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Design, synthesis, and evaluation of novel kazusamycin A derivatives as potent antitumor agents

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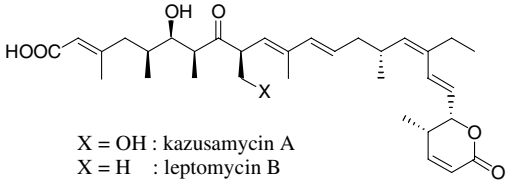
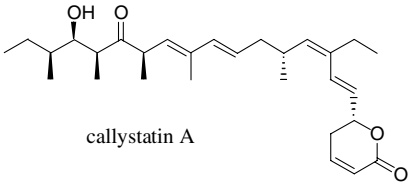
Abstract—Novel kazusamycin A derivatives were designed in the viewpoint of decrease of reactivity at the α,β-unsaturated δ-lactone moiety against Michael-type addition. Although 25–30 steps were required for the synthesis of each compound, their syntheses were achieved. Cytotoxicity against HPAC cell line was evaluated, and two of them exhibited comparable potency to kazusamycin A. Hepatic toxicity of these designed compounds was much lower than that of kazusamycin A.

Kazusamycin A was first isolated from the culture broth of *Streptomyces* sp. No. 81-484 in 1984,[1] and its absolute structure was determined by total synthesis in 2004.[2] Kazusamycin A exhibited potent antitumor activity against many kinds of leukemia and cell line of solid tumors both in vitro and in vivo,[3,4] but it has not been used in clinical setting by now[5] because of its hepatic and gastrointestinal toxicity.[6,7]



X = OH : kazusamycin A
X = H : leptomycin B

Kazusamycin A has a unique α,β-unsaturated δ-lactone ring. The mechanism of action of leptomycin B whose structure was very similar to that of kazusamycin A was studied, and it has become clear that the sulfhydryl group of Cys-529 in the target protein CRM1/exportin 1 causes irreversible Michael-type addition to this α,β-unsaturated δ-lactone moiety.[8]



callystatin A

A study on structure–activity relationship of callystatin A derivatives, which have an α,β-unsaturated δ-lactone ring and a lipophilic side chain, also gave us useful information.[9] Various transformation of the lipophilic side chain was investigated, and all compounds synthesized decreased antitumor activity. This result indicates that the lipophilic side chains of natural products are one of optimized structures. Accordingly, irreversible Michael-type addition of CRM1/exportin 1 is the main reason for extremely potent antitumor activity along with high affinity of lipophilic side chain to the target protein.

However, this reactive lactone moiety would also lead to high toxicity. It may react with side chains of cysteine, serine, lysine, or arginine moiety of proteins,

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or nucleophilic components of a living body. Then, this reaction would cause unfavorable effects.

In order to overcome this drawback of kazusamycin A, we have designed more stable α,β-unsaturated δ-lactone ring systems. They will decrease the reactivity against nucleophilic addition of CRM1/exportin 1, but its toxicity will also decrease because of its less reactivity. The decrease of antitumor activity and toxicity might be parallel in general, but we have expected that the safety margin would become wider since the lipophilic side chain showed high affinity to the target protein.[9]

There are two strategies to decrease the reactivity of α,β-unsaturated δ-lactone moiety to nucleophilic addition. One is to increase steric hindrance around the β-position at which nucleophiles attack, and the other is to deactivate the carbon–carbon double bond by adding electron-donating substituent at the α- or β-position.

We designed four compounds to confirm this hypothesis (Fig. 1). Compound **1a** is an example of more hindered compound by adding a methyl group at the γ-position, and **1b** is an example of deactivation of the carbon–carbon double bond by adding an electron-donating methyl group at the β-position. This methyl group would also affect steric hindrance around the β-position. Compound **1c** is an example of sterically less hindered but electrically deactivated compound by eliminating the γ-methyl group and adding a methyl group at the β-position of kazusamycin A. Compound **1d** is the least reactive compound in these four because of both steric and electrical factors arising from the addition of two methyl groups at the β- and γ-position of kazusamycin A.

Syntheses of the designed compounds should be achieved by using a similar route of total synthesis by Kuwajima.[2] Key intermediates of this project are compounds **2a**–**2d**, which would be transformed to the designed compounds easily by using similar reactions of total synthesis as shown in Scheme 1.

Coupling of aldehydes **2a**–**2d** with fragment **D** via Wittig reaction followed by two-step reactions would afford fragment **C**, which would be allowed to react with

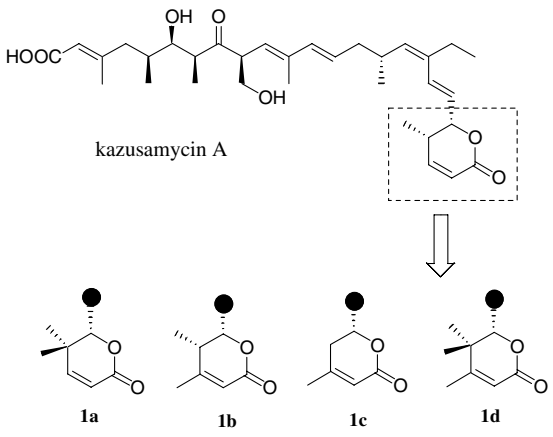
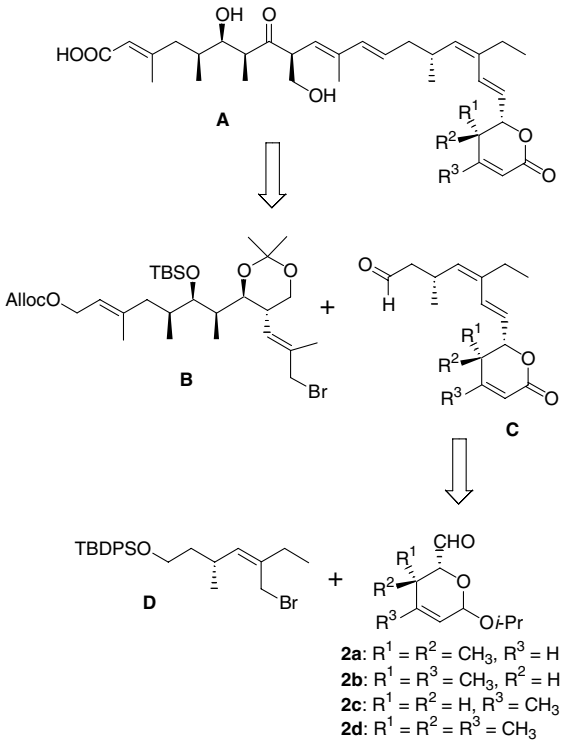


Figure 1. Design of kazusamycin A derivatives.

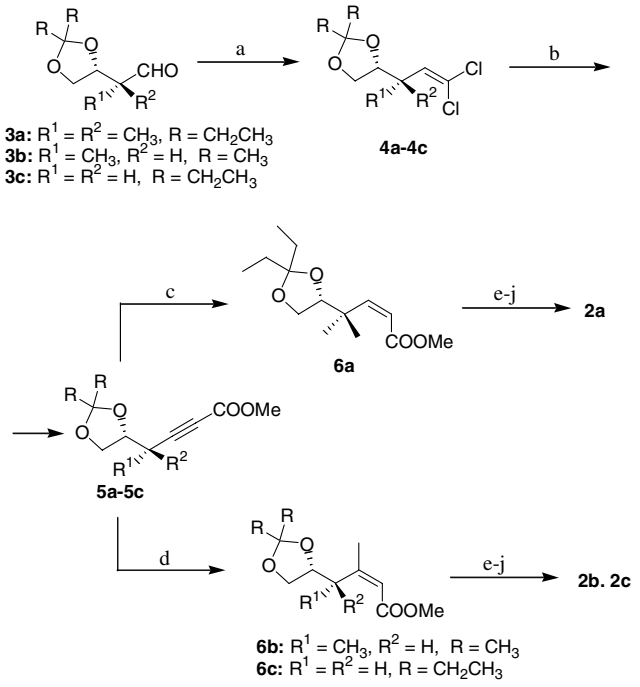


Scheme 1. Retrosynthetic analysis of the designed compounds.

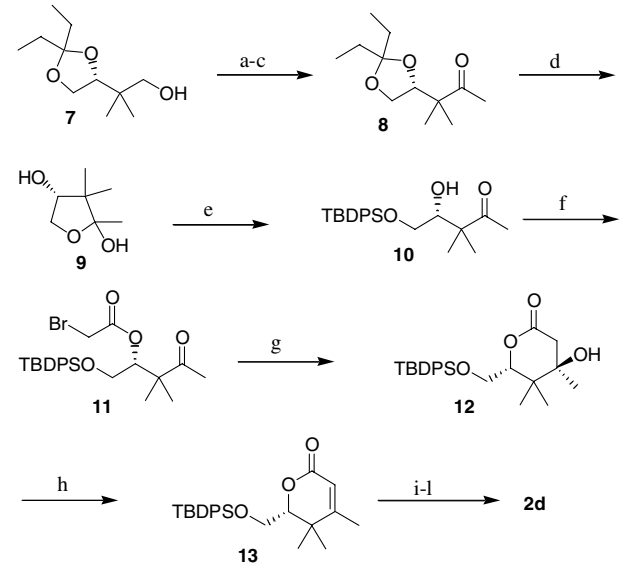
fragment **B** via Wittig reaction. Then, compound **A** would be synthesized in further eight-step reactions.[10,11]

As shown in Scheme 2, syntheses of **2a**–**2c** were analogous. Aldehydes **3a**–**3c**[12] were converted to the dichloroethylene derivatives **4a**–**4c** by Wittig reaction using $CCl_3PO(OEt)_2$. Treatment of **4a**–**4c** with BuLi afforded the lithiated acetylenes, which were allowed to react with methyl chloroformate to give the acetylenes **5a**–**5c**. Hydrogenation of **5a** by Lindlar catalyst poisoned by quinoline in toluene afforded *cis*-olefin **6a** in 87% yield. Trisubstituted olefins **6b** and **6c** were synthesized by addition of Me_2CuLi in THF at −78 °C (92% and 82% yield, respectively). Compounds **6a**–**6c** were converted to **2a**–**2c** in six steps each without any difficulty. Syntheses of **1a**, **1b**, and **1c** were achieved by similar reactions described in the literature.[2]

For synthesis of compound **2d** we developed a new synthetic route which is shown in Scheme 3. The starting alcohol **7**[14] was oxidized by sulfur trioxide–pyridine complex at rt in 97% yield, and methylmagnesium bromide was added to the resulting aldehyde at 0 °C. Then, the secondary alcohol formed was oxidized by sulfur trioxide–pyridine complex at rt to give the methylketone **8**. Treatment of **8** with 6 N HCl in THF furnished hydrolysis of the acetal moiety followed by cyclization to afford the hemiacetal **9**. The primary alcohol moiety which existed as a ring-opening form of **9** was protected by the reaction with TBDPSCl in the presence of DMAP. The total yield of these successive four steps was 26%. Then, the secondary alcohol moiety of **10** was allowed to react with bromoacetyl bromide in the presence of triethylamine to afford compound **11** in 98% yield. Treatment of **11** with samarium iodide gave



Scheme 2. Syntheses of the intermediates **2a**, **2b**, and **2c**. Reagents and conditions: (a) BuLi, $CCl_3PO(OEt)_2$, THF–Et_2O, −100 °C to −50 °C; (b) BuLi, THF, −78 °C to 0 °C, then ClCOOMe, −78 °C to 0 °C; (c) Lindlar catalyst, H_2, quinoline, toluene, rt, 87%; (d) Me_2CuLi, THF, −78 °C, 92% (**6b**), 82% (**6c**); (e) Dowex 50 W X8, MeOH, rt, then Amberlyst 15, CH_2Cl_2, rt; (f) TBDPSCl, imidazole, DMF, 0 °C to rt; (g) DIBAL, CH_2Cl_2, −78 °C; (h) *i*-PrOH, PPTS, benzene, rt; (i) TBAF, THF, rt; (j) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78 °C to 0 °C.



Scheme 3. Synthesis of the intermediate **2d**. Reagents and conditions: (a) SO_3·py, DMSO, rt, 97%; (b) MeMgBr, THF, 0 °C to rt; (c) SO_3·py, DMSO, rt; (d) 6 N HCl, THF, 0 °C to rt; (e) TBDPSCl, Et_3N, DMAP, CH_2Cl_2, 0 °C to rt, 26% (four steps); (f) bromoacetyl bromide, pyridine, CH_2Cl_2, 0 °C, 98%; (g) SmI_2, THF, −78 °C; (h) $SOCl_2$, pyridine, 0 °C, 86% (two steps); (i) DIBAL, CH_2Cl_2, −78 °C; (j) *i*-PrOH, PPTS, benzene, rt; (k) TBAF, THF, rt; (l) $(COCl)_2$, DMSO, CH_2Cl_2, −78 °C, then Et_3N, −78 °C to 0 °C.

Table 1. Cytotoxicity against HPAC cell line

Compound	R^1	R^2	R^3	IC_{50}[a] (nM)
Kazusamycin A	CH_3	H	H	0.0747
1a	CH_3	CH_3	H	0.0382
1b	CH_3	H	CH_3	179
1c	H	H	CH_3	0.309
1d	CH_3	CH_3	CH_3	16100

[a] IC_{50} values were calculated from the concentration–response curves, as the concentration of the test compounds elicited a decrease in the measured absorbance, equivalent to 50% of the vehicle group.

the hydroxylactone **12**, which was dehydrated via sulfinylation by thionyl chloride. Compound **12** could be converted to **2d** in five steps by referring to the reported method.[2]

Cytotoxicity of kazusamycin A[15] and four derivatives[16] against human HPAC cell line (ATCC CRL-2119) was tested,[17] and IC_{50} values of them are summarized in Table 1.

Compound **1a** exhibited similar cytotoxicity to kazusamycin A, and **1c** was slightly less potent than kazusamycin A. IC_{50} value of compound**1b** was much greater than that of kazusamycin A, and **1d** showed least potency.

This result is well explained by steric and electric effects. The high potency of **1a** indicates that the upper face of the lactone ring would not be important for both binding to CRM1/exportin 1 and addition of sulfhydryl group of Cys-529 in the target protein to the α,β-unsaturated δ-lactone moiety. Comparable potency of **1c** to kazusamycin A could be explained by balance of steric and electric factors. Namely, addition of a methyl group at the β-position gave unfavorable effect because of both deactivation of the carbon–carbon double bond and steric hindrance around the β-position, but removal of the methyl group at the γ-position gave quite favorable effect. Indeed, addition of a methyl group to the β-position of kazusamycin A caused marked decrease of potency (**1b**). The least potency of compound **1d** is reasonable since it is most hindered and deactivated compound of the four.

Finally, toxicity was evaluated to confirm our hypothesis. Compounds **1a**, **1c**, and kazusamycin A were intraperitoneally administered once daily to female BALB/c mice at dose levels of 0.125, 0.25, and 0.5 mg/kg for four days. Saline was injected to vehicle control mice.

As regards kazusamycin A, all mice given 0.5 mg/kg died, and higher value of ALT (GPT) was observed at 0.125 and 0.25 mg/kg in serum biochemistry. As regards compounds **1a** and **1c**, all mice survived, and value of ALT was a similar level to that of the control mice even at 0.5 mg/kg.[18,19]

Kazusamycin A exhibited fatal toxicity at higher dose and hepatic toxicity even at 0.125 mg/kg, whereas compounds **1a** and **1c** did not exhibit hepatic toxicity even at 0.5 mg/kg. This result indicates that our designed compounds with comparable potency to kazusamycin

A are much less toxic than kazusamycin A. So it would be possible to say that our hypothesis has been proved.

In summary, novel kazusamycin A derivatives were designed in the viewpoint of decrease of reactivity at the α,β-unsaturated δ-lactone moiety against Michael-type addition. Although 25–30 steps were required for the synthesis of each compound, their syntheses were achieved. Cytotoxicity against HPAC cell line was evaluated, and **1a** and **1c** exhibited comparable potency to kazusamycin A. Hepatic toxicity of these designed compounds was much lower than that of kazusamycin A.

Acknowledgments

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10. Furthermore, 33 and 10 steps were needed for the syntheses of fragments **B** and **D**, respectively.
11. Practical synthesis of fragments **B** and **D** has been investigated: Zhou, S.; Chen, H.; Liao, W.; Chen, S.-H.; Li, G.; Ando, R.; Kuwajima, I. *Tetrahedron Lett.* **2005**, *46*, 6341.
12. Synthesis of **3a** is described in Scheme 3. Compound **3b** is an intermediate for the total synthesis of kazusamycin A.[2] Dimethylacetal derivative of **3c** was described in a literature[13].
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15. This sample was synthesized by the method in Ref. 2.
16. These new four compounds were fully characterized. For example, **1a:** $[\alpha]_D^{27}$ −135.6 (*c* 0.106, MeOH); IR (KBr, neat, cm^{-1}) 3421, 2965, 1707, 1643, 1456, 1375, 1251, 1121, 967, 825; ^{1}HNMR (500 MHz, $CDCl_3$) δ = 0.78 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 6.4 Hz, 3H), 1.05 (t, *J* = 7.3 Hz, 3H), 1.07 (s, 3H), 1.09 (s, 3H), 1.19 (d, *J* = 7.3 Hz, 3H), 1.73–1.78 (m, 1H), 1.86 (s, 3H), 1.93 (dd, *J* = 13.3, 9.2 Hz, 1H), 2.05–2.15 (m, 2H), 2.13 (s, 3H), 2.19–2.23 (m, 3H), 2.62–2.69 (m, 1H), 2.77–2.82 (m, 1H), 3.58–3.64 (m, 2H), 3.85–3.90 (m, 2H), 4.61 (d, *J* = 7.8 Hz, 1H), 5.05 (d, *J* = 9.2 Hz, 1H), 5.23 (d, *J*= 9.6 Hz, 1H), 5.61–5.72 (m, 3H), 5.92 (d, *J* = 9.6 Hz, 1H), 6.00 (d, *J* = 15.6 Hz, 1H), 6.59 (d, *J* = 15.6 Hz, 1H), 6.69 (d, *J* = 9.6 Hz, 1H); ^{13}CNMR (125 MHz, $CDCl_3$) δ = 12.96, 13.31, 18.37, 24.54, 26.61, 32.12, 33.46, 35.60, 40.77, 45.29, 48.58, 54.15, 60.39, 62.14, 72.06, 86.98, 118.54, 121.27, 122.06, 128.63, 131.77, 135.05, 135.57, 136.94, 139.26, 156.97, 164.46, 172.52, 178.50, 215.26; HRMS (FAB) *m/z* ($M+Na^+$): calcd for $C_{34}H_{50}O_7Na$: 593.3454. Found: 593.3453.
17. A human pancreatic ductal carcinoma cell line HPAC CRL-2119 was obtained from the American Type Culture Collection (ATCC) and cultured in 1:1 mixture of Dulbecco's modified Eagle's medium and Ham's F-12 medium supplemented with 10% fetal bovine serum in a humidified atmosphere of 95% O_2 and 5% CO_2 at 37 °C. Cells were seeded at a concentration of 2×10^3 cells/well in culture medium into 96-well microtiter plates. Kazusamycin A and four derivatives were dissolved in DMSO. After a 24 h incubation period, treatment of test compounds was started. The final concentration of DMSO was 0.1%. After 72 h, 10 μL WST-1 reagent was added to each well, and the plate was incubated at 37 °C for 3 h. Absorbance of the culture supernatant at 450 nm was measured using a microplate reader.
18. ALT values (means ± SEM) of kazusamycin A (0.25 mg/kg), **1a** (0.5 mg /kg), **1c** (0.5 mg/kg), and vehicle control were 89.7 ± 9.9, 27.3 ± 7.2, 22.3 ± 2.0, and 27.7 ± 3.9, respectively.
19. As regards values of AST (GOT) and LDH, similar evidence was observed.