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Beta-carboline alkaloids derived from the ascidian *Synoicum* sp.

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ABSTRACT

Six β-carboline alkaloids (**1–6**) of the eudistomin Y class were isolated from the Korean ascidian *Synoicum* sp. These compounds were chemically converted to a known compound, eudistomin Y_1 (**7**) and six new derivatives, designated eudistomins Y_8–Y_{13} (**8–13**). Several of these natural and synthetic compounds exhibited moderate to significant antimicrobial activity, weak cytotoxic activity, and inhibitory activities toward sortase A, isocitrate lyase, and Na^+/K^+–ATPase. Structure–activity relationships were also deduced.

1. Introduction

Marine ascidians (phylum Chordata, class Ascidiacea) are widely recognized as prolific sources of structurally unique and biologically active alkaloids.[1,2] Of these metabolites, β-carboline compounds, represented by eudistomins, are frequently encountered and have been harvested from various ascidians from several taxonomical groups. Since the first isolation of eudistomins A–Q from *Eudistoma olivaceum*,[3] about thirty such compounds have been reported from the genera *Eudistoma*,[4–11] *Lissoclinum*,[12] *Pseudodistoma*,[13] *Ritterella*,[14] and *Synoicum*.[15] These compounds exhibited significant bioactivities, including antimicrobial,[6,16] antiviral,[3,14] calmodulin antagonistic,[5] and cytotoxic activities,[8,10] and have attracted considerable synthetic and biomedical interests.[17–21] In our search for the bioactive compounds from marine invertebrates, we encountered purple-colored ascidian *Synoicum* sp., which was harvested from Korean coastal waters. The organic extract obtained from the exhibited moderate cytotoxicity (LC_{50} 39.0 μg/mL) against the A549 cell line. Bioactivity-guided separation of the extract using multiple chromatographic methods yielded cytotoxic eudistomins Y_2–Y_7 (**1–6**). The biomedical importance of these compounds prompted us to pursue chemical derivatization as a means of determining structure–activity relationships. We report here the isolation and preparation of eudistomins Y_1 (**7**) and Y_8–Y_{13} (**8–13**). Several of these natural and synthetic compounds exhibited moderate to significant cytotoxicity and antibacterial activity as well as inhibitory activities toward enzymes, including sortase A (SrtA), isocitrate lyase (ICL), and Na^+/K^+–ATPase.

2. Results/discussion

The ascidian specimens were lyophilized, macerated, and repeatedly extracted with MeOH and CH_2Cl_2. The combined extracts were separated by solvent-partitioning followed by reversed-phase flash chromatography. The polar fractions were separated by reversed-phase HPLC to yield six compounds as yellow amorphous solids. The structures of the major (**3**, **4**, and **5**) and minor compounds (**1**, **2**, and **6**) were identified as eudistomins Y_2–Y_7 by combined spectroscopic analyses. These compounds were recently reported as antimicrobial β-carboline alkaloids from the ascidian *Eudistoma* sp. The spectroscopic data of these compounds were in good agreement with those reported in the literature.[11]

The significant bioactivity of eudistomin extracts prompted us to synthetically derivatize the major extract constituents. A direct attempt to debrominate **4** as a means of producing eudistomin Y_1 (**7**), a bromine-lacking eudistomin from *Eudistoma* sp., using H_2 and Pd/C resulted in concomitant debromination and reduction to yield eudistomin Y_8 (**8**), a new hydroxy-containing derivative. Compound **7** was subsequently prepared by oxidation of the hydroxy group of **8** (Scheme 1). The spectroscopic data of this compound agree with those in the literature.[11]

Compounds **3–5** were reduced to the corresponding hydroxy-containing eudistomins Y_9–Y_{11} (**9–11**) by treating with $NaBH_4$ (Scheme 2). The optical rotations were zero for compounds **8–11**,

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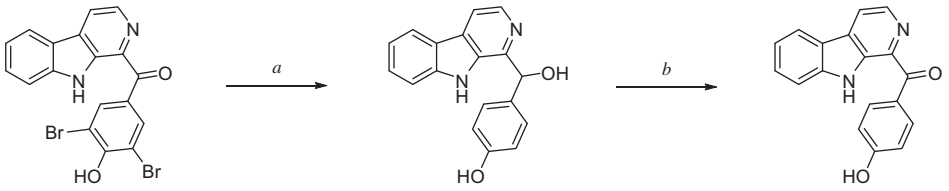
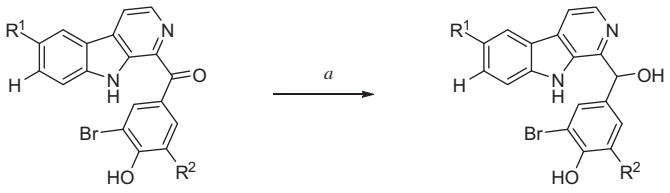


4 8 7

Scheme 1. For debromination and oxidation. Reagents and conditions: (a) H_2, Pd/C, MeOH, Et_3N, rt, 18 h; (b) PDC, CH_2Cl_2/THF, rt, 3 h.



3 R^1=Br, R^2=H
4 R^1=H, R^2=Br
5 R^1=Br, R^2=Br

9 R^1=Br, R^2=H
10 R^1=H, R^2=Br
11 R^1=Br, R^2=Br

Scheme 2. For reduction. Reagents and conditions: $NaBH_4$, THF, rt, 4 h.

which implies a non-stereoselective attack of the hydride at the carbonyl.

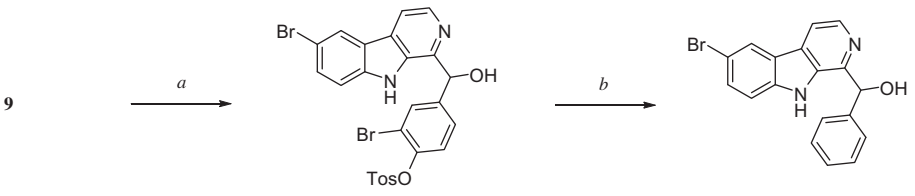
In addition, eudistomin Y_{13} (**13**), a new derivative that lacks substituents on its phenyl ring, was prepared from **9** by a two-step reaction using Tos-Cl and $LiAlH_4$. The tosylated eudistomin Y_{12} (**12**) was produced as an intermediate (Scheme 3). All the noble synthetic compounds were structurally identified using LRESIMS, HRFABMS, and NMR analyses. Protons and carbon atoms were adequately assigned by combined 1-D and 2-D NMR methods (Supplementary data).

To assess their potential as therapeutic agents, eudistomins (**1–6**) and their derivatives (**7–13**) were screened for bioactivity. They were first tested for in vitro cytotoxicity against the cancer cell line A549 using a MTT assay.[22] With the exception of **9** (LC_{50} 17.9 μM), all of the compounds reported herein exhibited weak to no activity against this cell line (LC_{50} 3.3 μM for doxorubicin) (Table 1). However, this indicates that a previous report, which stated that **1–7** showed no cytotoxicity, may need reconsideration.[11]

Sortase A (SrtA) is an enzyme that catalyzes the attachment of surface proteins to the peptidoglycan cell layer in Gram-positive bacteria, including *Staphylococcus aureus*.[23] Surface proteins not only promote interactions between invading pathogens and animal tissues, but also provide strategies for bacterial escape from the host's immune response. Therefore, SrtA has been regarded as a promising target for the development of efficient antibacterial agents.[24] The inhibitory activity of compounds **1–13** against recombinant SrtA from *S. aureus* ATCC 6538p were examined.[25] Compounds **3** and **4** exhibited marginal inhibitory activity (IC_{50} 163.2 and 146.4 μM, respectively) (Table 1).

Isocitrate lyase (ICL) is an enzyme that transforms isocitrate into glyoxylate in the glyoxylate cycle. Relative to the wild type, the microbial virulence of *Candida albicans* is significantly reduced in mutant strains that lack ICL.[26] The expression of glyoxylate cycle genes is detected during specific stages of the interaction between host and pathogen in a variety of pathogenic bacteria and fungi. Therefore, the development of specific inhibitors against ICL is an attractive prospect.[27] Compounds **1–13** were evaluated for inhibition of recombinant *C. albicans* ICL in accordance with a previous procedure.[28] 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-nitropropionic acid (IC_{50} 38.6 μM). Compounds **1**, **7**, and **8** showed moderate inhibition, with IC_{50} values of 50.2, 48.2, and 68.9 μM, respectively. Unfortunately, the inhibitory activities of the other seven compounds could not be determined (ND in Table 1). It is because the ICL assay spectrophotometrically measures the formation of glyoxylate phenylhydrazone by monitoring the solution absorbance at 324 nm in the presence of phenylhydrazine and isocitrate.[29] However, several of the evaluated compounds absorbed strongly at 324 nm, resulting in non-linear assay absorbance curves.

In addition, the inhibitory activities of compounds **1–13** against porcine cerebral cortex Na^+/K^+−ATPase were measured fluorometrically.[28] The method monitors the formation of fluorescent 3-*O*-methylfluorescein from the parent compound 3-*O*-methylfluorescein phosphate.[30] The inhibitory potencies (IC_{50}) of the tested compounds are shown in Table 1 and are compared to that of ouabain, a known Na^+/K^+−ATPase inhibitor (IC_{50} 6.3 μM). β-Carboline alkaloids **2–6** were strong inhibitors, with IC_{50} values of 7.5–22.5 μM. Compound **3** exhibited a similar activity (IC_{50} 7.5 μM) as that of ouabain. These findings revealed that the bromine group at the C-13 position of compound **3** was important (Fig. 1). For example, compound **7**, which does not contain a bromine at the C-13 position, was inactive with an IC_{50} of 346.9 μM. Bromination at the C-6 position of compound **3**, as in compounds **5**, **9**, and **11**, resulted in higher inhibitory activities than that of the debrominated compound **10**. Also, the presence of a hydroxy group at C-14 was important for the bioactivity of **9**, which exhibited a higher inhibitory activity (IC_{50} 33.3 μM) than **12** and **13**, which had a tosyl group and no substituent, respectively, at this position. These two compounds were inactive with IC_{50} values of 166.0 and 283.1 μM, respectively. Taken together, these results suggest that the Na^+/



12 13

Scheme 3. For tosylation and dehydroxlation. Reagents and conditions: Tos-Cl, CH_2Cl_2, Et_3N, rt, 2 h; $LiAlH_4$, THF, rt, 1 h.

Table 1
Results of bioactivity test

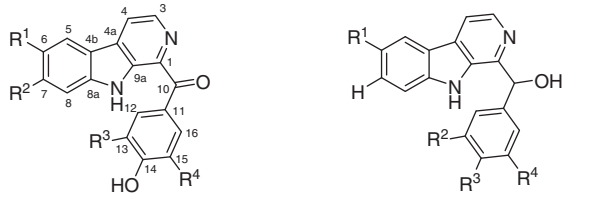
Compound	MIC(μg/mL)										A549	SrtA	ICL	Na^+/K^+−ATPase
	Gram (+) bacterium			Gram (−) bacterium			Fungus				LC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)	IC_{50} (μM)
	A	B	C	D	E	F	G	H	I	J	(*n* = 3)	(*n* = 3)	(*n* = 3)	(*n* = 3)
1	50	25	25	50	25	>100	>100	>100	>100	6.25	65.6 ± 0.6	>272.3	50.2 ± 0.7	209.3 ± 3.1
2	12.5	12.5	12.5	6.25	6.25	100	>100	>100	>100	>100	252.7 ± 0.1	>272.3	>272.3	22.5 ± 0.6
3	3.125	0.78	1.56	0.39	0.39	50	>100	>100	>100	>100	48.4 ± 3.1	163.2 ± 2.4	ND[c]	7.5 ± 0.3
4	6.25	3.125	3.125	0.78	1.56	100	>100	>100	>100	>100	85.4 ± 3.3	146.4 ± 1.9	ND	19.8 ± 0.5
5	1.56	1.56	1.56	0.39	0.78	50	>100	>100	>100	>100	60.0 ± 3.5	>190.5	ND	10.1 ± 0.4
6	3.125	0.78	1.56	0.78	0.78	50	>100	>100	>100	>100	59.0 ± 0.2	>190.5	ND	11.3 ± 0.8
7	>100	>100	>100	>100	>100	>100	50	50	100	50	59.0 ± 2.4	>346.9	48.2 ± 0.8	>346.9
8	100	100	100	>100	>100	>100	>100	>100	>100	>100	>344.4	>344.4	68.9 ± 1.1	>344.4
9	6.25	>100	>100	>100	>100	>100	>100	>100	>100	>100	17.9 ± 1.1	>223.2	ND	33.3 ± 0.9
10	25	25	12.5	12.5	12.5	>100	>100	>100	>100	>100	>223.2	>223.2	ND	122.1 ± 2.2
11	6.25	3.125	3.125	3.125	3.125	100	>100	>100	>100	>100	>189.8	>189.8	ND	32.8 ± 1.0
12	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100	63.3 ± 1.8	>166.0	>166.0	>166.0
13	>100	100	100	>100	>100	>100	>100	>100	>100	>100	49.0 ± 0.7	>283.1	>283.1	>283.1
Ampicillin	0.39	0.78	0.39	0.78	0.39	6.25								
Amphotericin B							0.78	0.78	0.39	0.39				
Doxorubicin											3.3 ± 0.1			
*p*HMB[a]												111.6 ± 1.5		
3-NP[b]													38.6 ± 0.9	
Ouabain														6.3 ± 0.1

A: *Staphylococcus aureus* (ATCC 6538p), B: *Bacillus subtilis* (ATCC 6633), C: *Micrococcus luteus* (IFO 12708), D: *Salmonella typhimurium* (ATCC 14028), E: *Proteus vulgaris* (ATCC 3851), F: *Escherichia coli* (ATCC 35270), G: *Aspergillus fumigatus* (HIC 6094), H: *Trichophyton rubrum* (IFO 9185), I: *Trichophyton mentagrophytes* (IFO 40996), J: *Candida albicans* (ATCC 10231).

[a] *para*-Hydroxymercuribenzoic acid.

[b] 3-Nitropropionic acid.

[c] Due to the high initial absorbance, inhibition was not accurately measured.



1 R^1=Br, R^2=H, R^3=H, R^4=H
2 R^1=H, R^2=H, R^3=Br, R^4=H
3 R^1=Br, R^2=H, R^3=Br, R^4=H
4 R^1=H, R^2=H, R^3=Br, R^4=Br
5 R^1=Br, R^2=H, R^3=Br, R^4=Br
6 R^1=H, R^2=Br, R^3=Br, R^4=Br
7 R^1=H, R^2=H, R^3=H, R^4=H
8 R^1=H, R^2=H, R^3=OH, R^4=H
9 R^1=Br, R^2=Br, R^3=OH, R^4=H
10 R^1=H, R^2=Br, R^3=OH, R^4=Br
11 R^1=Br, R^2=Br, R^3=OH, R^4=Br
12 R^1=Br, R^2=Br, R^3=O-Tos, R^4=H
13 R^1=Br, R^2=H, R^3=H, R^4=H

Figure 1. Structures of compounds **1**–**13**.

K^+−ATPase inhibitory activities of these eudistomins were altered by bromination at C-6 and C-13 and hydroxylation at C-14. The replacement of the C-10 carbonyl by a hydroxy group also affected inhibitory activity. Compounds **3**–**5**, which contain carbonyl groups, exhibited IC_{50} values much lower than those of their corresponding alcohols **9**–**11**.

The in vitro antimicrobial activities of eudistomins (**1**–**13**) were assessed against three Gram-positive bacteria, three Gram-negative bacteria, and four species of fungi.[31] The minimum inhibitory concentrations (MICs) of the tested compounds are shown in Table 1. Compounds **3**–**6** and **11** exhibited strong inhibitory activities against Gram-positive and Gram-negative bacteria, with the exception of *E. coli*, with MIC values ranging from 0.39 to 6.25 μg/mL, as shown compared with ampicillin. In the current series, an increase in the number of bromine atoms per molecule resulted in an increase in antibacterial activity. Compounds **3**–**6**, **10**, and **11**, which contain two or three bromine atoms, were more active than the other compounds. Interestingly, compounds **3**–**5**, which contain a carbonyl group at C-10, exhibited higher antibacterial activities than compounds **9**–**11**, which contain a hydroxy group at the same position. The reason for this increased activity is not clear. In assays for antifungal activity against medically important pathogenic fungi, fungal inhibition was observed only with compounds **1** and **7**, which exhibited potent anti-*Candida* and moderate antifungal activity, respectively.

3. Conclusion

Six β-carboline alkaloids (**1**–**6**) of the eudistomin Y class were isolated from the Korean ascidian *Synoicum* sp. These compounds were chemically converted to a known compound, eudistomin Y_1 (**7**) and six new derivatives, designated eudistomins Y_8–Y_{13} (**8**–**13**). Structure–activity relationships were determined for these compounds during the course of screening for bioactivity. These findings enhance the potential of these compounds for use as therapeutic leads.

4. Experimental

4.1. General experimental procedures

Optical rotations were measured on a JASCO P-1020 polarimeter using a 1 cm cell. IR spectra were recorded on a JASCO 4200 FT-IR spectrometer, using a ZnSe cell. UV spectra were acquired with HITACHI U-3010 spectrophotometer. NMR spectra were recorded in DMSO-d_6 solutions containing Me_4Si as an internal standard, on Bruker Avance 500, 600, Jeol JNM-LA 300, and Varian Gemini 2000 spectrometers. Electrospray ionization source (ESI) low resolution mass spectra were recorded on an Agilent Technologies 6130 Quadrupole mass spectrometer with an Agilent Technologies 1200 series HPLC. High resolution mass spectrometric data were obtained at the Korea Basic Science Institute (Daegu, Korea) and were acquired using a JEOL JMS 700 mass spectrometer with *meta*-nitrobenzyl alcohol as a matrix for the FABMS. Semi-preparative high performance liquid chromatography (HPLC)

was performed on a Spectrasystem p2000 equipped with a refractive index detector (Spectrasystem RI-150) and UV/Vis detector (Gilson UV/VIS 151). All solvents used were spectroscopic grade or distilled from glass prior to use.

4.2. Animal material

Specimens of *Synoicum* sp. (sample number 09CH-11) were collected by hand with scuba equipment at a depth of 20 m off the coast of Chuja-do, Korea on November 4, 2009. The morphological features of these purple-colored specimens were identical to those described for the *Synoicum* ascidian that was confirmed by B.J.R., an author of this paper. The colonial tunicates are up to 20 mm high and up to 50 mm in maximum dimention, rounded, cuchion-shaped, sessile and fixed by a small part of the basal surface, and zooids are said to have been bright dark red when alive. Zooids form circular system of 1 mm diameter with up 6–8 zooids around a central clonial cavity. Contracted zooids are nearly 7 mm long, of which the thorax 1 to 1.5 mm and the posterior abdomen is at least three times that length. Each branchial siphon has 6 lobes and the atrial siphon protruding, has small pointed lobes around its posterior rim. The thorox is especially large, with 16–18 stigmental rows, the gut loop is moderately short, the stomach is large almost spherical and smooth walled. Gonad unknown. The voucher specimens are deposited at the Natural History Museum, Ehwa Womans University under the curatorship of B.J.R.

4.3. Chemicals

Chemical reagents were obtained from commercial sources and used directly without further purification. Sodium borohydride was purchased from Fluka Organic Chemicals. *p*-Toluenesulfonyl chloride was purchased from Junsei Chemical Co. Lithium aluminum hydride (1.0 M solution in tetrahydrofuran), pyridinium dichromate, Pd/C (palladium, 10 wt % [dry basis] on activated carbon), and all other chemicals were purchased from Sigma-Aldrich Chemical Co.

4.4. Extraction and isolation

The fresh collected specimens were frozen immediately and kept at −25 °C until chemical investigations. The specimens were lyophilized (2.0 kg dry weight), macerated, and extracted repeatedly with MeOH (3 L × 3) and CH_2Cl_2 (3 L × 2). The combined organic extract (160.95 g) was partitioned between *n*-BuOH and H_2O, and then the *n*-BuOH layer (15.83 g) was repartitioned between 15% aqueous MeOH (12.27 g) and *n*-hexane (3.56 g). The 15% aqueous MeOH layer was subjected to C_{18} reversed-phase vacuum flash chromatography using sequential mixtures of MeOH and H_2O as eluents (6 fractions in gradient, H_2O–MeOH, from 50:50 to 0:100 and finally acetone. The H_2O–MeOH (20:80) fraction (0.39 g) was separated by C_{18} reversed-phase semi-preparative HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, H_2O–MeOH (35:65)) to yield, in order of elution, compounds **4**, **5**, **6**, and **2** at retention times 14, 24, 25, and 69 min, respectively. The H_2O–MeOH (10:90) fraction (0.59 g) was separated by C_{18} reversed-phase semi-preparative HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, H_2O–MeOH (20:80)) to yield, in order of elution, compounds **2**, **1**, and **3** at retention times 22, 23, and 39 min, respectively. Final purifications of these metabolites were then accomplished by reversed-phase HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, H_2O–MeOH (20:80, 0.01% TFA)) to yield compounds **1**, **2**, **3**, **4**, **5**, and **6**, at retention times 23, 22, 35, 34, 62, and 58 min, respectively. The overall purified yields were 2.7, 7.6, 119.0, 77.5, 78.3, and 5.0 mg for **1–6**, respectively.

4.4.1. Preparation of eudistomin Y_1 (7)

To a stirred mixture of 3.0 mg of **8** in 1.0 mL of dry CH_2Cl_2–THF (99:1) was added 7.5 mg of PDC. The mixture was stirred under N_2 for 3 h. Celite was added and the resulting mixture was stirred for 5 min. The mixture was filtered through Celite under vacuum. The residue was concentrated under reduced pressure and purified by HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, H_2O–MeOH (23:77 with 0.01% TFA)) to yield the pure compound **7** (2.1 mg) at retention time 12 min; LRESI-MS *m/z* 289.2 $[M+H]^+$; HRFABMS *m/z* 289.0978 $[M+H]^+$ (calcd for $C_{18}H_{13}O_2N_2$, 289.0977).

4.4.2. Preparation of eudistomin Y_8 (8)

To a stirred mixture of 10.0 mg of **4** and 0.02 mL of Et_3N in 2.0 mL of dry MeOH was added 10.0 mg of Pd/C under ice-cooling. The mixture was stirred and hydrogenated using H_2 at room temperature for 18 h. The mixture was filtered through Celite and silica gel under vacuum. The residue was concentrated under reduced pressure and purified by HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, gradient from H_2O–MeOH (50:50 with 0.01% TFA) to (0:100 with 0.01% TFA)) to yield the pure compound **8** (4.5 mg) at retention time 11 min; UV (MeOH) λ_{max} (log ε) 208 (2.93), 236 (2.93), 290 (2.59), 356 (2.01) nm; IR (ZnSe) ν_{max} 3232, 1509, 1241 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.22 (1H, s, NH), 8.22 (1H, d, *J* = 5.1 Hz, H-3), 8.18 (1H, br d, *J* = 7.8 Hz, H-5), 7.97 (1H, d, *J* = 5.1 Hz, H-4), 7.73 (1H, br d, *J* = 7.8 Hz, H-8), 7.51 (1H, dd, *J* = 7.8, 7.8 Hz, H-7), 7.36 (2H, d, *J* = 8.7 Hz, H-12, H-16), 7.20 (1H, dd, *J* = 7.8, 7.8 Hz, H-6), 6.65 (2H, d, *J* = 8.7 Hz, H-13, H-15), 6.03 (1H, s, H-10); ^{13}C NMR (DMSO-d_6) δ 156.3 (C-14), 148.0 (C-1), 140.6 (C-8a), 136.7 (C-3), 134.1 (C-11), 132.0 (C-9a), 128.5 (C-4a), 127.8 (C-7), 127.5 (C-12, C-16), 121.3 (C-5), 120.4 (C-6), 119.0 (C-4b), 114.7 (C-13, C-15), 113.4 (C-4), 112.5 (C-8), 75.6 (C-10); LRESI-MS *m/z* 291.2 $[M+H]^+$; HRFABMS *m/z* 291.1131 $[M+H]^+$ (calcd for $C_{18}H_{15}O_2N_2$, 291.1134).

4.4.3. Preparation of eudistomin Y_9 (9)

To a stirred mixture of 16.0 mg of **3** in 4.0 mL of THF was added 2.5 mg of $NaBH_4$ under ice-cooling. The mixture was stirred under N_2 gas at room temperature for 4 h. To quench the reaction, NH_4Cl and water were added under ice-cooling. The mixture was partitioned using EtOAc and water, the former was dried over anhydrous $MgSO_4$. The EtOAc fraction was filtered and the residue was concentrated under reduced pressure, purified by HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, gradient from H_2O–MeOH (50:50 with 0.01% TFA) to (0:100 with 0.01% TFA)) to yield the pure compound **9** (13.3 mg) at retention time 15 min; UV (MeOH) λ_{max} (log ε) 210 (2.91), 234 (2.97), 286 (2.63), 338 (2.12) nm; IR (ZnSe) ν_{max} 3225, 1482, 1247 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.43 (1H, s, NH), 8.47 (1H, d, *J* = 1.8 Hz, H-5), 8.25 (1H, d, *J* = 5.1 Hz, H-3), 8.05 (1H, d, *J* = 5.1 Hz, H-4), 7.70 (1H, d, *J* = 8.4 Hz, H-8), 7.69 (1H, d, *J* = 1.8 Hz, H-12), 7.64 (1H, dd, *J* = 8.4, 1.8 Hz, H-7), 7.31 (1H, dd, *J* = 8.4, 1.8 Hz, H-16), 6.84 (1H, d, *J* = 8.4 Hz, H-15), 6.03 (1H, s, H-10); ^{13}C NMR (DMSO-d_6) δ 153.0 (C-14), 147.8 (C-1), 139.3 (C-8a), 137.2 (C-3), 135.8 (C-11), 132.4 (C-9a), 130.5 (C-7), 130.4 (C-12), 127.7 (C-4a), 126.6 (C-16), 124.0 (C-5), 122.3 (C-4b), 115.9 (C-15), 114.6 (C-8), 114.0 (C-4), 111.1 (C-6), 108.8 (C-13), 74.9 (C-10); LRESI-MS *m/z* 447.0/449.0/451.0 $[M+H]^+$; HRFABMS *m/z* 448.9320 $[M+H]^+$ (calcd for $C_{18}H_{13}O_2N_2{}^{79}Br^{81}Br$, 448.9324).

4.4.4. Preparation of eudistomin Y_{10} (10)

To a stirred mixture of 3.8 mg of **4** in 1.0 mL of THF was added 0.6 mg of $NaBH_4$ under ice-cooling. The mixture was stirred under N_2 gas at room temperature for 4 h. To quench the reaction, NH_4Cl and water were added under ice-cooling. The mixture was partitioned using EtOAc and water, the former was dried over anhydrous $MgSO_4$. The EtOAc fraction was filtered and the residue was concentrated under reduced pressure, purified by HPLC (YMC ODS-A

column, 1 × 25 cm, 2.0 mL/min, gradient from H_2O–MeOH (50:50 with 0.01% TFA) to (0:100 with 0.01% TFA)), to yield the pure compound **10** (3.1 mg) at retention time 16 min; UV (MeOH) λ_{max} (log ε) 210 (2.80), 232 (2.68), 288 (2.34), 344 (1.91) nm; IR (ZnSe) ν_{max} 3438, 1627, 1476 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.34 (1H, s, NH), 8.26 (1H, d, J = 5.1 Hz, H-3), 8.21 (1H, br d, J = 7.8 Hz, H-5), 8.01 (1H, d, J = 5.1 Hz, H-4), 7.72 (1H, br d, J = 7.8 Hz, H-8), 7.71 (2H, s, H-12, H-16), 7.53 (1H, dd, J = 7.8, 7.8 Hz, H-7), 7.21 (1H, dd, J = 7.8, 7.8 Hz, H-6), 6.07 (1H, s, H-10); ^{13}C NMR (DMSO-d_6) δ 149.8 (C-14), 146.6 (C-1), 140.6 (C-8a), 137.7 (C-11), 137.0 (C-3), 132.1 (C-9a), 129.9 (C-12, C-16), 128.7 (C-4a), 128.0 (C-7), 121.4 (C-5), 120.3 (C-6), 119.2 (C-4b), 113.8 (C-4), 112.5 (C-8), 111.7 (C-13, C-15), 73.9 (C-10); LRESI-MS m/z 446.9/448.9/450.9 $[M+H]^+$; HRFABMS m/z 448.9320 $[M+H]^+$ (calcd for $C_{18}H_{13}O_2N_2{}^{79}Br^{81}Br$, 448.9324).

4.4.5. Preparation of eudistomin Y_{11} (11)

To a stirred mixture of 3.9 mg of **5** in 1.0 mL of THF was added 1.0 mg of $NaBH_4$ under ice-cooling. The mixture was stirred under N_2 gas at room temperature for 4 h. To quench the reaction, NH_4Cl and water were added under ice-cooling. The mixture was partitioned using EtOAc and water, the former was dried over anhydrous $MgSO_4$. The EtOAc fraction was filtered under vacuum, the residue was concentrated under reduced pressure and purified by HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, gradient from H_2O–MeOH (40:60 with 0.01% TFA) to (0:100 with 0.01% TFA)) to yield the pure compound **11** (3.1 mg) at retention time 14 min; UV (MeOH) λ_{max} (log ε) 208 (2.53), 240 (2.37), 292 (2.02), 358 (1.56) nm; IR (ZnSe) ν_{max} 3418, 1474, 1273 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.47 (1H, s, NH), 9.84 (1H, s, 14-OH), 8.49 (1H, d, J = 1.8 Hz, H-5), 8.28 (1H, d, J = 5.1 Hz, H-3), 8.08 (1H, d, J = 5.1 Hz, H-4), 7.71 (2H, s, H-12, H-16), 7.70 (1H, d, J = 8.7 Hz, H-8), 7.66 (1H, dd, J = 8.7, 1.8 Hz, H-7), 6.68 (1H, d, J = 4.5 Hz, 10-OH), 6.07 (1H, d, J = 4.5 Hz, H-10); ^{13}C NMR (DMSO-d_6) δ 149.6 (C-14), 147.0 (C-1), 139.3 (C-8a), 137.8 (C-11), 137.3 (C-3), 132.4 (C-9a), 130.5 (C-7), 129.9 (C-12, C-16), 127.8 (C-4a), 124.0 (C-5), 122.2 (C-4b), 114.6 (C-8), 114.2 (C-4), 111.6 (C-13, C-15), 111.2 (C-6), 74.1 (C-10); LRESI-MS m/z 524.9/526.9/528.9/530.9 $[M+H]^+$; HRFABMS m/z 528.8409 $[M+H]^+$ (calcd for $C_{18}H_{11}O_2N_2{}^{79}Br^{81}Br_2$, 528.8410).

4.4.6. Preparation of eudistomin Y_{12} (12)

To a stirred mixture of 10.5 mg of **9** and 0.025 mL of Et_3N in 3.0 mL of dry CH_2Cl_2 was added 7.0 mg of Tos-Cl. The mixture was stirred under N_2 for 2 h. After quenched by using 0.1 N HCl, $NaHCO_3$ and water, the mixture was partitioned using CH_2Cl_2 and water. The former layer was dried over anhydrous $MgSO_4$ and filtered under vacuum. The residue was concentrated under reduced pressure and purified by HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, gradient from H_2O–MeOH (30:70 with 0.01% TFA) to (0:100 with 0.01% TFA)) to yield the pure compound **12** (10.3 mg) at retention time 18 min; UV (MeOH) λ_{max} (log ε) 208 (2.77), 230 (2.79), 292 (2.29), 358 (1.73) nm; IR (ZnSe) ν_{max} 3425, 1480, 1175 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.55 (1H, s, NH), 8.49 (1H, d, J = 1.8 Hz, H-5), 8.27 (1H, d, J = 5.1 Hz, H-3), 8.08 (1H, d, J = 5.1 Hz, H-4), 7.94 (1H, d, J = 1.8 Hz, H-12), 7.76 (2H, d, J = 8.1 Hz, H-2', H-7'), 7.69 (1H, d, J = 8.7 Hz, H-8), 7.65 (1H, dd, J = 8.7, 1.8 Hz, H-7), 7.56 (1H, dd, J = 8.7, 1.8 Hz, H-16), 7.45 (2H, d, J = 8.1 Hz, H-3', H-6'), 7.13 (1H, d, J = 8.7 Hz, H-15), 6.16 (1H, s, H-10), 2.40 (1H, s, H-5'); ^{13}C NMR (DMSO-d_6) δ 146.6 (C-1), 146.1 (C-4'), 144.9 (C-14), 144.4 (C-11), 139.3 (C-8a), 137.3 (C-3), 132.5 (C-9a), 131.8 (C-1'), 131.2 (C-12), 130.5 (C-7), 130.2 (C-3', C-6'), 128.2 (C-2', C-7'), 127.9 (C-4a), 127.0 (C-16), 124.0 (C-5), 123.1 (C-15), 122.2 (C-4b), 115.4 (C-13), 114.5 (C-8), 114.2 (C-4), 111.2 (C-6), 74.3 (C-10), 21.1 (C-5'); LRESI-MS m/z 600.9/602.9/604.9 $[M+H]^+$; HRFABMS m/z 602.9409 $[M+H]^+$ (calcd for $C_{25}H_{19}O_4N_2S^{79}Br^{81}Br$, 602.9413).

4.4.7. Preparation of eudistomin Y_{13} (13)

To a stirred mixture of 8.0 mg of **12** in 1.0 mL of dry THF was add 0.25 mL of $LiAlH_4$ under ice-cooling. The mixture was stirred under N_2 for 1 h. To quench the reaction, 0.25 mL of water, 0.25 mL of 15% NaOH, and 0.75 mL of water were added step by step under ice-cooling and Celite was added. The resulting mixture was dried over anhydrous $MgSO_4$ and filtered through Celite under vacuum. The residue was concentrated under reduced pressure and purified by HPLC (YMC ODS-A column, 1 × 25 cm, 2.0 mL/min, gradient from H_2O–MeOH (60:40 with 0.01% TFA) to (0:100 with 0.01% TFA)), to yield the pure compound **13** (3.1 mg) at retention time 20 min; UV (MeOH) λ_{max} (log ε) 208 (2.67), 236 (2.76), 290 (2.36), 356 (1.80) nm; IR (ZnSe) ν_{max} 3300, 1623, 1274 cm^{-1}; ^1H NMR (DMSO-d_6) δ 11.43 (1H, s, NH), 8.45 (1H, d, J = 1.7 Hz, H-5), 8.26 (1H, d, J = 5.2 Hz, H-3), 8.04 (1H, d, J = 5.2 Hz, H-4), 7.70 (1H, d, J = 8.7 Hz, H-8), 7.63 (1H, dd, J = 8.7, 1.7 Hz, H-7), 7.59 (2H, d, J = 7.5 Hz, H-12, H-16), 7.28 (2H, dd, J = 7.5, 7.5 Hz, H-13, H-15), 7.18 (1H, t, J = 7.5 Hz, H-14), 6.14 (1H, s, H-10); ^{13}C NMR (DMSO-d_6) δ 148.0 (C-1), 143.5 (C-11), 139.3 (C-8a), 137.1 (C-3), 132.4 (C-9a), 130.4 (C-7), 128.0 (C-12, C-16), 127.7 (C-4a), 127.0 (C-14), 126.2 (C-13, C-15), 124.0 (C-5), 122.3 (C-4b), 114.6 (C-8), 113.9 (C-4), 111.1 (C-6), 76.1 (C-10); LRESI-MS m/z 353.1/355.1 $[M+H]^+$; HRFABMS m/z 353.0287 $[M+H]^+$ (calcd for $C_{18}H_{14}O_2N_2{}^{79}Br$, 353.0289).

4.5. Biological assays

Cytotoxicity assays were performed in accordance with literature protocols.[22] Antimicrobial assays were performed according to the method described previously.[31] Isocitrate lyase, sortase A, and Na^+/K^+–ATPase inhibition assays were performed according to previously described methods.[25,28,30]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.05.002.

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