Bioorganic & Medicinal Chemistry Letters 21 (2011) 1958–1961

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Sargachromanols as inhibitors of Na^+/K^+ ATPase and isocitrate lyase

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ARTICLE INFO

Article history:
Received 8 January 2011
Revised 8 February 2011
Accepted 9 February 2011
Available online 13 February 2011

Keywords:
Chromenes
Sargachromanols
Isocitrate lyase
Na^+/K^+ ATPase
Antibacterial activity

ABSTRACT

Sargachromanols A–P (**1**–**16**), 16 meroterpenoids of the chromene class isolated from the brown alga *Sargassum siliquastrum*, were evaluated for their inhibitory activities toward Na^+/K^+ ATPase from porcine cerebral cortex and isocitrate lyase (ICL) from *Candida albicans*. These studies led to the identification of compounds **4**, **6**, **8**, and **12** as potent Na^+/K^+ ATPase inhibitors. Compounds **12**, **13**, and **16** exhibited moderate ICL inhibitory activity. Compound **12** also showed weak antibacterial activity. The preliminary structure–activity relationship of these compounds is described to elucidate the essential structural requirements.

In recent years, many marine resources have attracted attention in the search for bioactive compounds to develop new drugs. Meroterpenoids of the chromene class, consisting of a polyprenyl chain attached to a hydroquinone or similar aromatic rings, are widely distributed among marine organisms.[1] Brown algal-derived chromenes exhibit antioxidant activity, cytotoxicity, antiviral activity, inhibitory effects on osteoporosis, and inducement of the larval settlement of a hydrozoan.[2–5] Chromenes from other marine organisms also possess diverse bioactive properties such as anticancer activities and inhibitory activities against various enzymes.[6–8] In continuation of our research for the discovery of inhibitors of enzymes from natural products, we have examined enzyme inhibitory activities of sargachromanols isolated from the brown alga *Sargassum siliquastrum*.[9] Na^+/K^+ ATPase and isocitrate lyase (ICL) were selected as target enzymes since they play crucial roles in cellular function.

Na^+/K^+ ATPase is an ubiquitous sodium pump in the membrane of most eukaryotic cells, which is essential to establish and maintain high K^+ and low Na^+ concentration in the cytoplasm. The establishment of an electrochemical gradient for Na^+ across the plasma membrane is vital for cell functions as diverse as the propagation of nerve signals, volume regulation, nutrient absorption, and pH regulation.[10] Since this pump is the only known receptor for toxic cardiac glycosides such as digoxin and ouabain, which are used to treat some heart diseases like congestive heart failure and cardiac arrhythmias, a new type of less toxic natural regulators of this pump might be useful for clinical purposes.[11–13]

The glyoxylate cycle is a reaction sequence in which acetates are converted to succinates during the energy production and biosynthesis of cell constituents; this cycle enables bacteria and fungi to grow on acetate in a hostile environment inside the macrophage where glucose is not available.[14,15] ICL is an enzyme that transforms isocitrate into glyoxylate in the glyoxylate cycle. It has been discovered that the microbial virulence of *Candida albicans* significantly decreased in the case of mutant strains lacking the ICL. Since expression of glyoxylate cycle genes is detected during specific stages of the interaction between host and pathogen in a variety of human-pathogenic bacteria and fungi, the development of specific inhibitors against ICL is an attractive prospect.[16–18]

In a previous work,[9] we had collected the brown alga *S. siliquastrum* (Mertens ex Turner, C. Agardh) (family Sargassaceae) from Jeju Island, Korea. The crude extract of these specimens exhibited significant antioxidant activity. Bioassay-guided separation of the crude extracts using various chromatographic techniques yielded 16 new chromenes, sargachromanols A–P (**1**–**16**) (Fig. 1). The structures of the polyprenyl portions of these chromanol-containing compounds were determined to be linear triprenyls (**1** and **2**) and tetraprenyls (**3**–**11**), while others were the corresponding rearranged (**12**–**15**) and cyclized (**16**) tetraprenyls, respectively. Herein we describe the bioactivity of these compounds toward Na^+/K^+ ATPase from porcine cerebral cortex, isocitrate lyase (ICL) from *C. albicans*, and antimicrobial activity. The preliminary structure–activity relationship of these compounds is described to elucidate the essential structural requirements.

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doi:10.1016/j.bmcl.2011.02.035

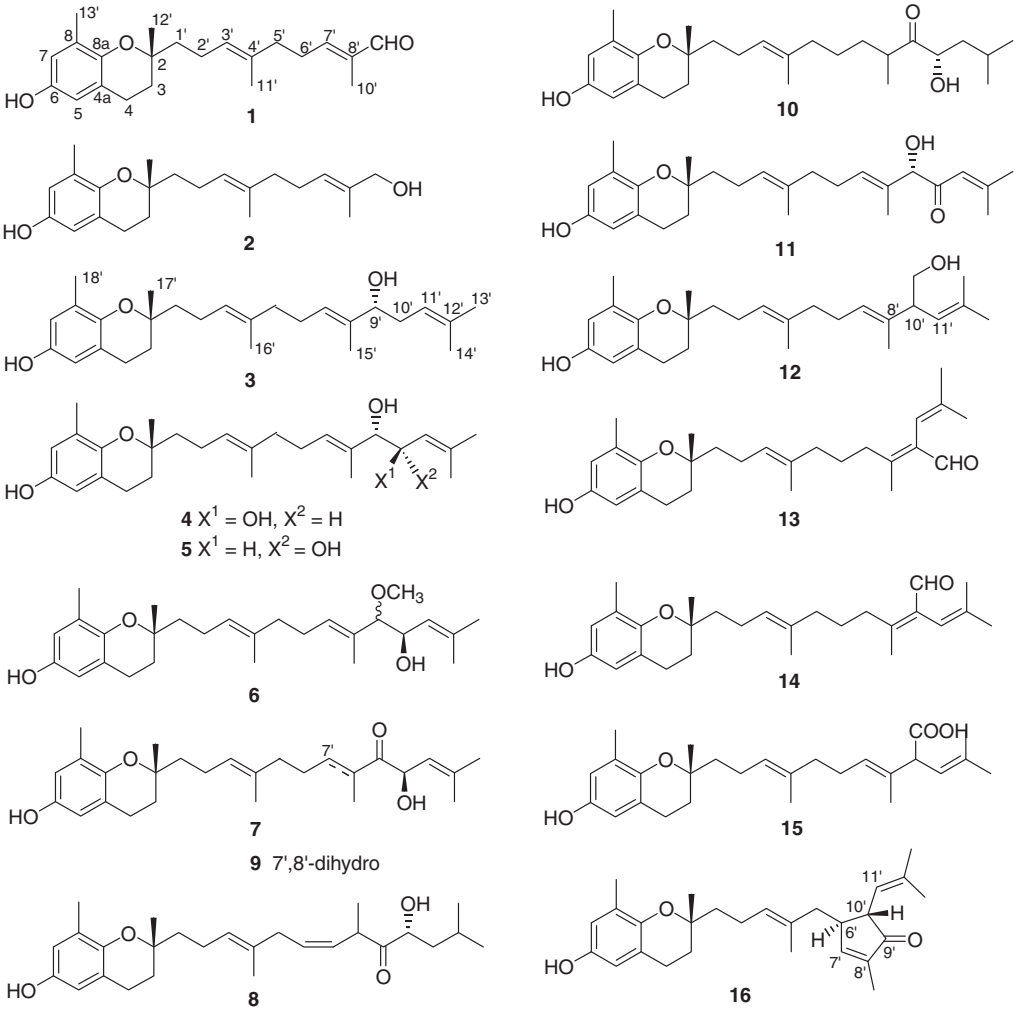

Figure 1. Chemical structures of sargachromanols A–P (**1**–**16**).

The inhibitory activity of compounds **1–16** against Na^+/K^+ ATPase from porcine cerebral cortex was measured by a fluorometric method.[13] The method is based on the on-line determination of change in fluorescence due to the formation of the fluorescent compound 3-*O*-methylfluorescein from the parent compound 3-*O*-methylfluorescein phosphate.[19] 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 Na^+/K^+ ATPase inhibitor, ouabain (IC_{50} 4.6 μM).[13] Among the tetraprenyl chromanols **3–11**, compound **4**, **6**, and **8** were found to be strong Na^+/K^+ ATPase inhibitor, with IC_{50} values of 3.6, 6.0, and 4.6 μM, respectively. Compound **12**, a tetraprenyl chromanol possessing a rearranged carbon skeleton, also showed potent inhibitory activity, with IC_{50} value of 7.0 μM. The Na^+/K^+ ATPase inhibitory activity study of these compounds revealed that the hydroxyl groups at the C-9′ and C-10′ position of compound **4** were important (Fig. 1). Compound **4** was 23 times more potent than that of compound **3**, which does not contain hydroxyl group at the C-10′ position. Compounds **4** and **5** were diastereomers of each other.[9] Carbonylation of the hydroxyl group at the C-9′ or C-10′ position of compound **4**, as in compounds **10** and **11**, respectively, exhibited lower inhibitory activities than compound **4**. These results suggest that the Na^+/K^+ ATPase inhibitory activities of tetraprenyl chromanols are altered by substitution at the C-9′ and C-10′ position.

The cloning and purification of ICL from the genomic DNA of *C. albicans* (ATCC 10231) were carried out as described previously.[20]

Table 1
ICL and Na^+/K^+ ATPase inhibitory activities of sargachromanols **1–16**[a]

Compound	ICL IC_{50} (μg/ml, μM)	Na^+/K^+ ATPase IC_{50} (μg/ml, μM)
1	63.4 (185.0)	10.4 (30.4)
2	>200	15.0 (43.6)
3	>200	36.2 (83.1)
4	>200	2.0 (3.6)
5	>200	7.8 (14.0)
6	>200	2.8 (6.0)
7	>200	10.4 (24.4)
8	>200	2.0 (4.6)
9	>200	25.9 (60.6)
10	>200	8.68 (20.2)
11	>200	13.0 (30.5)
12	48.4 (118.4)	2.9 (7.0)
13	71.0 (172.9)	59.3 (144.6)
14	>200	18.5 (45.0)
15	>200	53.5 (125.6)
16	60.8 (141.0)	86.6 (200.8)
3-NP[b]	4.2 (34.8)	—[d]
Ouabain[c]	—	3.37 (4.6)

[a] Enzyme inhibitory activities were measured as described in Refs. 19,22.
[b] 3-Nitropropionate, an ICL inhibitor used as a positive control.
[c] Ouabain, a Na^+/K^+ ATPase inhibitor used as a positive control.
[d] Not determined.

The compounds **1–16** were evaluated for their inhibitory activities toward *C. albicans* ICL according to a previously documented

procedure.[21,22] The inhibitory potencies (IC_{50}) of the tested compounds are shown in Table 1 and are compared to that of a known ICL inhibitor, 3-nitropropinate (IC_{50} 34.8 μM). Interestingly, tetraprenyl chromanols **3**–**11** were inactive against *C. albicans* ICL (IC_{50}s >200 μg/ml). Compounds **1**, **12**, **13**, and **16** showed moderate inhibitory activities, with IC_{50} values of 185.0, 118.4, 172.9, and 141.0 μM, respectively. By comparing chemical structures of tested compounds, it was found that the ICL inhibitory activities of tetraprenyl chromanols **12**–**15** were altered by substitution at the C-10′ position. The replacement of the C-10′ oxymethylene group of **12** (IC_{50} 118.4 μM) with an aldehyde (**13**) resulted in a decrease of ICL inhibitory activity (IC_{50} 172.9 μM). In this study, compound **14** showed little activity against *C. albicans* ICL (IC_{50} >200 μg/ml). Compounds **13** (*E* configuration) and **14** (*Z* configuration) were defined as aldehyde-bearing chromanols isomeric to each other.[9] The replacement of the C-10′ oxymethylene group of **12** with a carboxyl group (**15**) led to a total loss of activity (IC_{50} >200 μg/ml).

The in vitro antimicrobial activities of the sargachromanols **1**–**16** were assessed against three representative Gram-positive bacteria including *Staphylococcus aureus* (ATCC 6538p), *Bacillus subtilis* (ATCC 6633), and *Micrococcus luteus* (IFO 12708), three Gram-negative bacteria, *Proteus vulgaris* (ATCC 3851), *Salmonella typhimurium* (ATCC 14028), and *Escherichia coli* (ATCC 25922), and four fungi, *C. albicans* (ATCC 10231), *Aspergillus fumigatus* (HIC 6094), *Trichophyton rubrum* (IFO 9185), and *T. mentagrophytes* (IFO 40996).[23,24] The minimum inhibitory concentrations (MICs) of the tested compounds are displayed in Table 2. Among the sargachromanols tested, compound **12** exhibited only weak inhibitory activities against Gram-positive and Gram-negative bacteria except *E. coli*, with MIC values in the range of 12.5–25 μg/ml, as shown in comparison to ampicillin. In an antifungal activity assay using medically important pathogenic fungi, all of these compounds were inactive at 100 μg/ml.

In conclusion, 16 meroterpenoids of the chromene class were isolated from the brown alga *S. siliquastrum* and their inhibitory activities against Na^+/K^+ ATPase from porcine cerebral cortex and isocitrate lyase (ICL) from *C. albicans* were investigated. These studies led to the identification of compounds **4**, **6**, **8**, and **12** as new and promising lead compounds for the development of potent Na^+/K^+ ATPase inhibitors. Compounds **12**, **13**, and **16** also exhibited moderate ICL inhibitory activity. Since the enzymes of the glyoxylate cycle are not found in mammals, the isolated chromene compounds tested in this study are starting candidates for ICL inhibitor design.

Table 2
MIC of sargachromanols **1**–**16** against bacterial strains[a,b]

Compound	MIC (μg/ml)					
	SA	BS	ML	PV	ST	EC
1	>100	>100	>100	>100	>100	>100
2	100	50	50	50	100	>100
3	>100	>100	>100	>100	>100	>100
4	>100	25	>100	12.5	25	>100
5	>100	>100	>100	>100	>100	>100
6	>100	>100	>100	>100	>100	>100
7	>100	>100	>100	>100	>100	>100
8	>100	>100	>100	>100	>100	>100
9	>100	>100	>100	>100	>100	>100
10	>100	>100	>100	>100	>100	>100
11	>100	>100	>100	>100	>100	>100
12	25	25	25	12.5	12.5	>100
13	>100	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100	>100
15	>100	>100	>100	>100	>100	>100
16	>100	>100	>100	>100	>100	>100
Ampicillin	0.78	0.78	0.78	0.78	0.78	3.12

[a] MIC value represents concentration giving complete inhibition relative to the negative control.

[b] Microorganisms: SA, *Staphylococcus aureus* ATCC 6538p; BS, *Bacillus subtilis* ATCC 6633; ML, *Micrococcus luteus* IFC 12708; PV, *Proteus vulgaris* ATCC 3851; ST, *Salmonella typhimurium* ATCC 14028; EC, *Escherichia coli* ATCC 25922.

Acknowledgment

This study was supported by a grant of the Korea Healthcare Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (A080051).

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23. Three Gram-positive bacteria (*Staphylococcus aureus* ATCC 6538p, *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* IFO 12708) and three Gram-negative bacteria (*Proteus vulgaris* ATCC 3851, *Salmonella typhimurium* ATCC 14028 and *Escherichia coli* ATCC 25922) were used for antibacterial activity tests. Bacteria were grown overnight in Luria–Bertani (LB) broth at 37 °C, harvested by centrifugation, and then washed twice with sterile distilled water. Each test compound was dissolved in DMSO and diluted with Standard methods broth (Difco) to prepare serial twofold dilutions in the range of 100–0.05 μg/ml. Ten microliters of the broth containing approximately 10^5 colony-forming units (cfu)/ml of test bacteria was added to each well of a 96-well microtiter plate. Culture plates were incubated for 24 h at 37 °C. The minimum inhibitory concentration (MIC) values were determined as the lowest concentration of test compounds that inhibited bacterial growth. Ampicillin was used as a reference compound.
24. *Candida albicans* ATCC 10231, *Aspergillus fumigatus* HIC 6094, *Trichophyton rubrum* IFO 9185 and *T. mentagrophytes* IFO 40996 were used for antifungal activity tests. *C. 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 twice with sterile distilled water. *A. fumigatus*, *T. rubrum* and *T. mentagrophytes* were plated in potato dextrose agar (Difco) and incubated for 2 weeks at 28 °C.

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 100–0.05 μg/ml. Ten microliters of the broth containing approximately 10^4 cells/ml of test fungi was added to each well of a 96-well microtiter plate. Culture plates were incubated for 48–72 h at 28 °C. The MIC values were determined as the lowest concentration of test compounds that inhibited fungal growth. Amphotericin B was used as a reference compound.