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Biselyngbyasides, cytotoxic marine macrolides, are novel and potent inhibitors of the Ca^{2+} pumps with a unique mode of binding

Maho Morita [a,b], Haruo Ogawa [b], Osamu Ohno [a], Takao Yamori [c], Kiyotake Suenaga [a], Chikashi Toyoshima [b,*]

[a] Department of Chemistry, Keio University, Kohoku-ku, Yokohama, Kanagawa 223-8522, Japan
[b] Institute of Molecular and Cellular Biosciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-0032, Japan
[c] Cancer Chemotherapy Center, Japanese Foundation for Cancer Research, Ariake, Koto-ku, Tokyo 135-8550, Japan

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ABSTRACT

Biselyngbyasides (BLSs), macrolides from a marine cyanobacterium, are cytotoxic natural products whose target molecule is unknown. Here we report that BLSs are high affinity ($K_i \sim 10$ nM) inhibitors of Ca^{2+}-pumps with a unique binding mode. The crystal structures of the Ca^{2+}-pump in complex with BLSs at 3.2–3.5 Å-resolution show that BLSs bind to the pump near the cytoplasmic surface of the transmembrane region. The crystal structures and activity measurement of BLS analogs allow us to identify the structural features that confer high potency to BLSs as inhibitors of the pump.

1. Introduction

Natural products and their derivatives are rich sources of pharmaceutical lead compounds for the treatment of various diseases [1–3]. Especially in the area of cancer chemotherapy, more than half of anticancer agents are derived from or inspired by natural products [4]. Biselyngbyasides (BLSs, **1**–**4**, Fig. 1) are macrolides that display potent cytotoxicity against a variety of human cancer cells (e.g. GI_{50} of **1** = 36 nM for the central nervous system cancer cells SNB-78, 67 nM for lung cancer cells NCI H522) [5–8]. To date, six BLSs have been isolated from the marine cyanobacterium *Lyngbya* sp. The best studied is biselyngbyaside [5] (BLS, **1**), featuring a glycosylated 18-membered macrolactone containing a conjugated 1,3-diene, two olefins, and a side chain at C17. We previously showed that BLS (**1**) inhibits osteoclastgenesis and induces apoptosis of osteoclasts [9], and that the cytotoxicities of BLS analogs vary dramatically depending on their structures [6] (e.g. IC_{50} against HeLa cells: 2.5 μM for **1**, 0.0028 μM for **2**, 0.0039 μM for **3**, >10 μM for **4**). It is unknown why the cyanobacterium produces BLSs. In general, however, toxic secondary metabolites are hypothesized to protect cyanobacteria from predator such as mollusk and crustacean, or provide a competitive advantage for survival over other microorganisms.

To predict the target protein of BLSs, BLS (**1**) was submitted to Japanese Foundation for Cancer Research 39 (JFCR39) anticancer drug screening system (Table 1) [5]. The inhibition profile was similar to that of thapsigargin [10] (TG, **5**, Fig. 1), a potent inhibitor of sarco/endoplasmic reticulum (SR/ER) Ca^{2+}-ATPases (SERCA) [11], the calcium pump responsible for establishing the Ca^{2+} concentration gradient across the SR/ER membrane. The inhibition profile of BLS is also similar, though not to the same extent, to those of cyclopiazonic acid (CPA, **6**) [12–15] and 2,5-di-*tert*-butyl-1, 4-benzohydroquinone (BHQ, **7**) [16,17], known inhibitors of SERCA. However, BLSs have no structural resemblance to any of

Abbreviations: BHC, benzene hexachloride; BHQ, 2,5-di-*tert*-butyl-1, 4-benzohydroquinone; BLLA, biselyngbyolide A; BLLB, biselyngbyolide B; BLS, biselyngbyaside; BLSC, baselyngbyaside C; $C_{12}E_8$, octaethyleneglycol-*n*-dodecylether; CPA, cyclopiazonic acid; JFCR39, Japanese Foundation for Cancer Research 39; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SERCA, sarco/endoplasmic reticulum (SR/ER) Ca^{2+}-ATPases; SR, sarcoplasmic reticulum; TG, thapsigargin

Author contributions: C.T. supervised the study; M.M., C.T., O.O. and K.S. conceived the study; M.M., C.T. and H.O. performed experiments; C.T. and H.O. supervised structure refinement; M.M. and H.O. analyzed data; T.Y. analyzed the data from JFCR39 screening system; M.M. and C.T. wrote the manuscript.

* Corresponding author.
E-mail address: ct@iam.u-tokyo.ac.jp (C. Toyoshima).

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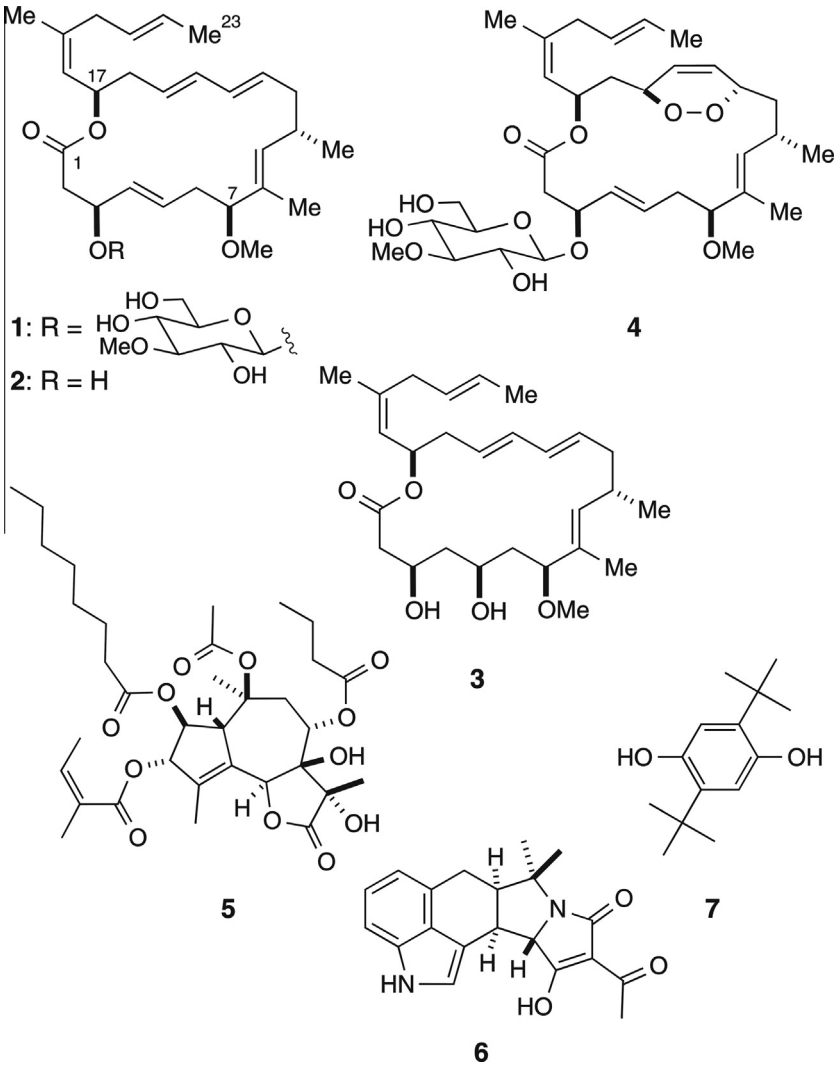

Fig. 1. Structures of biselyngbyasides and inhibitors of SERCA.

Table 1
Correlation between growth inhibition profiles of BLS and reference chemicals.

Rank	*r* value[a]	Reference chemical	Function
1	0.706[b,c]	TG	SERCA inhibitor
2	0.653[c]	Pyriproxyfen	Insecticide
3	0.622[b,c]	TG	SERCA inhibitor
4	0.590	γ-BHC[d]	Insecticide
5	0.589	CPA	SERCA inhibitor
6	0.571	Methoprene	Juvenile hormone analog
7	0.565	Dieldrin	Insecticide
8	0.559	BHQ	SERCA inhibitor
:	:		
:	:		

[a] Pearson correlation coefficient between the 50% growth inhibition (GI_{50}) profile of BLS and that of the reference chemical.
[b] Two values were obtained by independent tests.
[c] It is generally accepted that an *r* value of >0.6 suggests a mode of action similar to that of the reference chemical.
[d] γ-Benzene hexachloride.

them. Since inhibition of SERCA results in activation of ER-stress response and induction of apoptotic cell death [18,19], SERCA inhibitors are now of great interest as lead compounds for cancer chemotherapy [20,21]. For instance, a peptide-conjugated derivative of TG has been in human phase II evaluation as a chemotherapeutic agent against prostate cancer [20].

The SERCA family consists of SERCA1–3 [22,23], of which the best-studied member is SERCA1a, which is abundant in fast-twitch skeletal muscle. SERCA1a is composed of a large cytoplasmic headpiece consisting of A (actuator), N (nucleotide binding) and P (phosphorylation) domains, and 10 transmembrane helices (M1–M10) [24,25]. According to classical E1/E2 theory [26,27], in which SERCA1a has high affinity for Ca^{2+} in E1 but low affinity in E2, active transport of Ca^{2+} is achieved by alternating the affinity for Ca^{2+} and the sidedness of the binding sites [28].

2. Results and discussion

We evaluated the effect of BLSs (**1**–**4**) on the ATPase activity of SERCA1a and 2a by a coupled enzyme assay (Supplementary Fig. S2). BLSs (**1**–**3**) strongly inhibited SERCA1a (K_i = 19 nM for **1**, 17 nM for **2**, ~9 nM for **3**) similar to TG (**5**), whereas biselyngbyaside C (BLSC, **4**) was only weakly active (K_i = 18 μM) (Table 2). The low affinity of BLSC (**4**) to SERCA is consistent with the cell-based assessment of BLSs [6], but the comparable affinity of BLS (**1**) to those of biselyngbyolide B (BLLB, **2**) and biselyngbyolide A (BLLA, **3**) is markedly different.