 ICL activity assay

The enzyme activity of the purified ICL was determined by the method of Dixon and Kornberg(31). A 1-ml aliquot of the reaction mixture contained 20 mM sodium phosphate buffer (pH 7.0), 1.27 mM *threo*-DL (+) isocitrate, 3.75 mM $MgCl_2$, 4.1 mM phenylhydrazine, and 2.5 μg/ml purified ICL. The reaction was performed at 37 ℃ for 30 min with and without prescribed concentration of inhibitor dissolved in DMSO (final concentration, 1%). The formation of glyoxylate phenylhydrazone was followed spectrophotometrically at 324 nm. The effect of inhibitor on ICL was calculated as a percentage, relative to solvent-treated control, and the IC_{50} values were calculated using nonlinear regression analysis (percent inhibition *versus* concentration). 3-Nitropropionate is an ICL inhibitor used as positive control.

2.6 Strains, RNA and cDNA preparation

C. albicans strain SC5314 (wild-type), MRC10 (*Δicl*), and MRC11 (*Δicl +ICL*) were grown into the mid-log phase in YNB broth (2% glucose), collected by centrifugation, and washed twice with sterile distilled water. Cells were resuspended in YNB media containing 2% glucose, 2% acetate, or 2% acetate plus compound **1** (6.25 or 12.5 μg/ml) and grown for 4 h at 28 ℃. Total RNA from each sample was isolated by using RNeasy Mini Kit (Qiagen). One microgram of total RNA was reverse transcribed into cDNA by using Superscript III First-Strand Synthesis System (Invitrogen) according to the manufacturer's protocol. RT-PCR (Reverse transcription polymerase chain reaction) analysis was conducted with the *ICL*-specific primers: forward, 5'-ATGCCTTACACTCCTATTGACATTCAAAA-3'; reverse, 5'-TAGATTCAGCTTCAGCCATCAAAGC-3'. A housekeeping gene GAPDH (glyceraldehyde-3-phosphate dehydrogenase) was used as a loading control with the specific primers: forward, 5'-ATCACCATCTTCCAGGAG-3'; reverse, 5'-ATGGACTGTGGTCATGAG-3'. PCR amplification was started with an initial incubation at 98 ℃ for 10 min followed by 36 cycles of 30 s at 98 ℃ for, 30 s at 56 ℃, and 30 s at 72 ℃ and then performed at 72 ℃ for 5 min. *ICL* expression level was determined by densitometric analysis with ImageJ software

(NIH, Bethesda, MD, USA).

3. Results

3.1 Antibacterial activity

The antibacterial activities of compounds from specimens of *Synoicum* sp. were assessed against three representative Gram-positive bacteria; *Staphylococcus aureus* ATCC 6538p, *Bacillus subtilis* ATCC 6633, *Kocuria rhizophila* NBRC 12708, three representative Gram-negative bacteria *Salmonella enterica* ATCC 14028, *Proteus hauseri* NBRC 3851, *Escherichia coli* ATCC 35270. Compare to synoilides A and B (**5** and **6**), cadiolides E-I (**1-4**) displayed antibacterial activity against most tested strain excluding *Escherichia coli* ATCC 35270, with MIC averages ranging between 0.8-12.5 ㎍/ml. Among them, compound **4** displayed significant antibacterial activity against Gram-positive bacteria with MIC value of 0.8 ㎍/ml which are shown close to effect with positive control with MIC value of 0.4 ㎍/ml. Compound **2** has strong antibacterial activity against Gram-negative bacteria with MIC value of 0.8, 3.1 ㎍/ml, respectively.

Compound **6** is no activity against any tested strain (Table 1). Ampicillin and DMSO were used as positive and negative control (DMSO data not shown).

3.2 ICL inhibition activity

The effect of isolated compounds **1**–**6** on *C. albicans* ICL was evaluated. The inhibitory potencies (IC_{50}) of the tested compounds are shown in Table 2 and are compared to that of a known ICL inhibitor, 3-nitropropinate (IC_{50} 13.91 ± 2.29 μM). compounds **1**, **3**, and **4** were found to be strong ICL inhibitors, with IC_{50} values of 7.62 ± 0.30, 17.16 ± 0.45, and 10.36 ± 0.66 μM, respectively. In particular, compound **1** and **4** were more effective than 3-nitropropinate. ICL inhibitory ratio graph of 3-nitropropinate and each tested compounds shown in ;. 4.

As mentioned earlier, glyoxylate cycle which is utilized by pathogenic microorganisms during infection is progressed by C_2 carbon source. To investigate whether ICL inhibitors affected C_2 substrate use, *C. albicans* strain SC5314 was cultured in YNB broth containing either 2% glucose or 2% potassium acetate as the sole carbon source. MIC was assessed for checking inhibition effect. *C. albicans* strain SC5314 was used in this test since the ICL sequence alignment of this strain (GenBank accession number AF222905) showed 100% identity with that of *C. albicans* ATCC 10231 over its entire length (data not shown). As shown in Table 2, fungal growth inhibition tests indicated that the ICL inhibitors **1**–**6** had no inhibitory effects on SC5314 grown in glucose, but were inhibitory to SC5314 grown

in acetate. Especially, compound **1** showed potent inhibitory activity with MIC value of 12.5 μg/ml.

3.3 Growth phenotypes and RT-PCR analysis

Based on these findings, the effects of compound **1** on growth phenotype and *ICL* mRNA expression in *C. albicans* strain SC5314 (wild-type), MRC10 (Δ*icl*), and MRC11 (Δ*icl* + *ICL*) is investigated. As shown in Fig. 4A, these strains grew normally on YNB agar plates containing glucose or glucose plus 12.5 μg/ml of compound **1**. However, *ICL* deletion mutant MRC10 failed to grow on acetate as the sole carbon source. In addition, the growth of all tested strains was markedly inhibited on YNB agar plates containing acetate plus 12.5 μg/ml of compound **1**. The effects of compound **1** on *ICL* mRNA expression in *C. albicans* by RT-PCR is investigated. As can be seen in Fig. 4B, *ICL* transcript levels in SC5314 and MRC11 were undetectable when the YNB liquid medium contained glucose, but were strongly induced when the medium contained acetate. Interestingly, under the *ICL* expression condition, The transcript levels of *ICL* in both strains were diminished with the increase in concentration of compound **1**. At 12.5 μg/ml, compound **1** dramatically reduced (about 5-fold) the magnitude of *ICL* mRNA expression.

4. Discussion

Since cadiolide was first discovered in genus *Botryllus*(32), their derivatives is found up to date(33). Recently, some of bioactivity in cadiolides is reported(33). Because asidians secrete diverse useful secondary metabolites(26). isolated six compounds from asicidian *Synoicum* sp. are expected to be diverse bioactive compounds. Based on data above, antibacterial activity and ICL in *C. albicans* inhibitory activity of isolated compounds **1-6** from specimens of *Synoicum* sp. is demonstrated. Overall, antibacterial activity of cadiolides E-I (**1-4**) is more effective than synoilides A and B (**5** and **6**). Compound **4** is the strongest antibacterial activity among them. Compound **4** which is cadiolide derivative has a possibility used as a candidate for antibiotics. And the presence of the furanone moiety may be contributory in determining strong antibacterial activity. ICL inhibitory activity of compounds is significant differences between isolated cadiolides E-I (**1-4**) and synoilides A and B (**5** and **6**). Similar to antibacterial activity, cadiolides E-I (**1-4**) is stronger inhibition against ICL in *C. albicans* than synoilides A and B (**5** and **6**). In particular, compound **1** and **4** were more effective than 3-nitropropinate. By comparing chemical structures of tested compounds, it was found that bromine moiety at the aromatic ring proved to be an

important factor for effective inhibition of the ICL inhibitory activities of compounds **1**–**6** (Fig. 3). Compound **1** and **4** can be used as a candidate for ICL inhibitor design.

The microbial strategy for survival during growth in a nutrient-free environment entails a metabolic shift in the cell's carbon source to C_2 substrates generated by β-oxidation of fatty acids(15, 16, 34). Under these conditions, glycolysis is decreased and the glyoxylate shunt is significantly up-regulated to allow anaplerotic maintenance of the TCA cycle and assimilation of carbon via gluconeogenesis. Growth phenotypes and RT-PCR analysis indicated that compound **1** inhibited the *ICL* expression in *C. albicans* under C_2 carbon utilizing condition. Since the enzymes of the glyoxylate cycle are not found in mammals, cadiolides may be worthy of investigation as antifungal agents to suppress virulence in the *C. albicans*.

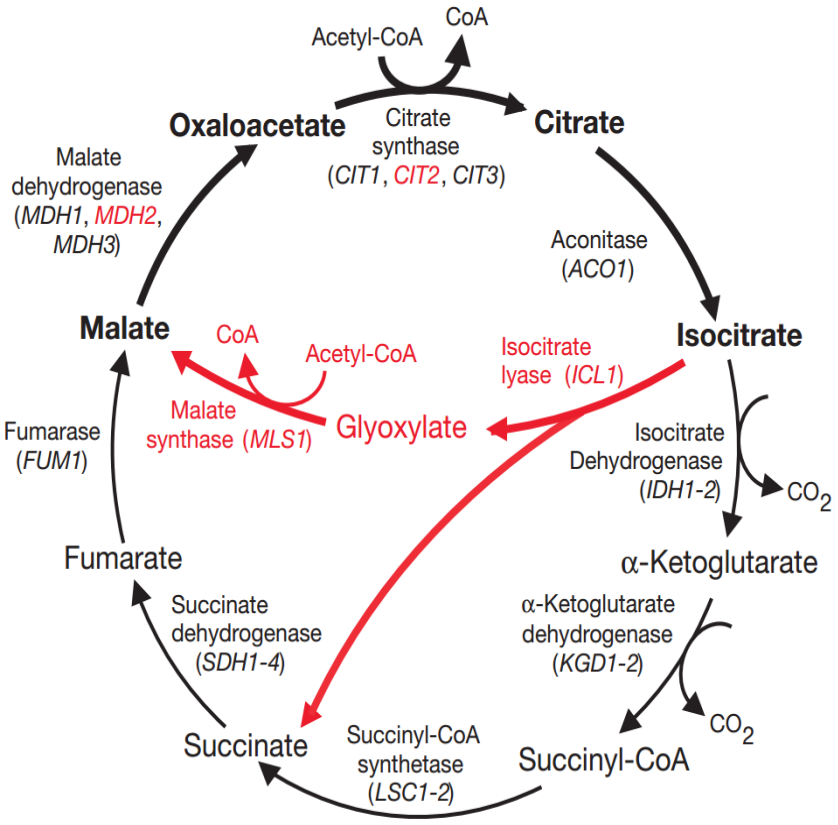


Fig. 1. Glyoxylate cycle(19)

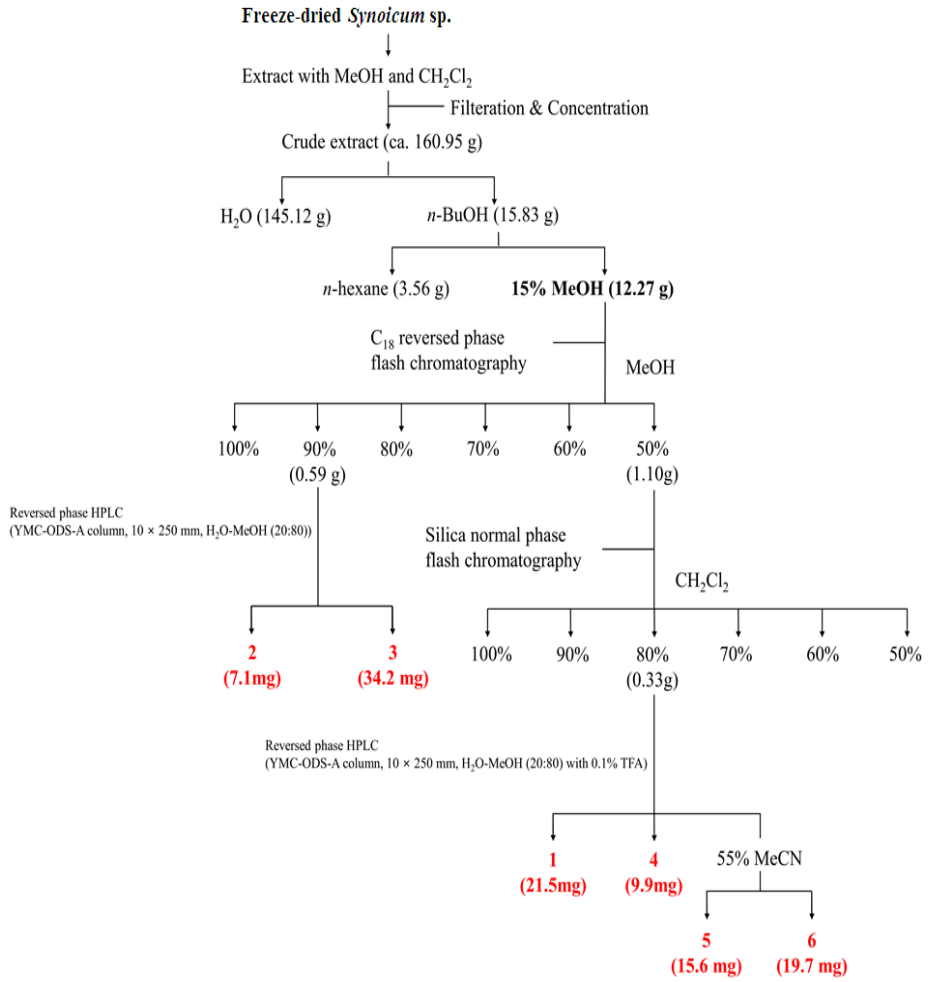


Fig. 2. Isolation procedure of compound 1–6

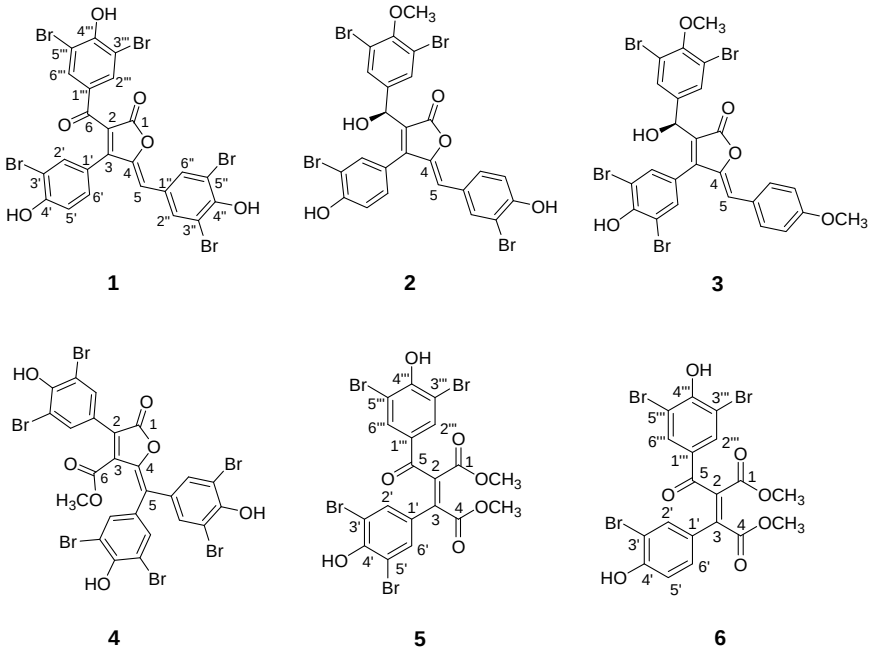
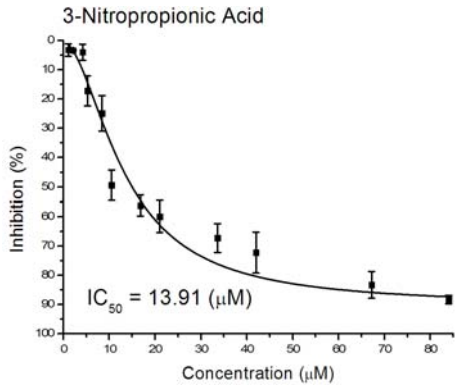
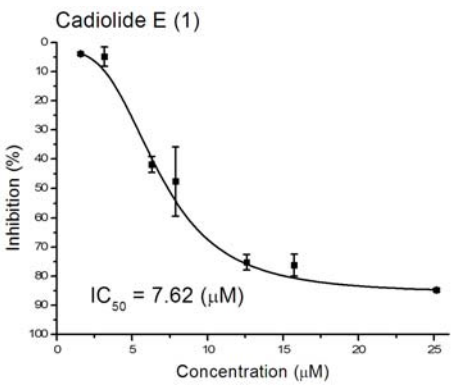
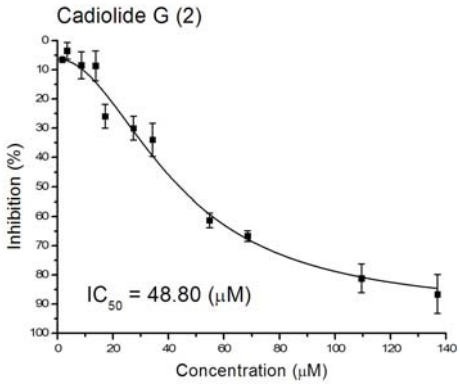
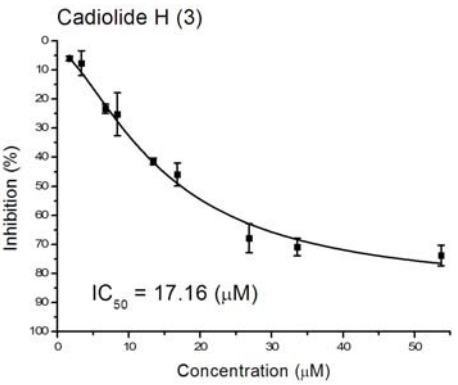


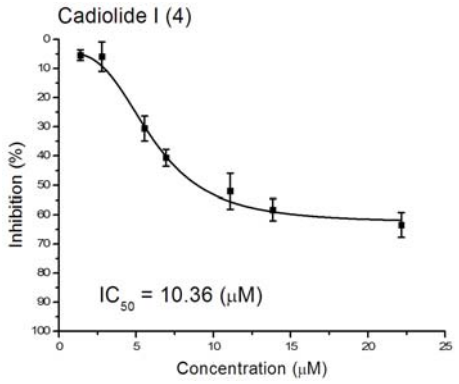
Fig. 3. Structures of brominated aromatic furanones and related esters from the ascidian *Synoicum* sp: cadiolide E (1); cadiolide G (2); cadiolide H (3); cadiolide I (4); synoilide A (5); synoilide B (6).

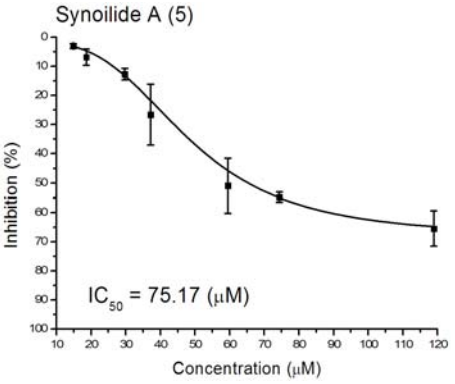

3-Nitropropionic Acid
Inhibition (%)
Concentration (μM)
IC50 = 13.91 (μM)


Cadiolide E (1)
Inhibition (%)
Concentration (μM)
IC50 = 7.62 (μM)


Cadiolide G (2)
Inhibition (%)
Concentration (μM)
IC50 = 48.80 (μM)


Cadiolide H (3)
Inhibition (%)
Concentration (μM)
IC50 = 17.16 (μM)


Cadiolide I (4)
Inhibition (%)
Concentration (μM)
IC50 = 10.36 (μM)


Synoilide A (5)
Inhibition (%)
Concentration (μM)
IC50 = 75.17 (μM)

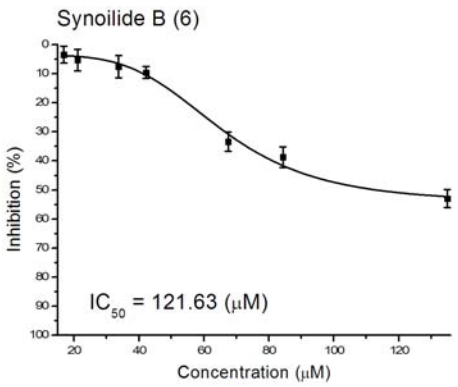


Fig. 4. IC_{50} data analysis of compound **1-6**

Plot of percent control activity against ICL inhibitor concentration. 3-nitropropionic acid was used as a positive control.

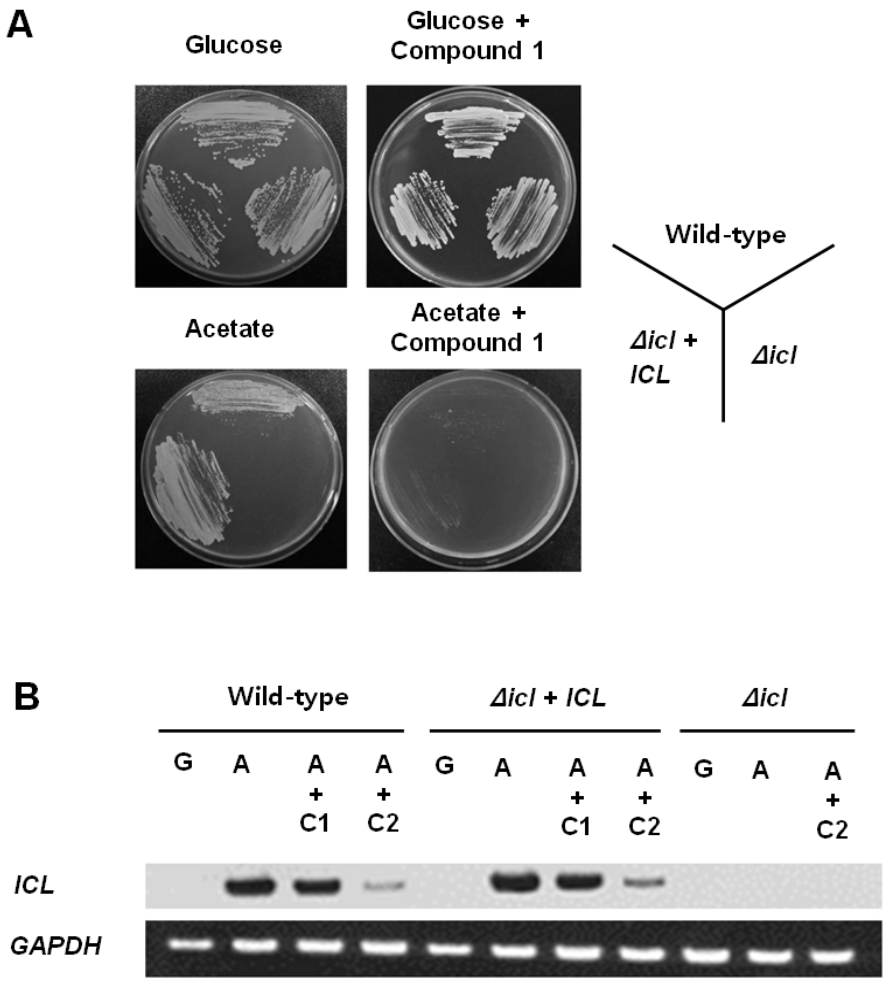


Fig. 5. Inhibitory activity of cadiolide E (1) against growth phenotypes and *ICL* mRNA expression of the wild-type and *Δicl* mutants.

(A) *C. albicans* strain SC5314 (wild-type), MRC10 (Δ*icl*), and MRC11 (Δ*icl* + *ICL*) were grown on YNB agar plates containing the indicated carbon source (2% glucose or 2% sodium acetate) with or without 12.5 μg/ml of compound **1** for 3 days at 28℃.

(B) Strains were grown to mid-log phase in minimal YNB liquid medium containing 2% glucose. Cells were collected by centrifugation and shifted to the same media containing 2% glucose (G), 2% sodium acetate (A), or 2% sodium acetate (A) plus compound **1** (C1, 6.25 μg/ml; C2, 12.5 μg/ml) for 4 h at 28℃. Total RNA was prepared from these cells and the *ICL* mRNA expression was determined by RT-PCR analysis. *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase), a housekeeping gene, was used as a loading control.

Table 1. Antibacterial activity of compounds 1-6

Compound	MIC (μg/ml)					
	Gram(+) bacteria			Gram(-) bacteria		
	A	B	C	D	E	F
1	3.1	1.6	0.8	1.6	3.1	>100
2	3.1	12.5	3.1	0.8	3.1	>100
3	6.3	1.6	3.1	3.1	3.1	>100
4	0.8	0.8	0.8	1.6	6.3	>100
5	100	50	100	50	100	>100
6	>100	>100	>100	>100	>100	>100
ampicillin	0.4	0.4	0.4	0.4	1.6	6.3

A : *Staphylococcus aureus* ATCC 6538p, B: *Bacillus subtilis* ATCC 6633, C: *Kocuria rhizophila* NRBC 12708, D: *Salmonella enteriaca* ATCC 14028 E: *Proteus hauseri* NRBC 3851, F: *Escherichia coli* ATCC 35270

Table 2. Inhibitory activity of compounds 1–6 against ICL enzyme from *C. albicans* ATCC 10231 and cell growth of *C. albicans* SC5314

Compound	ICL IC_{50}, (μg/ml, μM)	MIC (μg/ml)	
		Glucose	Acetate
1	6.05 ± 0.23 (7.62 ± 0.30)	>200	12.5
2	35.66 ± 4.73 (48.80 ± 6.47)	>200	50
3	12.78 ± 0.34 (17.16 ± 0.45)	>200	50
4	9.35 ± 0.60 (10.36 ± 0.66)	>200	50
5	50.57 ± 5.37 (75.17 ± 7.98)	>200	100
6	72.10 ± 4.23 (121.63 ± 7.13)	>200	100
3-Nitropropinate	1.66 ± 0.27 (13.91 ± 2.29)	>200	200
Amphotericin B	ND	1.56	1.56

3-Nitropropinate and amphotericin B inhibitors of ICL and fungal growth, respectively, were used as positive controls; ND, not determined.

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초 록

Synocium sp. 유래의 cadiolides와 synoilides의 항균활성 연구

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해양은 지구 표면적의 75%를 차지하며 수많은 종이 서식하는 것으로 알려져 있다. 해양에 서식하고 있는 수많은 생물종 중에서 1% 미만이 연구되고 있다. 해양생물이 분비하는 각종 천연물들은 항암, 항바이러스, 항진균, 항박테리아에 관여하는 다양한 생리활성 및 독특한 구조를 가지는 것으로 확인되고 있다. 이러한 의미에서 해양천연물은 연구가치가 상당히 높은 편이다.

이전 연구에서 해양생물 중의 하나인 asicidan *Synoicum* sp. 로부터 cadiolides E, G, H, I와 synoilides A, B를 분리하였다. 분리한 cadiolides E-I와 synoilides A, B에 대해 항미생물 활성 및 그에 관련된 필수 효소인 isocitrate lyase(ICL), sortase A, Na^+/K^+-ATPase의 저해활성을 측정하였다. 그 결과 cadiolides E-I와 synoilide A에서 항균활성 및 ICL 저해 활성을 나타내는 것을 확인할 수 있었다. 그 중에서도 cadiolide I는 그람 양성세균에 가장 강한 항균활성을 나타내었으며 (MICs: *S. aureus*, *B. subtilis*, *K.rhizophila*; 0.8, 0.8, 0.8 μg/ml) cadiolide E는 그람 음성세균에

가장 강한 저해활성을 나타내었다 (MICs; *S. enteriaca, P. hauseri* ; 1.6, 6.3 μg/ml). 또한 cadiolides E, I의 ICL 저해활성은 (IC_{50}: 6.05 ± 0.23 μg/ml (7.62 ± 0.30 μM); 9.35 ± 0.60 μg/ml (10.36 ± 0.66 μM)) 비교 물질인 3-nitropropionate (IC_{50}: 1.66 ± 0.27 μg/ml (13.91 ± 2.29 μM))보다 더욱 강력한 것을 확인하였다.

Glyoxlate cycle의 탄소원으로 이용되는 C_2 조건하에서 cadiolides와 synoilides의 *Candida albicans* 최소저해농도를 측정하였다. 그 결과 가장 강한 활성을 가지는 물질은 cadiolide E임을 확인하였다 (MICs: 12.5 μg/ml). 그리고 glyoxylate cycle의 주요 효소인 ICL 유전자의 야생형, 돌연변이의 성장 표현형과 유전자 발현수준을 확인한 결과 cadiolide E가 ICL의 발현을 강하게 저해함을 확인하였다.

당이 부족한 환경하에서 병원성 미생물인 *C. albicans*는 대사경로로 glyoxylate cycle을 사용한다. 그리고 glyoxylate cycle은 식물이나 미생물에서는 발견되는 반면 포유동물에서는 발견되지 않는다고 보고 되었다. 이러한 점에 있어 ICL 저해 활성 물질은 인간에게는 아무런 영향없이 병원성 미생물을 효과적으로 제거할 것이다. 본 연구를 통해 cadiolide는 우수한 항진균제로서 개발 가능한 후보 물질로 사료된다.

주요어 : Antibacterial activity, Isocitrate Lyase(ICL), Glyoxylate cycle, Ascidian *Synoicum* sp., ICL inhibition

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