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# Inhibition of *Candida albicans* isocitrate lyase activity by sesterterpene sulfates from the tropical sponge *Dysidea* sp.

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ABSTRACT

Seven sesterterpene sulfates (**1**–**7**) were isolated from the tropical sponge *Dysidea* sp. and their inhibitory activities against isocitrate lyase (ICL) from *Candida albicans* were evaluated. Among the isolated natural products compound **6** and **7** were found to be strong ICL inhibitors. The isolated compounds (**1**–**7**) also showed potent antibacterial effect against *Bacillus subtilis* and *Proteus vulgaris*, but did not display antifungal activity.



The glyoxylate cycle is a modified form of the tricarboxylic acid cycle; it was initially discovered in microorganisms and plays a fundamental role in nature by providing the means for microorganisms to grow on $C_2$ carbon sources such as acetate, ethanol, or fatty acids.[1] This function has been confirmed by the analysis of mutants of pathogenic microorganisms that lack isocitrate lyase (ICL) and malate synthase (MLS), key enzymes in the glyoxylate cycle.[2–5] The carbon-conserving glyoxylate pathway is present in most prokaryotes, lower eukaryotes, and plants, but it has not been observed in vertebrates.[6]

The role of the glyoxylate cycle in microbial virulence has been reported in many pathogens including *Candida albicans* and *Mycobacterium tuberculosis*.[2,3] Recently, it has been reported that the genes of the glyoxylate cycle are highly induced when *C. albicans* is phagocytized by macrophage.[2] 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. In addition, mutant strains of *C. albicans* lacking ICL are markedly less virulent in a mouse model of systemic candidiasis and less persistent in internal organs than wild-types.[2,7] 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. The inhibitors of ICL would be especially effective in treating the persistent infections like candidiasis and tuberculosis.

Only several ICL inhibitors are reported to date; these include 3-nitropropionate,[8] 3-bromopyruvate,[9] 3-phosphoglycerate,[10] mycenon,[11] oxalate,[12] and itaconate.[12] However, these are not pharmacologically suitable for testing in vivo because of their toxicity and low activity. We have recently isolated two sesterterpene sulfates, halisulfate 1 and halisulfate 5 from tropical sponge *Hippospongia* sp.[13] Both sesterterpene sulfates exhibited stronger inhibitory activity against ICL of the rice blast fungus *Magnaporthe grisea* than 3-nitropropionate, a prototype inhibitor of ICL.

As part of our efforts to discover novel ICL inhibitors, we encountered the marine sponge *Dysidea* sp. (family *Dysididea*) from the Chuuk island of Federated States of Micronesia whose crude extract exhibited significant inhibitory activity against ICL of *C. albicans*. Bioassay-guided separation of the crude extract using various chromatographic techniques yielded five halisulfates and two coscinosulfate analogs. Herein, we report the isolation and biological activities of these compounds.

The specimens of *Dysidea* sp. (family *Dysididea*) were collected by hand using SCUBA at 10–20 m depth from Chuuk Atoll, Federated States of Micronesia. The freeze-dried sponge (112.27 g) was

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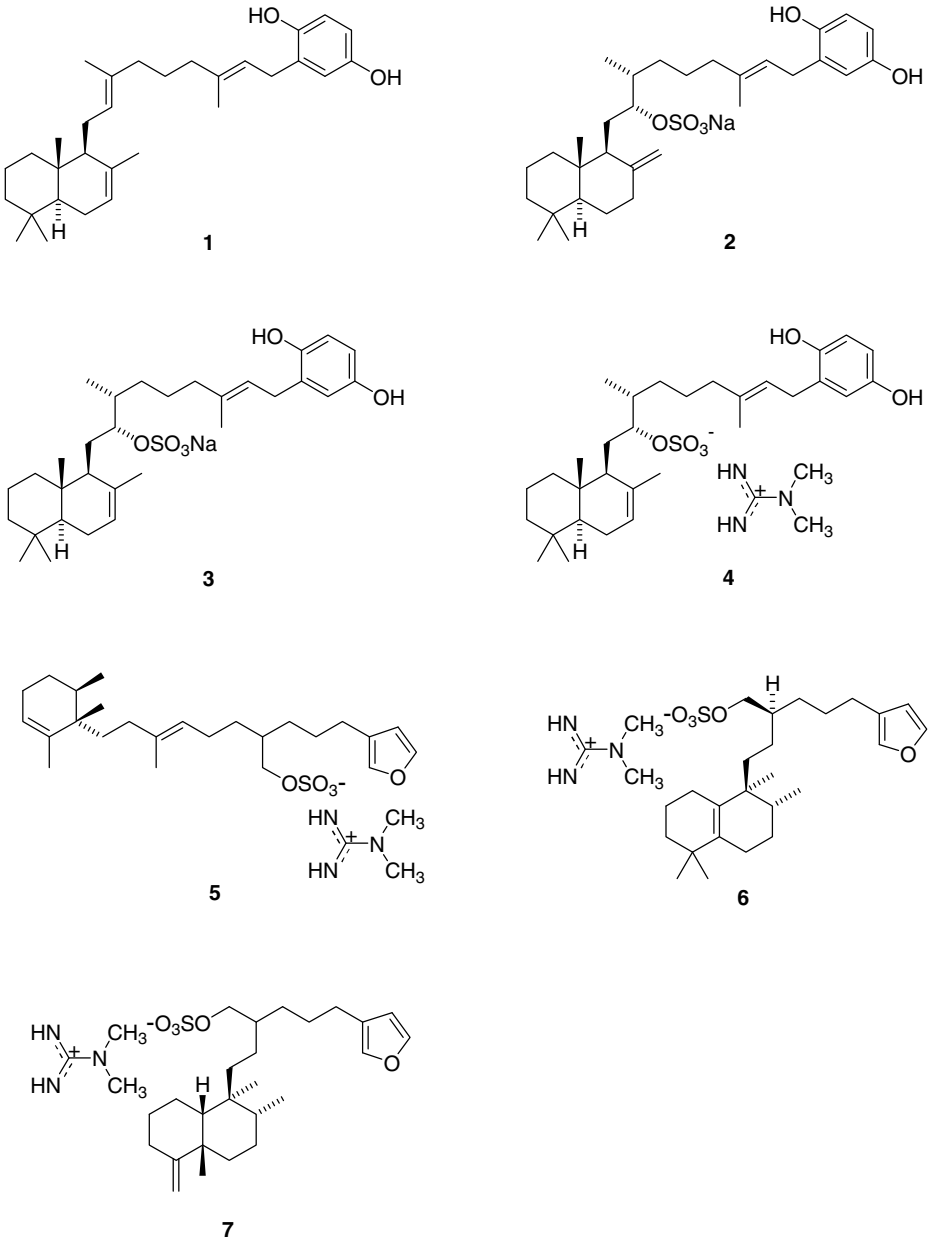


**Figure 1.** Chemical structures of sesterterpene sulfates: coscinoquinol (**1**); coscinosulfate analog (**2**); halisulfate 1 (**3**); halisulfate 1 (**4**), 2 (**5**), 3 (**6**), and 5 (**7**) possessing *N*,*N*-dimethylguanidinium as a common counter ion.

sliced and repeatedly extracted with MeOH (1 L ×5) and $CH_2Cl_2$ (1 L ×2). The extract was filtered and concentrated under reduced pressure to afford 27.03 g of crude extract. The residue was partitioned between $H_2O$ (16.93 g) and *n*-BuOH (9.90 g), then the organic layer was dried and re-partitioned between 15% aqueous MeOH (8.03 g) and *n*-hexane (1.64 g). The residue of aqueous MeOH layer (3.88 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% aqueous MeOH, and 100% MeOH). The fractions eluted with 30% (0.11 g), 20% (1.17 g), 10% aqueous MeOH (1.19 g), and 100% methanol (0.42 g) were dried and separated by reversed phase HPLC (YMC-ODS-A $C_{18}$ column, 250 × 10 mm; 5% aqueous MeOH for **1**, 30% aqueous MeOH for **4**, 50% aqueous $CH_3CN$ for **5**, **6**, and **7**, and 60% aqueous $CH_3CN$ for **2** and **3**) to yield 3.4, 4.8, 26.4, 22.2, 10.0, 5.4, and 12.7 mg of **1**–**7**, respectively.

Compounds **1** and **3** were determined to be coscinoquinol and halisulfate 1, respectively, by combined spectroscopic analyses and comparison of NMR data with those reported previously.[14–16] The molecular formula of compound **2** was deduced to be $C_{31}H_{47}SO_6Na$ by HRFABMS. The NMR data were close to those of

**Table 1**
Inhibitory effect of sesterterpene sulfates on the activity of ICL enzyme and fungal growth of *Candida albicans* ATCC 10231[a]

| Compound | ICL $IC_{50}$, μg/ml (μM) | MIC, μg/ml (μM) |
|---|---|---|
| **1** | 34.6 (76.8) | >100 (222.2) |
| **2** | 54.1 (71.0) | >100 (131.2) |
| **3** | 53.5 (70.2) | >100 (131.2) |
| **4** | >100 (157.5) | >100 (157.5) |
| **5** | >100 (185.5) | >100 (185.5) |
| **6** | 18.2 (33.8) | >100 (185.5) |
| **7** | 16.9 (31.3) | >100 (185.5) |
| 3-Nitropropinate | 6.0 (50.7) | >100 (845.0) |
| Amphotericin B | ND[b] | 1.6 (1.7) |

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

**Table 2**
Antibacterial activity of sesterterpene sulfates

| Compound | Antibacterial activity (MIC, μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | *S. aureus* | *B. subtilis* | *M. luteus* | *P. vulgaris* | *S. typhimurium* | *E. coli* |
| **1** | 100 | 3.25 | 25 | 6.25 | 25 | >100 |
| **2** | 25 | 1.56 | 50 | 6.25 | 50 | >100 |
| **3** | 25 | 1.56 | 50 | 6.25 | 50 | >100 |
| **4** | 50 | 6.25 | >100 | 12.5 | 50 | >100 |
| **5** | >100 | 6.25 | >100 | 25 | >100 | >100 |
| **6** | 50 | 6.25 | 50 | 12.5 | 100 | >100 |
| **7** | 50 | 12.5 | 25 | 6.25 | 25 | >100 |
| Ampicillin | 3.12 | 1.56 | 1.56 | 1.56 | 1.56 | 6.25 |

Microorganisms: *Staphylococcus aureus* ATCC6538p; *Bacillus subtilis* ATCC6633; *Micrococcus luteus* IFC12708; *Proteus vulgaris* ATCC3851; *Salmonella typhimurium* ATCC14028; *Escherichia coli* ATCC11775.

**3** except for the presence of an exo-methylene: two protons at 4.75 and 4.39 ppm, $^{13}C$ $\delta$ 106.2 and 151.2 ppm. Chemicals shifts of all protons and carbons were interpreted through HMBC and HMQC experiments and led to assign coscinosulfate analog (**2**).[15] A closely related congener, compound **4**, was isolated as a white solid. The spectroscopic data for this compound were also very similar to those of compound **3**.[16] However, the presence of *N,N*-dimethylguanidinium as the counter ion of a sulfate group at C-12 was revealed by NMR: $\delta_H$ 3.02 (6H, s); $\delta_C$ 158.7 (C), 38.3 ($CH_3$). Although the sulfates were usually isolated as the sodium salts from marine organisms, the isolation of few *N,N*-dimethylguanidinium sulfates were reported so far.[17–19] The $^1H$ and $^{13}C$ NMR data of compounds **5**, **6**, and **7** were very similar to those of halisulfate 2, 3, and 5, respectively, with the presence of *N,N*-dimethylguanidinium as the only difference in the NMR data, confirmed by a combination of 2D NMR experiments (Fig. 1).[16,20] Some of these metabolites were previously reported as inhibitors of several enzymes (phospholipase A2, serine protease, PMA-induced inflammation, CDC25A phosphatase, and glutathione reductase) and antimicrobial constituents against some microorganisms.[14–16,21]

The cloning and purification of ICL from the genomic DNA of *C. albicans* (ATCC 10231) were carried out as described previously.[12] The compounds **1**–**7** were evaluated for their inhibitory activities toward *C. albicans* ICL according to a previously documented procedure.[12,22,23] 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).[24] Among the compounds tested, the furan moiety containing compounds **6** ($IC_{50}$: 33.8 μM) and **7** ($IC_{50}$: 31.3 μM) showed higher inhibitory activities than 3-nitropropinate. In addition, compounds **6** and **7** were almost two times more potent against ICL than the hydroquinone moiety containing compounds **1**–**3**. The compound **1**, which does not contain the sulfate ester group at the C-12 position, has similar ICL inhibitory activity with **2** and **3**. These observations suggested that the sulfate ester group of sesterterpene sulfates might not be a crucial pharmacophore for ICL inhibition. Interestingly, compounds **4** and **5** were inactive against *C. albicans* ICL ($IC_{50}$ values of >157.5 and >185.5 μM, respectively). By comparing chemical structures of compounds **4**–**7**, it was found that the ICL inhibitory activity of sesterterpene sulfates are markedly decreased when the *N,N*-dimethylguanidinium which serves as the counter ion of a sulfate group is in the immediate vicinity of either the hydroquinone **4** or furan moiety **5**. These observations suggest that ICL inhibitory activity of sesterterpene sulfates is greatly affected by two stereochemical features; distance and orientation. In particular, not only the distance, the spatial orientation of the sulfate ester group relative to that of the hydroquinone or furan moiety also plays a critical role in ICL inhibitory activity. Taken together, our data indicate that the furan moiety of sesterterpene sulfates might be important for effective inhibition of the ICL activity of *C. albicans*.

The in vitro antimicrobial activities of the sesterterpene sulfates **1**–**7** were assessed against three representative Gram-positive bacteria viz. *Staphylococcus aureus* (ATCC6538p), *Bacillus subtilis* (ATCC6633), and *Micrococcus luteus* (IFC12708), three Gram-negative bacteria viz. *Proteus vulgaris* (ATCC3851), *Salmonella typhimurium* (ATCC14028), and *Escherichia coli* (ATCC11775), and four fungal organisms viz. *C. albicans* (ATCC10231), *Aspergillus fumigatus* (HIC6094), *Trichophyton rubrum* (IFO9185), and *Trichophyton mentagrophytes* (IFO40996).[25,26] In an antifungal activity assay using medically important pathogenic fungi including *C. albicans* (Table 1) all of the sesterterpene sulfates **1**–**7** were inactive at 100 μg/ml (data not shown). However, these compounds displayed antibacterial activity against Gram-positive and Gram-negative bacteria except *E. coli* (Table 2). Especially, compounds **1**–**7** exhibited potent inhibitory activities against *B. subtilis* with minimum inhibitory concentration (MIC) values in the range of 1.56–12.5 μg/ml, as shown in comparison to ampicillin.

In conclusion, we isolated sesterterpene sulfates as ICL inhibitors of *C. albicans* from the tropical sponge *Dysidea* sp. based on a bioassay. The furan moiety of sesterterpene sulfates proved to be an important factor for effective inhibition of the ICL activity of *C. albicans*, changing $IC_{50}$s, when comparing the structures of the isolated sesterterpene sulfates. The isolated compounds exhibited significant antibacterial activity against Gram-positive and Gram-negative bacteria, but did not display antifungal activity against pathogenic fungi. Since the enzymes of the glyoxylate cycle are not found in mammals, sesterterpene sulfates are good starting candidates for ICL inhibitor design.

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26. *Candida albicans* ATCC10231, *Aspergillus fumigatus* HIC6094, *Trichophyton rubrum* IFO9185, and *Trichophyton mentagrophytes* IFO40996 were used for antifungal activity tests. *Candida albicans* was grown for 48 h at 28 °C in YPD broth (1% yeast extract, 2% peptone, and 2% dextrose), harvested by centrifugation, and then washed twice with sterile distilled water. *Aspergillus fumigatus*, *T. rubrum*, and *T. mentagrophytes* were plated in potato dextrose agar (PDA) (Difco), incubated at 28 °C for 2 weeks. Spores were washed three times with sterile distilled water and resuspended in distilled water to obtain an initial inoculum size of $10^5$ spores/ml. Each test compound was dissolved in DMSO and diluted with potato dextrose broth (Difco) to prepare serial twofold dilutions in the range of 100–0.4 μg/ml. Ten microliters of the broth containing about $10^3$ (for yeast) and $10^4$ (for filamentous fungi) cells/ml of test fungi were added to each well of a 96-well microtiter plate. Culture plates were incubated for 48–72 h at 28 °C.