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Apoptosis-inducing effect of epolactaene derivatives on BALL-1 cells

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Abstract—Epolactaene, a neuritogenic compound in human neuroblastoma SH-SY5Y, induces apoptosis in a human leukemia B-cell line, BALL-1. The apoptosis-inducing activities of 34 epolactaene derivatives, including those of the newly synthesized α-alkyl-α,β-epoxy-γ-lactam derivative and cyclopropane derivatives, were also tested. The structure–activity relationships of the epolactaene derivatives as an inducer of apoptosis are described. The α-acyl-α,β-epoxy-γ-lactam moiety as well as the hydrophobicity derived from the long alkyl side chain are both important for activity. Compound **1e** displayed the strongest activity among all the synthesized compounds with an IC_{50} value of 0.70 μM.
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1. Introduction

Apoptosis, the process of programmed cell death, proceeds with several biochemical and cytological features, including the condensation and fragmentation of nuclei, apoptotic body formation, and chromosomal DNA fragmentation into ca. 180 bp oligomers.[1–3] Apoptosis is tightly regulated with the cell cycle by a cascade of initiator and effector caspsases that are activated sequentially.[4–8] Inappropriate control of apoptosis plays a role in many human diseases.[9–11] Abnormal inhibition of apoptosis is a hallmark of cancer and autoimmune diseases,[12–14] while excessive apoptosis is implicated in neurodegenerative disorders such as Alzheimer's disease.[9,15]

Epolactaene (**1a**) is a microbial metabolite that was originally isolated by Kakeya and Osada et al. from the cultured mycelium of *Penicillium* sp. BM 1689-P.[16] Epolactaene was found to induce neurite outgrowth and cell cycle arrest at the G1 phase in a human neuroblastoma cell line, SH-SY5Y.[16–19] The potent biological properties of epolactaene, along with its structural complexity, have initiated intensive synthetic research efforts.[20–24] We are particularly interested in the unique biological activity of epolactaene and have undertaken a series of both synthetic and biological studies.[25–31]

We have previously described the synthesis of epolactaene and several of its analogues (**1**–**12**) (Figs. 1 and 2).[25–27,30] Mizushina et al. reported that epolactaene **1a** and its analogue **1b** selectively inhibited the activities of mammalian DNA polymerases α (pol α), β (pol β) and human DNA topoisomerase II (topo II).[28] More recently, analogues **1e**, **7b**, **8b**, and **12a** have also been reported to inhibit the activities of mammalian pol α and pol β.[30] Among these analogues, compound **1e** was found to be the most

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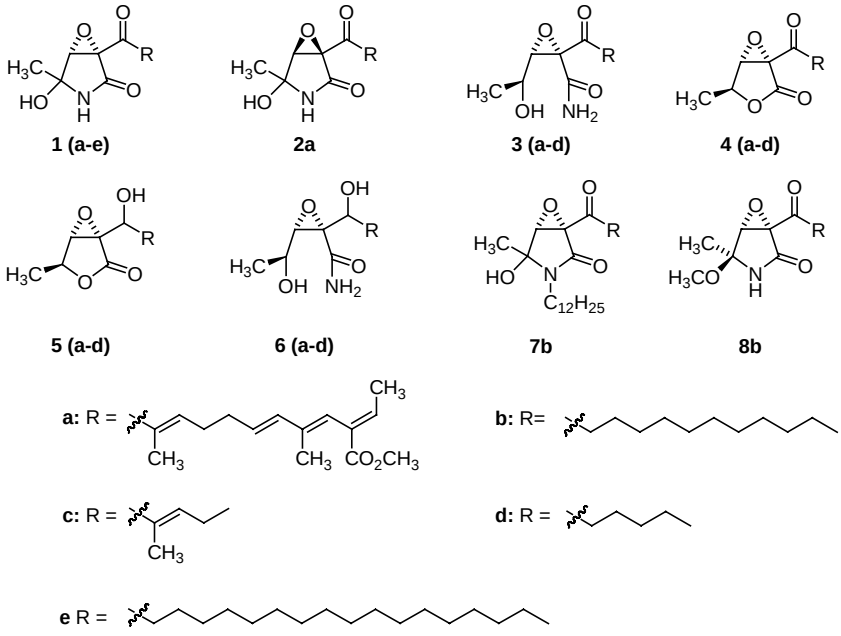


Figure 1. Structure of epolactaene and its derivatives.

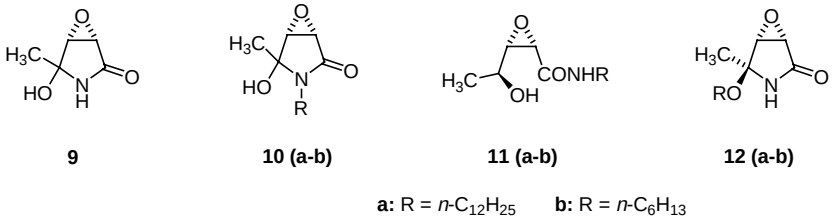


Figure 2. Structure of α,β-epoxy-γ-lactams (**9**–**12**).

potent inhibitor of DNA pols. The same group has recently reported that some epolactaene derivatives inhibit TPA (12-*O*-tetradecanoylphorbol-13-acetate)-induced inflammation in the mouse inflammatory test.[31] Because epolactaene derivatives possess anti-inflammatory activity in vivo, these agents have potential therapeutic utility.

We are interested in compounds that induce apoptosis as potential anti-cancer agents, because many cancer cells exhibit abnormal inhibition of apoptosis.[13,32] Thus, we investigated the effect of epolactaene derivatives on several cancer cell lines. We found that synthetic analogues **1**(**a**–**d**) induced apoptosis in a human leukemia B-cell line, BALL-1, in a dose- and time-dependent manner.[29] The α,β-epoxy-γ-lactam at the core of the molecule and the linear extended alkyl group in the side chain were both very important for maintaining biological activities.

It has recently been reported that epolactaene binds to Hsp60 as a Michael acceptor, thereby inhibiting Hsp60 chaperone activity.[33,34] However some epolactaene derivatives, which lack an α,β-unsaturated ketone, also inhibit growth in cancer cell lines. These results suggest that alternative protein targets exist. To investigate the mode of action, we examined the structure–activity relationships of these epolactaene derivatives as inducers of apoptosis.

2. Chemistry

We have previously reported the preparation of **1**–**6**, based on the bridgehead oxiranyl anion strategy,[27] and the preparation of **7**–**12**.[30] Here, we describe the preparation of α-alkyl-α,β-epoxy-γ-lactam and cyclopropane analogues in order to examine the influence of epoxyketone on the biological activity of the derivatives (Fig. 3).

Scheme 1 outlines the preparation of α-alkyl-α,β-epoxy-γ-lactam **15**. A solution of **13** in THF was added to a solution of LDA and dodecyl trifluoromethanesulfonate in THF at −78 °C to give the alkylated product, along with several unknown byproducts. The formation of

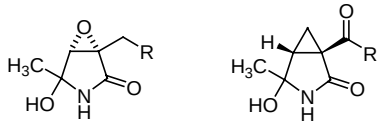
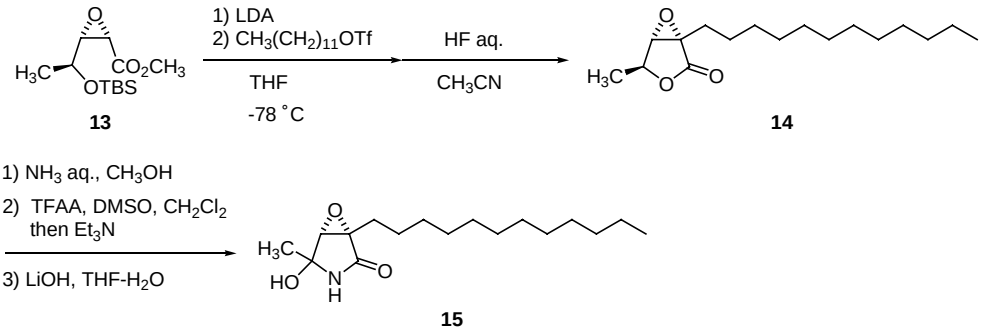


Figure 3. Structure of newly designed epolactaene derivatives.

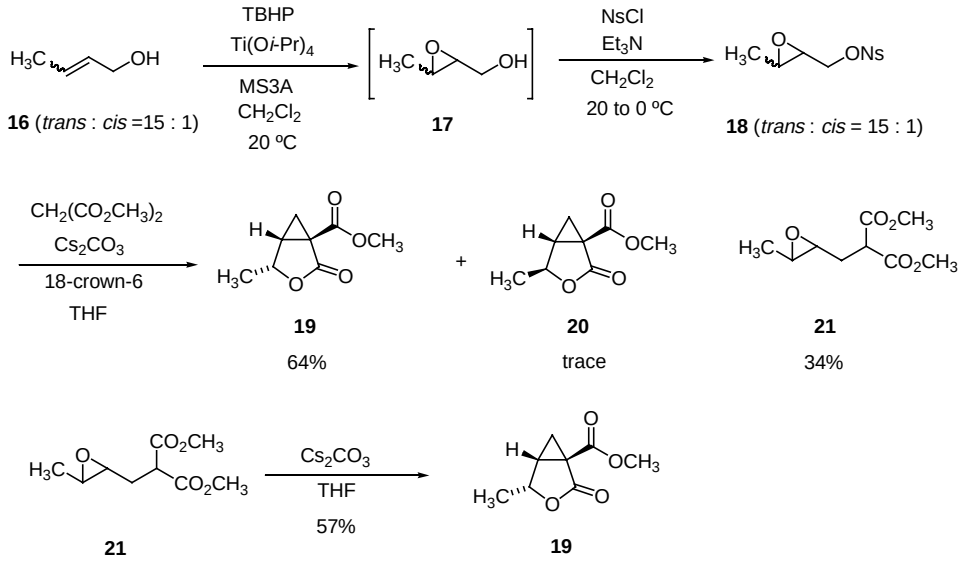


Scheme 1. Synthesis of α-alkyl-α,β-epoxy-γ-lactam **15**.

byproducts presumably results from both the instability of dodecyl triflate during silica gel purification and also the high reactivity of the oxiranyl anion derived from **13**. Decomposition of **13** was observed when dodecyl iodide was used in this reaction. No significant improvement was observed when the reaction was carried out at a lower temperature (−100 °C). Thus without any purification, treatment of the alkylated product with aqueous HF in CH_3CN, desilylation, and lactonization proceeded to give **14**. After ammonolysis of **14**, the resulting hydroxyamide was oxidized by Swern oxidation.[27] Since O-trifluoroacetylated **15** was observed, the crude oxidized product was hydrolyzed to give **15**.

We also aimed to synthesize cyclopropane epolactaene analogues. Construction of the cyclopropane ring was conducted according to Temnikova's procedure (Scheme 2).[35] Crotyl alcohol (**16**) was treated with *tert*-butylhydroperoxide (TBHP) and $Ti(O\text{-}i\text{-}Pr)_4$ in the presence of MS3A, followed by *p*-nitrosulfonate (NsCl) and Et_3N in one pot to give **18** in a racemic form.[36,37] Treatment of **18** with dimethyl malonate and Cs_2CO_3 in the presence of 0.1 equivalent amount of 18-crown-6 afforded **19**, **20**, and **21**.[38] The stereochemistry of **19** and **20** was determined by ^1H NMR spectra and NOE experiments (Fig. 4).[39] The coupling constant (J_{4-5} = 4.6 Hz) between H-4 and H-5 in **19** is greater than that (J_{4-5} = ~0 Hz) in **20**. NOEs between H-6 and methyl group and NOEs between H-4 and H-5 in **19** were observed. These results suggest that the cyclopropane ring and methyl group in **19** exist on the same face. The major isomer **19** will be derived from *trans*-epoxide, whereas the minor **20** will be derived from *cis*-epoxide. The product **20** was non-cyclic α-alkylated malonate, which can be converted into **19** by treatment with Cs_2CO_3 in THF.

Hydrolysis of methyl ester in **19** gave a carboxylic acid **22**, which was transformed into Weinreb amide **23** (Scheme 3).[40] After ammonolysis of **23**, the resulting hydroxy group was protected as TBS ether to give **25**. Treatment of **25** with *n*-BuLi in THF gave **26** in 50% yield (29% of **25** was recovered). Deprotection of the TBS group with 3HF-Et_3N gave hydroxyamide **27** and lactone **28**. Oxidation of **27** with Dess–Martin periodinane[41] afforded **29** as a 1.2:1 tautomeric mixture and lactone **28**. Compound **29** is one of the desired cyclopropane epolactaene analogues. The mechanism for the formation of lactone **28** is unclear, although it was also observed by treatment of **27** with Dess–Martin periodinane in the presence of $NaHCO_3$ or pyridine. Thus, the results suggest that lactone formation does not occur under acidic conditions. Although **28** was formed, we



Scheme 2. Synthesis of compound **19**.

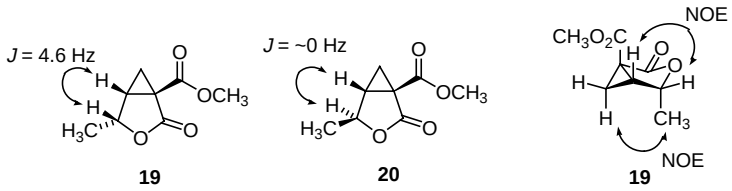
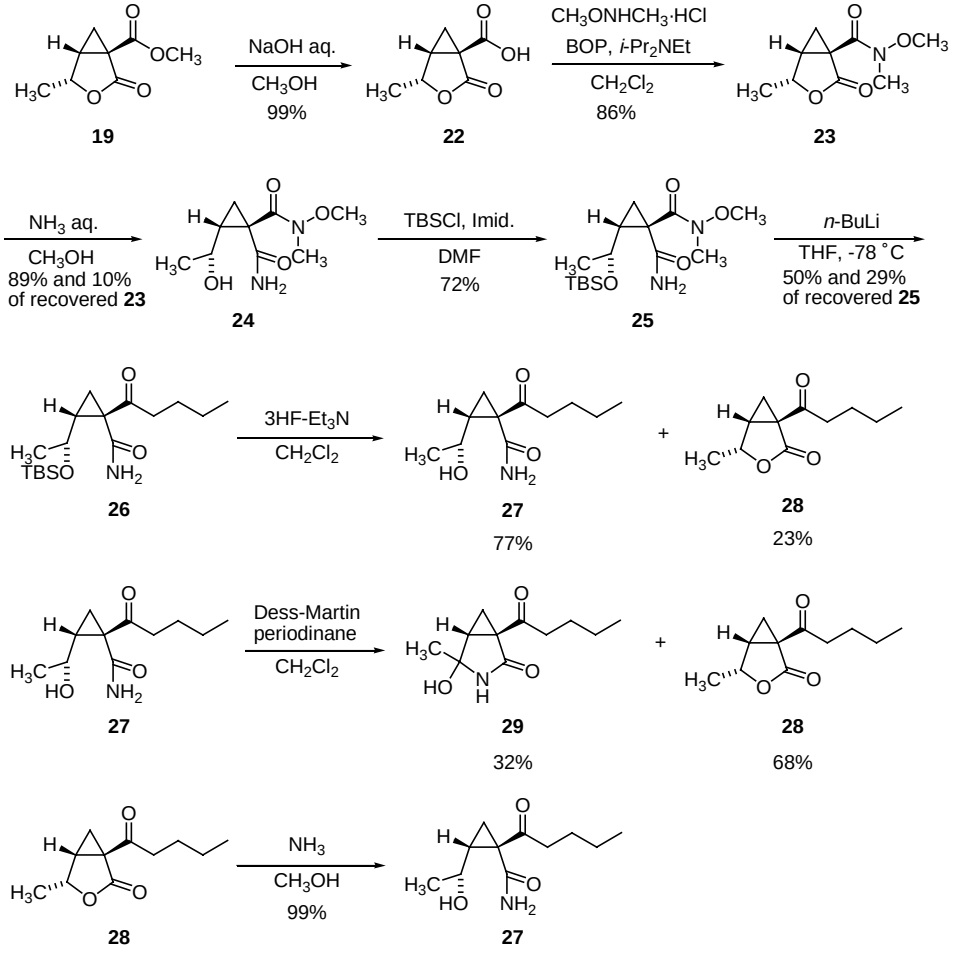


Figure 4. Determination of the stereochemistry of **19** and **20**.



Scheme 3. Synthesis of cyclopropane derivative **29**.

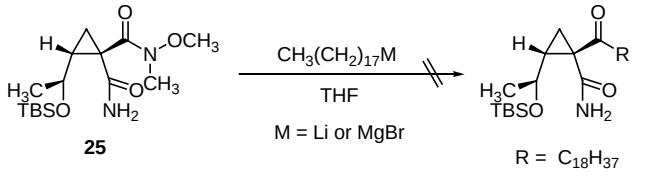
found that ammonolysis of **28** in MeOH regenerated the hydroxyamide **27**.

Since we have already established that a long alkyl group in the side chain is important for the apoptosis-inducing activity, we attempted to obtain other cyclopropane analogues. However, the addition of *n*-octadecyl lithium or *n*-octadecyl magnesium bromide to **25** did not proceed (Scheme 4). When **25** was treated with excess amount (30–50 equiv) of *n*-octadecyl lithium or *n*-octadecyl magnesium bromide under higher temperature (from room temperature to reflux), none of the desired products was obtained and **25** was recovered in 76–86% yield. The lack of reaction is presumably due to the low reactivity of *n*-octadecyl lithium and *n*-octadecyl magnesium bromide. Although the addition of 1-heptadec-1-enyl lithium to **25** was investigated, almost all of the starting material **25** was recovered.

Thus, we attempted to introduce an *N*-alkyl-*N*-methylamide moiety into the side chain to act as a bioisostere of the acyl group (Fig. 5).[42] Condensation of **22** and hexadecanoic acid with BOP in the presence of *i*-Pr_2NEt gave **30** as a 1.7:1 rotational mixture in 92% yield. After ammonolysis of **30**, the resulting hydroxy group was oxidized with the Dess–Martin reagent to give **32** as a 1.1:1 rotational mixture. Judging from the ^{1}H NMR spectrum (singlet at 2.35 ppm) and IR spectrum (absorption at 1712 cm^{-1}), **32** appears to exist as a ketone. The formation of γ-lactam was not observed in the ^{1}H NMR spectrum (see Scheme 5).

3. Apoptosis-inducing activity in BALL-1 cells

We examined the cell death-inducing action of epolactaene derivatives in BALL-1 cells by means of the



Scheme 4. The addition of alkyl metal reagents to Weinreb amide **25**.

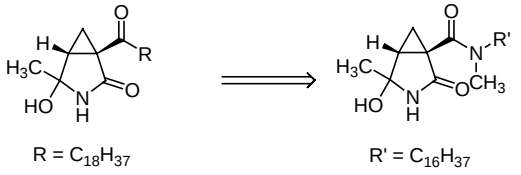


Figure 5. Modification at the side chain of cyclopropane derivative.

MTT assay.[29] The assay can indirectly calculate the cell survival rate by determining the action of a mitochondrial dehydrogenase on MTT in metabolically active cells.[43] The 50% growth inhibitory concentrations (IC_{50}'s) are summarized in Table 1.

Compounds**1** (**a**–**e**), **2a**, **7b**, **8b**, and **10**(**a**–**b**) induced cell death in BALL-1 cells at concentrations lower than 10 μM. Each compound induced cell death in a dose- and time-dependent manner. The formation of DNA ladder on agarose gels in DNA fragmentation assay was observed after treatment with 5 μM of each compound for 6 h. These results indicate that these compounds induced apoptosis.[29]

Because the hydroxyamides **3**, **6** and lactones **4**, **5** did not inhibit BALL-1 cells at concentrations lower than 10 μM, the γ-lactam moiety appears to be very important for activity. There was no difference in the biological action of (+)-epolactaene (**1a**) and (−)-epolactaene (**2a**), signifying that the stereochemistry of the epoxide does not influence the apoptosis-inducing activity.

Of all the compounds tested in this study, **1e** was the most potent inducer of apoptosis. Interestingly, **1e** had the greatest calculated log *P* (i.e., octanol/water partition coefficient) value among **1**(**a**–**e**), inferring that hydrophobicity affects the activity. We reasoned that the hydrophobic side chain of epolactaene might enhance the apoptosis-inducing effect by increasing the permeability of the compound across the cell membrane.[44]

Table 1. Fifty percent inhibitory concentrations (IC_{50}s) acting against BALL-1 cell viability and the calculated octanol/water partition coefficients (C log *P*) of epolactaene and its derivatives

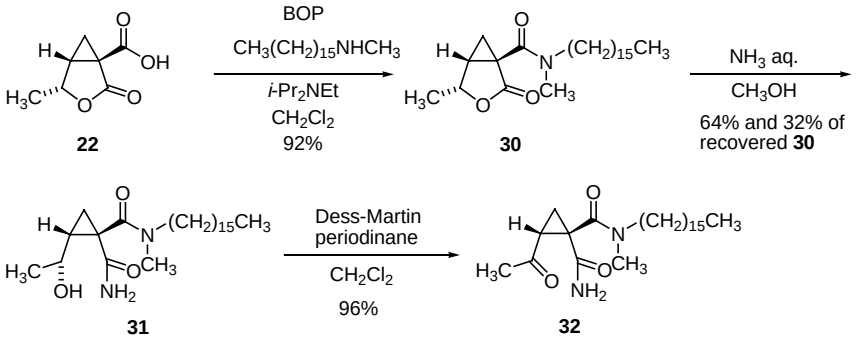
Compound	IC_{50} (μM)	C log *P*[b]
1a	3.82[a]	1.85
1b	1.65[a]	2.80
1c	5.04[a]	0.20
1d	7.40[a]	0.29
1e	0.70	5.30
2a	3.26	1.85
3(**a**–**d**)	>10	—
4(**a**–**d**)	>10	—
5(**a**–**d**)	>10	—
6(**a**–**d**)	>10	—
7b	6.51	7.61
8b	3.50	3.16
9	>10	−1.13
10a	3.96	3.69
10b	8.34	1.18
11(**a**–**b**)	>10	—
12(**a**–**b**)	>10	—
15	>10	3.96
29	>10	0.78
32	>10	5.28

[a] Ref. 29.
[b] The C log *P* values were obtained using CS ChemDraw version 7.0 software (Cambridge Soft, USA).

Although the *N*-dodecyl derivative (**7b**) and *O*-methyl derivative (**8b**) both have greater C log *P* values than **1b**, their biological activities were slightly lower. Thus, substitution at the N- or O-atoms appears to influence the activity.

Compound **9**, which lacks the acyl side chain at the α-position, had no effect on BALL-1 cells. We reasoned that compound **9**, being very hydrophilic, might not readily permeate the cell membrane. Intriguingly, the *N*-alkyl-substituted compounds **10**(**a**–**b**) possess apoptosis-inducing activity, whereas the hydroxyamides **11**(**a**–**b**) and *O*-alkyl-substituted compounds **12**(**a**–**b**) had no activity at concentrations lower than 10 μM.

Compounds **15** had no effect on BALL-1 cells at concentrations lower than 10 μM. Compound **15** lacks the carbonyl group at the side chain of **1b**. The carbonyl



Scheme 5. Synthesis of cyclopropane derivative **32**.

group at the side chain affected the activity, because compound **1b** was found to induce apoptosis with an IC_{50} value of 1.65 μM. Compounds **29** and **32** did not have any effect on BALL-1 cells at concentrations lower than 10 μM, either. Compounds **29** and **32** have a cyclopropane ring instead of an epoxide ring. Because the epoxide derivatives **1(a–e)** induced apoptosis at concentrations lower than 10 μM, the activity of the cyclopropane derivatives was less potent than those of the epoxide derivatives. These results indicate that the α-acyl-substituted α,β-epoxy-γ-lactam moiety is important for the apoptosis-inducing activity.

4. Discussion

Epolactaene (**1a**) was originally reported to possess a potent neurite outgrowth activity in a human neuroblastoma cell line, SH-SY5Y.[16,17] In our precedent paper, we found that epolactaene and some of its derivatives induced apoptosis in a human leukemia B-cell line, BALL-1, in a dose- and time-dependent manner.[29] In this study, we describe the structure–activity relationships of epolactaene derivatives as inducers of apoptosis. We tested the apoptosis-inducing activity of 34 epolactaene derivatives, including some newly synthesized derivatives. We found that compounds **1(a–e)**, **2a**, **7b**, **8b**, and **10(a–b)** induced apoptosis in BALL-1 cells at concentrations lower than 10 μM. Compound **1e** was the most potent inducer of apoptosis with an IC_{50} value of 0.70 μM. We also found that the hydrophobicity of epolactaene derivatives, which was calculated as C log *P*, affected the apoptosis-inducing activity. We propose that the side-chain moiety of the molecule may facilitate permeation across the cell membrane.

Our structure–activity relationship studies suggest that the α-acyl-α,β-epoxy-γ-lactam moiety is important for activity, because neither hydroxy amides (**3** and **6**), lactones (**4** and **5**), α-alkyl-α,β-epoxy-γ-lactam (**15**) nor cyclopropanes (**29** and **32**) affected BALL-1 cells at concentrations lower than 10 μM. Substitution at the N-or O-position of the α-acyl-α,β-epoxy-γ-lactam (compounds **7b** and **8b**) caused a slight decrease in activity. Although neither α,β-epoxy-γ-lactam **9** nor O-substituted analogues **12(a–b)** induced apoptosis at concentrations lower than 10 μM, N-substituted analogues **10(a–b)** did show the activity. However, the differences between α-acyl-α,β-epoxy-γ-lactams **1(a–e)** and **10(a–b)** in terms of the mode of action were not examined in detail.

Mizushina et al. reported that epolactaene and its derivatives inhibit the activity of mammalian pol α, β, and λ, and human Topo II.[28,30,31] It is known that inhibition of these enzymes can result in cell cycle arrest, apoptosis, and anti-inflammatory activity.[45–47] Indeed, Mizushina et al. have recently reported that the anti-inflammatory effect of epolactaene might be related to the inhibition of pol λ. In addition, the structure–activity relationships of epolactaene derivatives as apoptosis-inducers are similar to those of DNA polymerase inhibitors. However, epolactaene derivatives weakly inhibit the activity of DNA polymerases at the concentrations required to induce apoptosis.[28,30,31] Thus, the inhibition of these DNA-modifying enzymes may play some part in the apoptosis-inducing effect of epolactaene and its derivatives, but is not the primary mechanism of action. Nagumo et al. have recently reported that epolactaene covalently binds Hsp60 and inhibits Hsp60 chaperone activity.[33,34] The reports demonstrate that Micheal addition of Hsp60 through Cys-442 to the α,β-unsaturated ketone moiety of epolactaene is responsible for the inhibition of chaperone activity. However, some epolactaene derivatives that lack the α,β-unsaturated ketone also inhibit the growth of cancer cell lines (e.g., compounds **1(b–e)**, **7b**, and **8b**). These results suggest that the apoptosis-inducing effect of epolactaene is not always explained by the inhibition of Hsp60. In addition to these results, we have also found that epolactaene derivative **1b** induces apoptosis more effectively in B lymphocytes than T lymphocytes. Epolactaene derivatives did not induce apoptosis in attached cells, HepG2, HeLa, and A549.[29] These results also suggest that additional target proteins are involved in the biological action of epolactaene.

In conclusion, we have described the structure–activity relationships of epolactaene derivatives as inducers of apoptosis. We have demonstrated that the α-acyl-α,β-epoxy-γ-lactam moiety as well as the length of the side chain (i.e., the hydrophobicity) are important for the activity. Based on the structure–activity relationships of these derivatives, we are currently attempting to identify the target molecules of epolactaene.

5. Experimental procedures

5.1. General

^1H and ^{13}C NMR were recorded on a JEOL JNM-LD400 or JNM-LD500, or on a BRUKER DXR400 or DRX600. Chemical shifts were reported in δ, parts per million (ppm), relative to TMS as an internal standard or calibrated using residual undeuterated solvent as an internal reference. IR spectra were recorded on a JASCO FT/IR-410 spectrometer. Mass spectra were obtained on an API QSTAR Pulsar i spectrometer. Optical rotations were measured on a JASCO P-1030 digital polarimeter. Melting points were determined with a Yanaco MP-3S melting point apparatus. Column chromatography was carried out on Fuji Silisia PSQ100B. Analytical thin-layer chromatography (TLC) was performed on precoated Merck silica gel 60 F_{254} plates, and compounds were visualized by UV illumination (254 nm) or heating 150 °C after spraying phosphomolybdic acid in ethanol. THF was distilled from sodium/benzophenone. CH_2Cl_2 was distilled from P_2O_5. DMSO was distilled from CaH_2. All other solvent and reagents were obtained from commercial sources and used without further purification. Organic extracts were dried over Na_2SO_4 or $MgSO_4$, filtered, and concentrated using a rotary evaporator. Involatile oils and solids were vacuum-dried.

Fetal bovine serum (FBS), kanamycin sulfate, dimethyl sulfoxide (DMSO) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Wako Pure Chemical Industries (Tokyo, Japan). RPMI 1640 was purchased from Nissui (Tokyo, Japan). 2-Mercaptoethanol was purchased from Nakalai Tesque (Kyoto, Japan).

BALL-1 cells were purchased from Riken Cell Bank (Tsukuba, Japan). BALL-1 cells were maintained at 37 °C with 5% CO_2 in RPMI 1640 supplemented with kanamycin sulfate (64 mg/L), 2-mercaptoethanol (3.5 μL/L), sodium bicarbonate (2 g/L), and heat-inactivated 10% (v/v) FBS. These cells were routinely diluted with the above medium to the appropriate concentrations ($1.0–4.0 \times 10^5$ cells/mL).

5.1.1. (1*S*,4*S*,5*S*)-1-Dodecyl-4-methyl-3,6-dioxabicyclo-[3.1.0]hexan-2-one (14). To a solution of diisopropylamine (0.8 mL, 5.71 mmol) in THF (30 mL) was added *n*-BuLi (3.7 mL of a 1.56 M solution in hexane, 5.8 mmol) at 0 °C. The mixture was stirred at 0 °C for 10 min. A solution of dodecyl trifluoromethanesulfonate (1.2 g, 3.77 mmol) in THF (10 mL) was added to the mixture at 0 °C and the mixture was cooled to −78 °C. A solution of **13** (1.0 g, 3.84 mmol) in THF (40 mL) was added to the mixture in portions for 20 min. Then the mixture was quenched by the addition of H_2O and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated.

To a solution of the residue in CH_3CN (10 mL) was added a solution of 5% aqueous HF in CH_3CN (10 mL) at rt. After stirring for 3 h, the mixture was quenched by the addition of a saturated aqueous solution of $NaHCO_3$ and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography (1:5, EtOAc/hexane) to yield **14** (14.8 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 4.59 (1H, q, J = 6.7 Hz, H-4), 3.79 (1H, s, H-5), 2.09 (1H, m, H-1′), 1.84 (1H, m, H-1′), 1.38 (3H, d, J = 6.8 Hz, 4-CH_3), 1.38–1.22 (16H, m, H-2′–H-11′), 0.88 (3H, t, J = 7.0 Hz, H-12′). No further characterization was attempted on this compound, which was used directly in the next step.

5.1.2. (1*R*,5*R*)-1-Dodecyl-4-hydroxy-4-methyl-6-oxa-3-azabicyclo-[3.1.0]hexan-2-one (15). A solution of **14** (14.6 mg, 0.052 mmol) and NH_3 (0.1 mL, a 28% solution in H_2O) in MeOH (2 mL) was stirred at rt for 30 min. The solvent was removed under reduced pressure.

To a solution of DMSO (25 μL, 0.35 mmol) in CH_2Cl_2 (0.5 mL) was added TFAA (25 μL, 0.18 mmol) at −78 °C. After 10 min at −78 °C, a solution of the residue in CH_2Cl_2 (2 mL)–DMSO (20 μL) was added to the mixture and the mixture was stirred at −78 °C for 45 min. Then Et_3N (70 μL, 0.50 mmol) was added and the mixture was stirred at rt for 10 min. The mixture was quenched by the addition of H_2O and extracted with CH_2Cl_2. The extract was washed with brine, dried ($MgSO_4$), and concentrated.

A solution of the residue and LiOH (2.5 mg, 0.10 mmol) in THF-H_2O (4:1, 2.5 mL) was stirred at 0 °C for 10 min. The mixture was quenched by the addition of H_2O and extracted with EtOAc. The extract was washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by silica gel chromatography (1:2 EtOAc/hexane) to afford **15** (10.8 mg, 67% in 2 steps) as a ca. 17:1 tautomeric mixture, as a white solid. Mp = 35 °C; $[\alpha]_D^{23}$ −12.6 (c 0.10, MeOH); ^{1}H NMR (600 MHz, $CDCl_3$) δ: 6.40 (1H, br s, NH), 3.67 (1H, s, H-5), 1.96 (1H, m, H-1′), 1.87 (1H, m, H-1′), 1.73 (1H, br s, OH), 1.54 (3H, s, 4-CH_3), 1.42 (1H, m, H-2′), 1.36 (1H, m, H-2′), 1.33–1.25 (18H, m, H-3′–H-11′), 0.88 (3H, t, J = 6.9 Hz); ^{13}C NMR (150 MHz, $CDCl_3$) δ: 173.2, 83.5, 62.8, 61.7, 31.9, 29.7, 29.7, 29.6, 29.6, 29.5, 29.4, 29.3, 24.7, 24.1, 22.7, 21.9, 14.9; IR (KBr) cm^{-1}: 3425, 3224, 2918, 2852, 1705, 1469, 1432, 1372, 1161, 951, 779, 717, 626; HRMS, calcd for $C_{17}H_{31}NO_3Na$ $(M+Na)^+$ 320.2201. Found 320.2200.

5.1.3. (±)-2,3-Epoxy-1-butyl *p*-nitrobenzenesulfonate (18). To a solution of crotyl alcohol (3.4 g, 47.2 mmol, E:Z = 15:1) and Ti(O*i*-Pr)$_4$ (1.4 mL, 4.72 mmol) in CH_2Cl_2 (50 mL) was added TBHP (14 mL of a 3.7 M solution in CH_2Cl_2, 51.9 mmol) at −20 °C in portions for 30 min. After stirring at −20 °C for 2 h, $P(OCH_3)_3$ (8.9 mL, 75.4 mmol) was added to the mixture. The mixture was warmed to −10 °C. Then Et_3N (9.7 mL, 69.8 mmol), DMAP (0.69 g, 5.7 mmol), followed by a solution of *p*-nitrobenzensulfonyl chloride (10.5 g, 47.2 mmol) in CH_2Cl_2 (70 mL), were added to the mixture. The mixture was filtered through Celite, and the filtrate was washed with a 10% solution of tartaric acid in H_2O, satd $NaHCO_3$ aq, brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (1:5 → 1:1 EtOAc/hexane) to afford **18** (9.6 g, 75%) as a 15:1 mixture of E and Z isomers, as a yellow solid. Mp = 83–86 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.41 (2H, d, J = 8.5 Hz, Ar), 8.13 (2H, d, J = 8.5 Hz, Ar), 4.41 (1H, dd, J = 11.4 Hz, 3.20 Hz, H-1), 4.05 (1H, dd, J = 11.4 Hz, 6.3 Hz, H-1), 2.96–2.94 (1H, m, H-2), 2.90 (1H, qd, J = 5.2 Hz, 1.8 Hz, H-3), 1.32 (3H, d, J = 5.2 Hz, 3-CH_3); IR ($CHCl_3$) cm^{-1}: 3106, 3028, 3001, 1608, 1536, 1380, 1351, 1312, 1291, 1210, 1187, 1096, 1016, 961, 857; HRMS, calcd for $C_{10}H_{12}O_6NS$ $(M+H)^+$ 274.0385. Found 274.0386.

5.1.4. (1*SR*,4*RS*,5*RS*)-Methyl 2-oxo-3-oxa-bicyclo[3.1.0]-hexane-1-carboxylate (19). To a solution of Cs_2CO_3 (6.2 g, 19.0 mmol) and 18-crown-6 (167.1 mg, 0.63 mmol) in THF (59 mL) was added dimethyl malonate (0.87 mL, 7.6 mmol) at rt. After stirring for 15 min, a solution of **18** (1.7 g, 6.3 mmol) in THF (20 mL) was added to the mixture in portions for 30 min. The mixture was stirred for 30 h. Then the mixture was quenched by the addition of H_2O and extracted with EtOAc (3×). The combined extract was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (1:5 → 1:1 EtOAc/hexane) to afford **19** (690 mg, 64%), **20** (trace), and **21** (433 mg, 34%). **19**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ: 3.57 (1H, qd, J = 6.3 Hz, 4.6 Hz, H-4), 3.82 (3H, s, $COOCH_3$), 2.73 (1H, ddd, J = 9.5 Hz, 4.8 Hz, 4.6 Hz, H-5), 1.96 (1H, dd,

J = 9.5 Hz, 4.6 Hz, H-6), 1.46 (1H, dd, J = 4.8 Hz, 4.6 Hz, H-6), 1.37 (3H, d, J = 6.3 Hz, 4-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 171.1, 169.2, 73.6, 56.7, 32.6, 30.9, 17.9, 17.5; IR ($CHCl_3$) cm^{-1}: 3026, 2989, 2956, 1780, 1728, 1441, 1387, 1340, 1320, 1110, 1093, 1051, 1024, 943, 760; HRMS, calcd for $C_8H_{10}O_4$ $(M)^+$ 170.0579. Found 170.0568.

5.1.5. (1*RS*,4*RS*,5*SR*)-Methyl 2-oxo-3-oxa-bicyclo[3.1.0]-hexane-1-carboxylate (20). ^1H NMR ($CDCl_3$, 500 MHz) δ: 4.45 (1H, q, J = 6.3 Hz, H-4), 3.82 (3H, s, CO_2CH_3), 2.53 (1H, dd, J = 8.2 Hz, 5.2 Hz, H-5), 2.05 (1H, dt, J = 8.2 Hz, 5.2 Hz, H-6), 1.42 (1H, dd, J = 5.5 Hz, 5.2 Hz, H-6), 1.37 (1H, d, J = 6.3 Hz, 4-CH_3).

5.1.6. Dimethyl 3,4-epoxypentane-1,1-dicarboxylate (21). ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.77 (6H, s, CO_2CH_3), 3.57 (1H, dd, J = 5.8 Hz, 3.1 Hz, H-1), 2.79 (1H, m, H-3), 2.73 (1H, m, H-4), 2.27 (1H, m, H-1), 1.98 (1H, m, H-1), 1.29 (3H, d, J = 6.8 Hz, H-5); ^{13}C NMR ($CDCl_3$, 125 Hz) δ: 169.2, 169.2, 56.7, 52.6, 52.2, 48.4, 31.2, 17.3; IR ($CHCl_3$) cm^{-1}: 3028, 3007, 2955, 1733, 1438, 1344, 1287, 1159, 1022, 838, 701; HRMS, calcd for $C_9H_{14}O_5$ $(M)^+$ 202.0841. Found 202.0849.

5.1.7. (1*SR*,4*RS*,5*RS*)-4-Methyl-2-oxo-3-oxa-bicyclo[3.1.0]-hexane-1-carboxylic acid (22). To a solution of **19** (144 mg, 0.85 mmol) in methanol (2 mL) was added a 3 M aqueous NaOH solution (1.2 mL, 3.4 mmol) at 0 °C, and the mixture was stirred at 0 °C for 1 h. Then the solvent was removed, and a 1 M aqueous solution of HCl was added to the residue. The mixture was extracted with $CHCl_3$ (10×). The combined organic layer was dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography (1:20 MeOH/$CHCl_3$) to give **22** (130 mg, 99%) as a white solid. Mp = 162–164 °C; ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.92 (1H, qd, J = 6.3 Hz, 4.6 Hz, H-4), 2.99 (1H, ddd, J = 8.0 Hz, 5.1 Hz, 4.6 Hz, H-5), 1.96 (1H, dd, J = 8.0 Hz, 4.6 Hz, H-6), 1.63 (1H, dd, J = 5.1 Hz, 4.6 Hz, H-6), 1.45 (1H, d, J = 6.3 Hz, 4-CH_3); IR ($CHCl_3$) cm^{-1}: 3606, 3304, 3027, 2990, 2935, 1772, 1732, 1442, 1400, 1354, 1336, 1130, 1092, 1054, 1019, 944, 907; HRMS, calcd for $C_7H_8O_4$ $(M)^+$ 156.0423. Found 156.0424.

5.1.8. (1*RS*,4*RS*,5*RS*)-*N*-Methoxy-*N*-methyl-4-methyl-2-oxo-3-oxa-bicyclo[3.1.0]hexane-1-carboxamide (23). To a solution of **22** (149 mg, 0.96 mmol), *i*-Pr_2NEt (0.39 mL, 2.3 mmol), and *N*,*O*-dimethylhydroxylamine hydrochloride (186 mg, 1.9 mmol) in CH_2Cl_2 (5 mL) was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (634 mg, 1.4 mmol) at 0 °C. The mixture was stirred at rt for 1 h. Then the mixture was quenched by the addition of H_2O and extracted with CH_2Cl_2 (3×). The combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by PTLC (2:1 EtOAc/hexane) to yield **23** (164 mg, 86%) as a colorless oil. ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.85 (1H, qd, J = 6.1 Hz, 4.6 Hz, H-4), 3.73 (3H, s, OCH_3), 3.24 (3H, s, NCH_3), 2.41 (1H, m, H-5), 1.84 (1H, m, H-6), 1.37 (3H, d, J = 6.1 Hz, 4-CH_3), 1.23 (1H, br t, J = 5.2 Hz, H-6); IR ($CHCl_3$) cm^{-1}: 3030, 3019, 2985, 2948, 2896, 2872, 2825, 1777, 1656, 1528, 1459, 1422, 1391, 1350, 1335, 1290, 1217, 1112, 1092, 1022, 771, 669; HRMS, calcd for $C_9H_{13}O_4N$ $(M)^+$ 199.0845. Found 199.0847.

5.1.9. (1*RS*,2*RS*,1′*RS*)-*N*-Methoxy-*N*-methyl-2-(1′-hydroxyethyl)cyclopropane-1,1-dicarboxamide (24). To a solution of **23** (113 mg, 0.57 mmol) in MeOH (2 mL) was added NH_3 (2 mL, 28% solution in H_2O), and the mixture was stirred overnight. Then the solvent was removed, the residue was purified by silica gel chromatography (1:50–1:20 EtOH/EtOAc) to yield **24** (109 mg, 89%) as a colorless oil and recovered **23** (11 mg, 10%). ^1H NMR ($CDCl_3$, 500 MHz) δ: 5.90 (1H, br, NH), 5.65 (1H, br, NH), 3.83 (1H, m, H-1′), 3.73 (3H, s, OCH_3), 3.26 (3H, s, NCH3), 1.83 (1H, m, H-2), 1.80 (1H, m, H-3), 1.36 (1H, dd, J = 5.0 Hz, 4.3 Hz, H-3), 1.34 (3H, d, J = 6.4 Hz, H-2′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 170.3, 169.6, 65.1, 61.2, 34.6, 33.4, 32.9, 23.2, 15.6; IR ($CHCl_3$) cm^{-1}: 3685, 3599, 3498, 3413, 3010, 2938, 1766, 1671, 1587, 1458, 1421, 1390, 1335, 1112, 1092, 1048, 1022, 1001, 939; HRMS, calcd for $C_9H_{13}O_4N$ $(M-NH_3)^+$ 199.0845. Found 199.0836.

5.1.10. (1*RS*,2*RS*,1′*RS*)-*N*-Methoxy-*N*-methyl-2-{1′-(*tert*-butyldimethylsilyloxy)ethyl}cyclopropane-1,1-dicarboxamide (25). To a solution of **24** (78 mg, 0.36 mmol) and imidazole (73 mg, 1.1 mmol) in DMF (5 mL) was added TBSCl (81 mg, 0.54 mmol) at 0 °C. The mixture was stirred at rt for 3 h. Then the mixture was quenched by the addition of H_2O and extracted with EtOAc (3×). The combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (1:50 EtOAc/hexane) to yield **25** (86 mg, 72%) as a colorless oil. ^1H NMR ($CDCl_3$, 500 MHz) δ: 3.71 (3H, s, OCH_3), 3.52 (1H, dq, J = 9.2 Hz, 6.1 Hz, H-1′), 3.24 (3H, s, NCH_3), 1.92 (1H, td, J = 9.2 Hz, 7.6 Hz, H-2), 1.72 (1H, dd, J = 7.6 Hz, 4.8 Hz, H-3), 1.26 (3H, d, J = 6.1 Hz, H-2′), 1.22 (1H, dd, J = 9.2 Hz, 4.8 Hz, H-3), 0.89 (9H, s, *t*-Bu), 0.06 (3H, s, $SiCH_3$), 0.05 (3H, s, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 168.7, 168.7, 67.8, 61.1, 35.1, 33.5, 32.9, 25.8 (3×), 24.0, 18.0,17.7,−4.4, −4.6; IR ($CHCl_3$) cm^{-1}: 3512, 3404, 3012, 2957, 2930, 2856, 1686, 1649, 1592, 1471, 1375, 1256, 1117, 1096, 1016, 780; HRMS, calcd for $C_{15}H_{29}O_4N_2Si$ $(M-H)^+$ 320.1897. Found 329.1895.

5.1.11. (1*SR*,2*RS*,1′*RS*)-2-{1″-(*tert*-butyldimethylsilyloxy)ethyl}-1-pentanoylcyclopropane-1-carboxamide (26). To a solution of **25** (50 mg, 0.15 mmol) in THF (2 mL) was added *n*-BuLi (0.48 mL, 1.6 M solution in hexane, 0.76 mmol) at −78 °C. The mixture was stirred at −78 °C for 5 h. The mixture was quenched by the addition of saturated NH_4Cl aq and extracted with Et_2O. The organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (1:7–2:1 EtOAc/hexane) to yield **26** (25 mg, 50%) as a colorless oil and recovered **25** (14 mg, 29%). ^1H NMR ($CDCl_3$, 500 MHz) δ: 8.24 (1H, br s, NH), 5.63 (1H, br s, NH), 3.83 (1H, dq, J = 8.3 Hz, 6.1 Hz, H-1″), 2.26 (2H, t, J = 7.4 Hz, H-2′), 2.08 (1H, dd, J = 8.3 Hz, 4.9 Hz, H-3), 1.76 (1H,

dt, J = 9.2 Hz, 8.3 Hz, H-2), 1.66 (1H, dd, J = 9.2 Hz, 4.9 Hz, H-3), 1.54 (2H, m, H-3′), 1.28 (2H, m, H-4′), 1.16 (3H, d, J = 6.1 Hz, H-2″), 0.89 (3H, s, J = 6.8 Hz, H-5′), 0.89 (9H, s, *t*-Bu), 0.09 (3H, s, $SiCH_3$), 0.07 (3H, s, $SiCH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 202.8, 169.3, 67.3, 40.0, 38.6, 36.9, 26.3, 25.9 (3×), 23.8, 22.2, 19.6, 18.1, 13.9,−4.4, −4.6; IR ($CHCl_3$) cm^{-1}: 3649, 3487, 3154, 3058, 2996, 2958, 2930, 2857, 1682, 1563, 1465, 1379, 1097, 1005, 903, 836; HRMS, calcd for $C_{17}H_{33}O_3NSi$ $(M)^+$ 327.2230. Found 327.2252.

5.1.12. (1*SR*,2*RS*,1′*RS*)-2-(1″-hydroxyethyl)-1-pentanoylcyclopropane-1-carboxamide (27). To a solution of **26** (24 mg, 73 μmol) in THF (1 mL) was added $3HF{\cdot}Et_3N$ (28 mL, 180 μmol). After the mixture was stirred for 3 days, the solvent was removed. The residue was purified by silica gel chromatography (1:5 EtOAc/hexane) to yield **27** (12 mg, 77%) as a colorless oil and **28** (3.3 mg, 23%) as a colorless oil. **27**: ^1H NMR ($CDCl_3$, 500 MHz) δ: 8.19 (1H, br s, NH), 5.78 (1H, br s, NH), 3.92 (1H, quin, J = 6.4 Hz, H-1″), 2.29 (2H, m, H-2′), 2.14 (1H, dd, J = 7.7 Hz, 5.2 Hz, H-3), 1.81 (1H, ddd, J = 9.5 Hz, 7.7 Hz, 5.2 Hz, H-2), 1.71 (1H, dd, J = 9.5 Hz, 5.2 Hz, H-3), 1.55 (2H, m, H-3′), 1.33 (2H, m, H-2′), 1.30 (3H, d, 3H, J = 6.4 Hz, H-1″), 0.91 (3H, t, H-5′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 205.5, 164.2, 64.8, 38.5, 37.1, 36.5, 26.5, 23.3, 22.2, 18.4, 13.8; IR ($CHCl_3$) cm^{-1}: 3649, 3005, 2957, 1682, 1559, 1466, 1382, 1271, 1175, 1096, 993, 822; HRMS, calcd for $C_{11}H_{16}O_3$ $(M-NH_3)^+$ 196.1099. Found 196.1095.

5.1.13. (1*SR*,4*RS*,5*RS*)-4-Methyl-1-pentanoyl-3-oxa-bicyclo[3.1.0]hexan-2-one (28). ^1H NMR ($CDCl_3$, 500 MHz) δ: 4.75 (1H, dq, J = 6.1 Hz, 4.9 Hz, H-4), 3.11 (1H, m, H-2′), 2.86 (1H, m, H-2′), 2.73 (1H, ddd, J = 7.7 Hz, 5.2 Hz, 4.6 Hz, H-5) 1.92 (1H, dd, J = 7.7 Hz, 4.6 Hz, H-6), 1.59 (2H, m, H-3′), 1.43 (1H, dd, J = 5.2 Hz, 4.6 Hz, H-6), 1.36 (3H, d, J = 6.1 Hz, 4-CH_3), 1.34 (2H, m, H-4′), 0.92 (3H, t, J = 7.3 Hz, H-5′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 203.0, 162.2, 73.8, 41.4, 34.3, 26.8, 25.5, 22.2, 20.7, 17.6, 13.9; IR ($CHCl_3$) cm^{-1}: 3660, 3591, 3052, 2298, 2957, 1768, 1696, 1552, 1489, 1459, 1302, 1250, 1188, 1112, 1091, 1039, 996, 940, 918, 893, 863, 839; HRMS, calcd for $C_{11}H_{16}O_3$ $(M)^+$ 196.1099. Found 196.1092.

5.1.14. (1*SR*,5*RS*)-4-Hydroxy-4-Methyl-1-pentanoyl-3-aza-bicyclo[3.10]hexan-2-one (29). To a solution of **27** (20 mg, 0.12 mmol) in CH_2Cl_2 (5 mL) was added Dess–Martin periodinane (149 mg, 0.35 mmol), and the mixture was stirred at rt for 6 h. The mixture was quenched by the addition of satd Na_2SO_3 aq and diluted with EtOAc. The layers were separated, and the organic layer was washed with satd $NaHCO_3$ aq, H_2O, brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (1:5–2:1 hexane/EtOAc) to yield **29** (7.9 mg, 32%) as a 1.2:1 tautomeric mixture, as a colorless oil, and **28** (15.6 mg, 68%). ^1H NMR ($CDCl_3$, 400 MHz) δ: 5.61 (1H, br s, NH), 5.42* (1H, br s, NH), 3.08 (2H, m, H-2′), 2.88* (2H, m, H-2′), 2.56 (1H, m, H-5), 2.51* (1H, m H-5), 1.94 (1H, dd, J = 8.0 Hz, 4.4 Hz, H-6), 1.88* (1H, dd, J = 8.0 Hz, 4.4 Hz, H-6), 1.66* (3H, s, 4-CH_3), 1.57 (2H, m, H-3′), 1.57* (2H, m, H-3′), 1.53 (1H, m, H-6), 1.50* (1H, m, H-6), 1.52 (3H, s, 4-CH_3), 1.32 (2H, m, H-4′), 1.32* (2H, m, H-4′), 0.91 (3H, t, J = 7.3 Hz, H-5′), 0.90* (3H, t, J = 7.3 Hz, H-5′); IR ($CHCl_3$) cm^{-1}: 3642, 3568, 3425, 3025, 3007, 2957, 2929, 2858, 1699, 1587, 1458, 1388, 12203, 1124,963, 850; HRMS, calcd for $C_{11}H_{17}NO_3$ $(M+Na)^+$ 234.1106. Found 234.1123.

5.1.15. (1*RS*,4*RS*,5*RS*)-*N*-Hexadecyl-*N*-methyl-4-methyl-2-oxo-3-oxa-bicylco[3.1.0]hexane-1-carboxamide (30). To a solution of **22** (84 mg, 0.54 mmol), *i*-Pr_2NEt (0.22 mL, 1.29 mmol), and *N*-methylhexadecylamine (275 mg, 1.08 mmol) in CH_2Cl_2 (9 mL) was added benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate (BOP) (357 mg, 0.81 mmol) at 0 °C. The mixture was stirred at rt for 2 h. Then the mixture was quenched by the addition of H_2O and extracted with CH_2Cl_2 (3×). The combined organic layer was washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (1:1 hexane/EtOAc) to yield **30** (195 mg, 92%) as a 1.7:1 rotational mixture, as a colorless oil. ^1H NMR ($CDCl_3$, 400 MHz) δ: 4.88 (1H, m, H-4), 4.88* (1H, m, H-4), 3.39 (2H, m H-3′), 3.39* (2H, m, H-3′), 3.17 (3H, s, NCH_3), 2.94 (3H, s, NCH_3), 2.63 (1H, m, H-5), 2.63* (1H, m, H-5), 1.56 (2H, m, H-4′), 1.56 (1H, m, H-6), 1.56* (2H, m, H-4′), 1.56* (1H, m, H-6), 1.37 (3H, d, 4-CH_3), 1.37* (3H, d, 4-CH_3), 1.33 (1H, m, H-6), 1.33* (1H, m, H-6), 1.26 (26H, H-5′–H-17′), 1.26* (26H, m, H-5′–H-17′), 0.88 (3H, t, J = 7.1 Hz, H-18′), 0.88* (3H, t, J = 7.1 Hz, H-18′); IR ($CHCl_3$) cm^{-1}: 3028, 2958, 2928, 2854, 1771, 1764, 1718, 1638, 1625, 1494, 1457, 1409, 1385, 1352, 1210, 1110, 1048, 1018, 938; HRMS, calcd for $C_{24}H_{43}NO_3$ $(M)^+$ 393.3243. Found 393.3242.

5.1.16. (1*RS*,2*RS*)-*N*-Hexadecyl-*N*-methyl-2-(1′-hydroxyethyl)clopropane-1,1-dicarboxamide (31). A solution of **30** (136 mg, 0.35 mmol) and NH_3 (8 mL, 28% solution in H_2O) was stirred at rt overnight. The solvent was removed, and the residue was purified by silica gel chromatography (2:3 EtOAc/hexane and 1:20 EtOH/EtOAc) to yield **31** (91 mg, 64%) as a 1.7:1 rotational mixture, as a colorless oil, and recovered **30** (44 mg, 32%). ^1H NMR ($CDCl_3$, 300 MHz) δ: 6.19 (1H, br s, NH), 6.19* (1H, br s, NH), 5.59 (1H, br s, NH), 5.59* (1H, br s, NH), 3.80 (1H, m, H-1′), 3.80* (1H, m, H-1′), 3.38 (2H, m, H-3″), 3.38* (2H, m, H-3″), 3.18 (3H, s, NCH_3), 2.94* (3H, s, NCH_3), 1.83* (1H, m, H-3), 1.75 (1H, m, H-3), 1.56 (2H, m, H-4″), 1.56 (1H, m, H-3), 1.56* (2H, m, H-4″), 1.56* (1H, m, H-3), 1.35 (3H, d, J = 6.3 Hz, H-2′), 1.35* (3H, d, J = 6.3 Hz, H-2′), 1.25 (26H, m, H-5″-H-12″), 1.25* (26H, m, H-5″-H-12″), 0.88 (3H, t, J = 7.2 Hz, H-18″), 0.88* (3H, t, J = 7.2 Hz, H-18″). No further characterization was attempted on this compound, which was used directly in the next step.

5.1.17. (1*RS*,2*RS*)-*N*-Hexadecyl-*N*-methyl-2-acetylcyclopropane-1,1-dicarboxamide (32). To a solution of **31** (88 mg, 0.21 mmol) in CH_2Cl_2 (3 mL) was added Dess–Martin periodinane (271 mg, 0.64 mmol). The mixture was stirred for 1 h. Then the mixture was quenched by the addition of satd Na_2SO_3 aq and diluted with EtOAc. The layers were separated, and the organic layer was

washed with satd $NaHCO_3$ aq, H_2O, brine, dried (Na_2SO_4), and concentrated. The residue was purified by silica gel chromatography (1:8–1:1 hexane/EtOAc) to yield **32** (83 mg, 96%) as a 1.1:1 rotational mixture, as a colorless oil. ^1H NMR ($CDCl_3$, 400 MHz) δ: 6.25 (1H, br d, NH), 6.25* (1H, br d, NH), 5.90 (1H, br d, NH), 5.90* (1H, br d, NH), 3.51* (1H, m, H-3), 3.37 (2H, m, H-3″), 3.31* (2H, m, H-3″), 3.12 (3H, s, NCH_3), 2.91* (3H, s, NCH_3), 2.74 (1H, m, H-2), 2.74* (1H, m, H-2), 2.35 (3H, s, $COCH_3$), 2.35* (3H, s, $COCH_3$), 2.14 (1H, m, H-3), 1.51 (2H, m, H-4″), 1.51* (2H, m, H-4″), 1.50* (1H, m, H-3), 1.41 (1H, dd, $J = 8.3$ Hz, 4.9 Hz, H-3), 1.25 (26H, m, H-5″-H-17″), 1.25* (26H, m, H-5″-H-17″), 0.87 (3H, t, $J = 6.8$ Hz, H-18″); ^{13}C NMR (100 MHz, $CDCl_3$) δ: 201.9, 201.8, 165.8, 165.7, 48.1, 46.7, 40.2, 39.8, 33.7, 32.8, 32.5, 31.6, 30.2, 29.4, 28.0, 28.0, 27.9, 27.7, 26.2, 25.0, 25.0, 21.0, 16.5, 16.3, 12.4., 12.4 (rotational mixture); IR ($CHCl_3$) cm^{-1}: 3007, 2927, 2855, 1712, 1692, 1633, 1590, 1489, 1466, 1407, 1386, 1309, 1239, 1176, 1090, 991, 908; HRMS, calcd for $C_{24}H_{44}N_2O_3$ $(M)^+$ 408.3352. Found 408.3350.

5.2. Detection of cell death by MTT assay

BALL-1 cells were plated onto a 96-well plate (Sumitomo Bakelite Co., Tokyo, Japan) at a concentration of 2.0×10^4 cells/well and preincubated at 37 °C for 1 h. The cells were then treated with epolactaene or its derivatives. After incubation at 37 °C for 24 h, cell viability was determined by the MTT assay, a method for determining cell viability by measuring the mitochondrial dehydrogenase action. In this assay, 11 μL MTT stock solution (5 mg/mL in phosphate-buffered saline (PBS)) was added to the cells, and the plate was incubated 37 °C for 1 h. After centrifugation for 5 min at 1500 rpm, the supernatant was discarded, and 100 μL DMSO was added to dissolve MTT formazan. Absorbance at 570 nm was measured with a microplate reader (Bio-Rad Model 550, Bio-Rad, Tokyo, Japan), and the percentage of cell viability was taken as the percentage absorbance at 570 nm of epolactaene-treated cells and control.

References and notes

1. Kerr, J. F.; Wyllie, A. H.; Currie, A. R. *Br. J. Cancer* **1972**, *26*, 239.
2. Wyllie, A. H.; Kerr, J. F.; Currie, A. R. *Int. Rev. Cytol.* **1980**, *68*, 251.
3. Lin, C.-F.; Yan, J.-S.; Chang, C.-Y.; Kuo, S.-C.; Lee, M.-R.; Huang, L.-J. *Bioorg. Med. Chem.* **2005**, *13*, 1537.
4. O'Driscoll, L.; Linehan, R.; Clynes, M. *Curr. Cancer Drug Target* **2003**, *3*, 131.
5. Denault, J. B.; Salvesen, G. S. *Chem. Rev.* **2002**, *102*, 4489.
6. Thornberry, N. A. *Chem. Biol.* **1998**, *5*, R97.
7. Cai, S. X.; Guan, L.; Jia, S.; Wang, Y.; Yang, W.; Tseng, B.; Drewe, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5295.
8. Sun, H.; Nikolovska-Coleska, Z.; Yang, C.-Y.; Xu, L.; Tomita, Y.; Krajewski, K.; Roller, P. P.; Wang, S. *J. Med. Chem.* **2004**, *47*, 4147.
9. Thomoson, C. B. *Science* **1995**, *267*, 1456.
10. Nicholson, D. W. *Nature* **2000**, *407*, 810.
11. Reed, J. C. *Nat. Rev. Drug. Discovery* **2002**, *1*, 111.
12. Rathmell, J. C.; Thompson, C. B. *Cell* **2002**, *109*, 97.
13. Reed, J. C. *J. Clin. Oncol.* **1999**, *17*, 2941.
14. Reed, C. J. *Semin. Hematol.* **2000**, *37*, 9.
15. Robertson, G. S.; Crocker, S. J.; Nicholson, D. W.; Schulz, J. B. *Brain Pathol.* **2000**, *10*, 283.
16. Kakeya, H.; Takahashi, I.; Okada, G.; Isono, K.; Osada, H. *J. Antibiot.* **1995**, *48*, 733.
17. Kakeya, H.; Onozawa, C.; Sato, M.; Arai, K.; Osada, H. *J. Med. Chem.* **1997**, *40*, 391.
18. Watabe, M.; Kakeya, H.; Osada, H. *Oncogene* **1999**, *18*, 5211.
19. Watabe, M.; Machida, K.; Osada, H. *Cancer Res.* **2000**, *60*, 5214.
20. Hayashi, Y.; Narasaka, K. *Chem. Lett.* **1998**, 313.
21. Hayashi, Y.; Kanayama, J.; Yamaguchi, J.; Shoji, M. *J. Org. Chem.* **2002**, *67*, 9443.
22. Marumoto, S.; Kogen, H.; Naruto, S. *J. Org. Chem.* **1998**, *63*, 2068.
23. Marumoto, S.; Kogen, H.; Naruto, S. *Tetrahedron* **1999**, *55*, 7129.
24. Marumoto, S.; Kogen, H.; Naruto, S. *Tetrahedron* **1999**, *55*, 7145.
25. Kuramochi, K.; Itaya, H.; Nagata, S.; Takao, K.; Kobayashi, S. *Tetrahedron Lett.* **1999**, *40*, 7367.
26. Kuramochi, K.; Nagata, S.; Itaya, H.; Takao, K.; Kobayashi, S. *Tetrahedron Lett.* **1999**, *40*, 7371.
27. Kuramochi, K.; Nagata, S.; Itaya, H.; Matsubara, Y.; Sunoki, T.; Uchiro, H.; Takao, K.; Kobayashi, S. *Tetrahedron* **2003**, *59*, 9743.
28. Mizushina, Y.; Kobayashi, S.; Kuramochi, K.; Nagata, S.; Sugawara, F.; Sakaguchi, K. *Biochem. Biophys. Res. Commun.* **2000**, *273*, 784.
29. Nakai, J.; Kawada, K.; Nagata, S.; Kuramochi, K.; Uchiro, H.; Kobayashi, S.; Ikekita, M. *Biochim. Biophys. Acta* **2002**, *1581*, 1.
30. Kuramochi, K.; Mizushina, Y.; Nagata, S.; Sugawara, F.; Sakaguchi, K.; Kobayashi, S. *Bioorg. Med. Chem.* **2004**, *12*, 1983.
31. Mizushina, Y.; Kuramochi, K.; Ikawa, H.; Kuriyama, I.; Shimazaki, N.; Takemura, M.; Oshige, M.; Yoshida, H.; Koiwai, O.; Sugawara, F.; Kobayashi, S.; Sakaguchi, K. *Int. J. Mol. Med.* **2005**, *15*, 785.
32. Kemnitzer, W.; Drewe, J.; Jiang, S.; Zhang, H.; Wang, Y.; Zha, J.; Jia, S.; Herich, J.; Labreque, D.; Storer, R.; Meerovitch, K.; Bouffard, D.; Rej, R.; Denis, R.; Blais, C.; Lamothe, S.; Attardo, G.; Gourdeau, H.; Tseng, B.; Kasibhatla, S.; Cai, S. X. *J. Med. Chem.* **2004**, *47*, 6299.
33. Nagumo, Y.; Kakeya, H.; Yamaguchi, J.; Uno, T.; Shoji, M.; Hayashi, Y.; Osada, H. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4425.
34. Nagumo, Y.; Kakeya, H.; Shoji, M.; Hayashi, Y.; Dhomae, N.; Osada, H. *Biochem. J.* **2005**, *387*, 835.
35. Temnikova, T. I.; Semenova, S. N. *USSR J. Org. Chem. Engl. Trans.* **1965**, *1*, 2076.
36. Klunder, J. M.; Onami, T.; Sharpless, K. B. *J. Org. Chem.* **1989**, *54*, 1295.
37. Gao, Y.; Hanson, R. M.; Klunder, J. M.; Ko, S. Y.; Masamune, H.; Sharpless, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 5765.
38. Kitaori, K.; Mikami, M.; Furukawa, Y.; Yoshimoto, H.; Otera, J. *Synlett* **1988**, 499.
39. Meyers, A. I.; Romine, J.; Fleming, S. A. *J. Am. Chem. Soc.* **1988**, *110*, 7245.
40. Nahm, S.; Weinreb, S. M. *Tetrahedron Lett.* **1981**, *22*, 3815.
41. Dess, D. B.; Martin, J. C. *J. Am. Chem. Soc.* **1991**, *113*, 7277.

42. Tanatani, A.; Yamaguchi, K.; Azumaya, I.; Fukutomi, R.; Shudo, K.; Kagechika, H. *J. Am. Chem. Soc.* **1998**, *120*, 6433.
43. Alley, M. C.; Scudiero, D. A.; Monks, A.; Hursey, M. L.; Czerwinski, M. J.; Fine, D. L.; Abbott, B. J.; Mayo, J. C.; Schoemaker, R. H.; Boyd, M. R. *Cancer Res.* **1988**, *48*, 589.
44. Pouillart, P.; Douillet, O.; Scappini, B.; Villa, P. *Eur. J. Pharmacol. Sci.* **1998**, *7*, 93.
45. Kaufmann, S. H. *Cancer Res.* **1989**, *49*, 5870.
46. Mizushina, Y.; Miyazaki, S.; Ohta, K.; Hirota, M.; Sakaguchi, K. *Biochim. Biophys. Acta* **2000**, *1475*, 1.
47. Mizushina, Y.; Hirota, M.; Murakami, C.; Ishidoh, T.; Kamisuki, S.; Shimazaki, N.; Takemura, M.; Perelescu, M.; Suzuki, M.; Yoshida, H.; Sugawara, F.; Koiwai, O.; Sakaguchi, K. *Biochem. Pharmacol.* **2003**, *66*, 1935.