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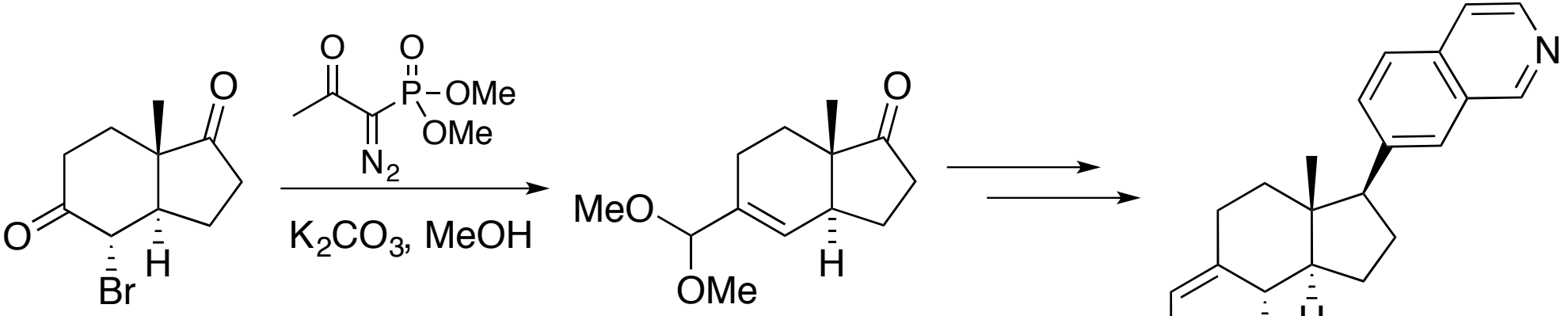


- Second-generation synthetic method considering step- and redox-economy
- Generation of novel cortistatin A analog with dramatically enhanced bioactivity

Short-step Synthesis and Structure-activity Relationship of Cortistatin A Analogs

Naoyuki Kotoku [*, †], Aoi Ito [†], Shunichi Shibuya, Kanako Mizuno, Aki Takeshima, Masaki Nogata, and Motomasa Kobayashi [*]

Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan

[*]Corresponding authors: Tel.: +81-6-6879-8215; Fax: +81-6-6879-8219;
E-mail: kotoku@phs.osaka-u.ac.jp and kobayasi@phs.osaka-u.ac.jp

[†] These authors contributed equally to this work.

Abstract

An improved method for synthesizing structurally simplified analogs of cortistatin A (**1**), a novel anti-angiogenic steroidal alkaloid from a marine sponge, was developed. In contrast to previous methods, step- and redox-economical synthesis was achieved using a known α-bromoketone as the starting material. The structure-activity relationship study revealed that the isoquinoline portion was strictly recognized by the target molecule. Surprisingly, the introduction of the acetamide moiety on the A-ring structure dramatically enhanced the selective antiproliferative activity against endothelial cells. This new method can be easily applied to gram-scale synthesis and enabled us to prepare various analogs, which were focused on the participation of the side chain and A-ring structure.

Keywords: Cortistatin A; Anti-angiogenesis; Marine sponge; Analog synthesis; Structure-activity relationship.

1. Introduction

Marine natural products have garnered considerable attention as a rich and promising source of drug candidates, especially in the field of anticancer drug discovery. [1,2] In most cases, however, the sustainable supply of active compounds has been a challenge for further evaluation and drug development. Generally, only small amounts of bioactive compounds can be isolated from the extracts of marine organisms such as sponges and tunicates. Chemical synthesis of bioactive natural products and their analog compounds can often overcome this drawback. The syntheses of truncated natural products based on structure-activity relationship (SAR) studies would be expected to facilitate the development of more accessible and promising drug leads with optimized activity or chemical stability. [3,4]

In our study of bioactive substances from marine organisms, we have focused on identifying anti-angiogenic substances and isolated cortistatins,[5] a family of novel *abeo*-9(10-19)-androstane-type steroidal alkaloids, from the Indonesian marine sponge *Corticium simplex*. Cortistatin A (**1**, Figure 1), a major constituent of *C. simplex*, showed a potent and highly selective antiproliferative activity against human umbilical vein endothelial cells (HUVECs). Cortistatin A (**1**) was also revealed to exhibit a potent inhibitory activity against *in vitro* migration and tubular formation of HUVECs induced by vascular endothelial growth factor (VEGF) or basic fibroblast growth factor (bFGF). [5d] Because of their unique chemical structure and intriguing biological properties, cortistatins are expected to be an emerging novel chemical entity for use as anti-angiogenic drug leads.

However, the extremely scarce supply of natural cortistatins has hampered our validation of the feasibility of their use as potential practical drug leads. There have been a number of reports of the synthesis of cortistatins including six total syntheses [6,7]; however, the yields were low in most cases. Therefore, we engaged in a synthetic study of structurally simplified analog compounds and produced a useful analog, **2** (Figure 1**)**. [8,9] Analog **2** exhibited a comparable antiproliferative activity to that of cortistatins against HUVECs, good selectivity, and potent *in vivo* antitumor activity following oral administration. To develop a more practical and promising anticancer drug lead based on the core structure of analog **2** as a scaffold, we focused on establishing a more efficient synthetic method than the existing methods. In this report, our second-generation synthetic method for producing cortistatin analogs and the generation of a novel and potent analog **30** are presented.

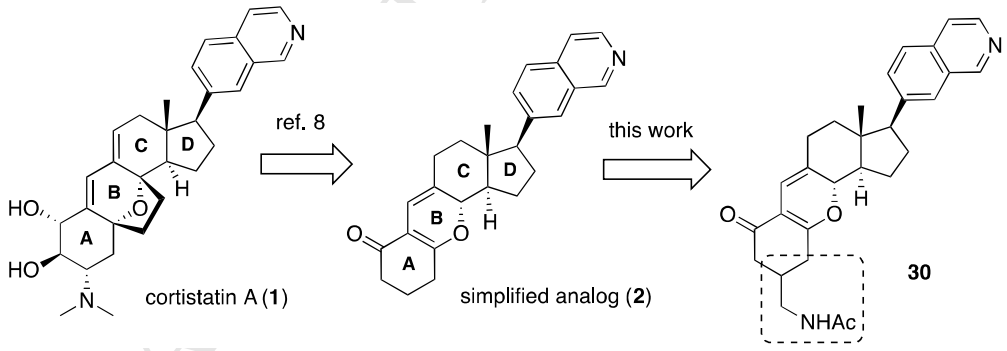
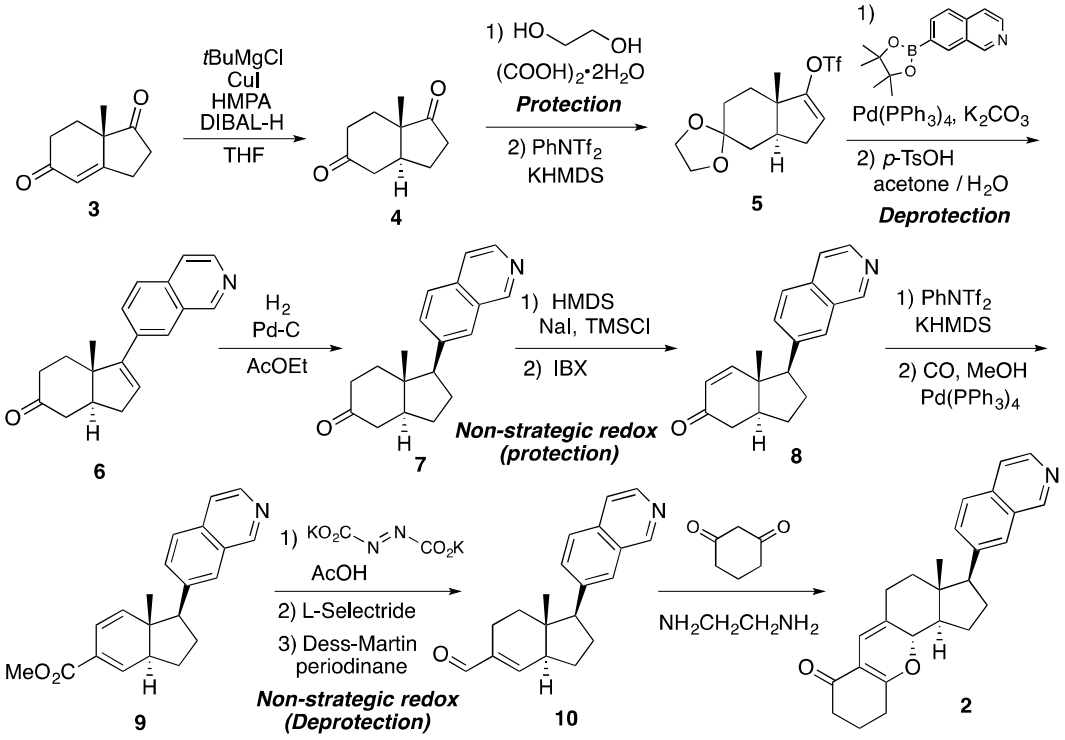


Figure 1 (revised)

2. Results and Discussion

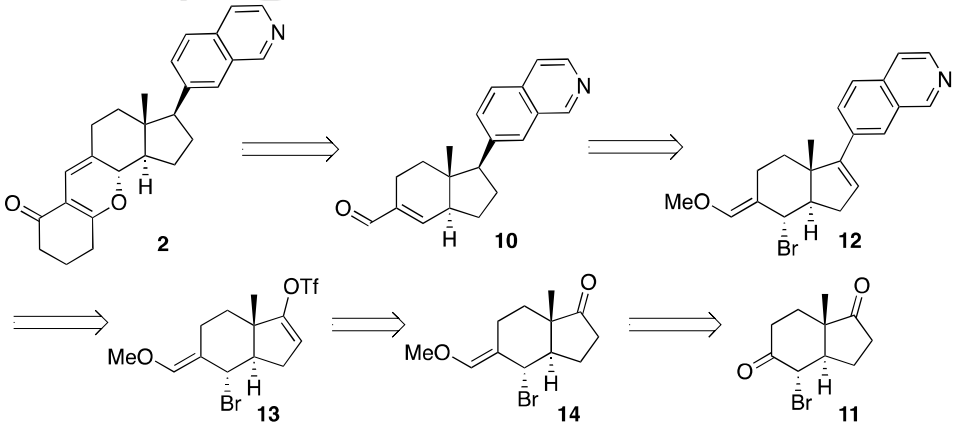
The first-generation synthetic method for producing the cortistatin analog **2**

developed by our group is depicted in Scheme 1.[8] We were able to prepare >100 mg of analog **2** using this method without any difficulty to examine its *in vivo* efficacy. However, it was far from being an efficient method[10,11] because it had the following drawbacks: (1) The number of reaction steps and total yield were not satisfactory; in particular, six reaction steps are needed for converting the cyclohexanone **7** to the α,β-unsaturated aldehyde **10** (Scheme 1). (2) The aforementioned problematic steps include iterative redox reactions. (3) The side chain portion of the structure was introduced in an earlier step of the synthesis, and eight reaction steps were needed to prepare each analogous compound for the optimization study of the side chain structure. Therefore, we developed a second-generation synthesis of the cortistatin analog **2** to modify the process for mass production and structural optimization.



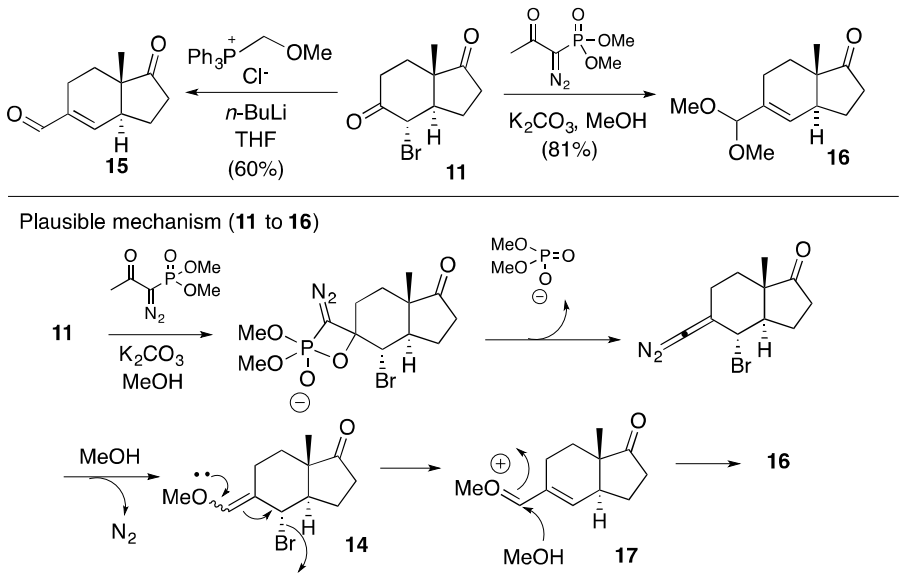
Scheme 1

The retrosynthetic analysis of the second-generation synthesis is depicted in Scheme 2. The most significant modification was the use of the α-bromoketone **11** as a starting material. Compound **11** can be obtained from Hajos-Parrish ketone (**3**) [12] at a large scale (>100 g) through a *tert*-butyl cuprate (*t*-BuCu)- or SiCu-catalyzed stereoselective conjugate reduction and subsequent bromination using a known method, which was previously used to generate a precursor for the synthesis of vitamin-D_2 and its analogs. [13] The problematic functionalizations encountered in the first-generation synthesis, such as the one-carbon homologation and regioselective introduction of a double bond (from **7** to **10** in Scheme 1), could be resolved through a shortened sequence. Specifically, the one-carbon homologation through the Wittig reaction giving vinyl ether **14**, and subsequent β-elimination of the bromide (from **12** to **10**) could provide the requisite α,β-unsaturated aldehyde functionality (Scheme 2). The protection/deprotection steps for the carbonyl group were also eliminated, which reduced the overall number of steps.



Scheme 2 (revised)

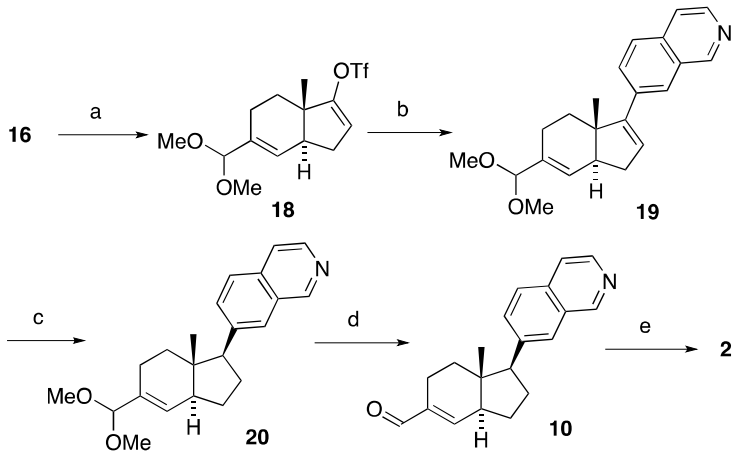
Initially, a one-carbon homologation reaction of the α-bromoketone **11** was attempted (Scheme 3). A Wittig reaction using (methoxymethyl)triphenylphosphonium chloride provided an α,β-unsaturated aldehyde **15** in place of the desired vinyl ether in moderate yield. In contrast, the use of the Ohira-Bestmann reagent in methanol in the presence of potassium carbonate [14] provided another product in good yield. Spectral analysis revealed that this product was α,β-unsaturated acetal **16**. A plausible reaction mechanism leading to **16** is shown in Scheme 3. The homologation product of the Ohira-Bestmann reaction, methyl vinyl ether **14**, might be formed *in situ*, from which the bromide group would be eliminated as depicted, thereby providing an oxocarbenium ion (**17**). Finally, the nucleophilic addition of the solvent MeOH would yield the α,β-unsaturated acetal **16**. The serendipitous but elegant reaction performed here achieved the requisite transformation around the carbonyl group in a single reaction step.



Scheme 3 (revised)

Next, the introduction of an isoquinoline moiety with the desired stereochemistry was successfully achieved using the Suzuki-Miyaura cross-coupling reaction between isoquinolin-7-yl boronate and the enol triflate **18**, obtained from **16** in good yield, followed by subsequent hydrogenation of the double bond in **19** using the palladium on carbon (Pd-C) catalyst (Scheme 4). To our delight, a complete chemoselectivity between the two double bonds in **19** was fortuitously achieved in this hydrogenation step, providing **20** as the sole product. Following the hydrolysis of the acetal moiety, treatment of the resulting aldehyde **10** with 1,3-cyclohexanedione in the presence of ethylenediamine yielded the desired analog **2**. Thus, the second-generation synthetic method for the generation of cortistatin analogs was developed. All the non-strategic transformation steps in the first-generation synthesis were eliminated. This redox-, atom-, and step-economical synthetic method can be applied to sub-gram or gram-scale

synthesis without substantially decreasing the yield of each reaction.

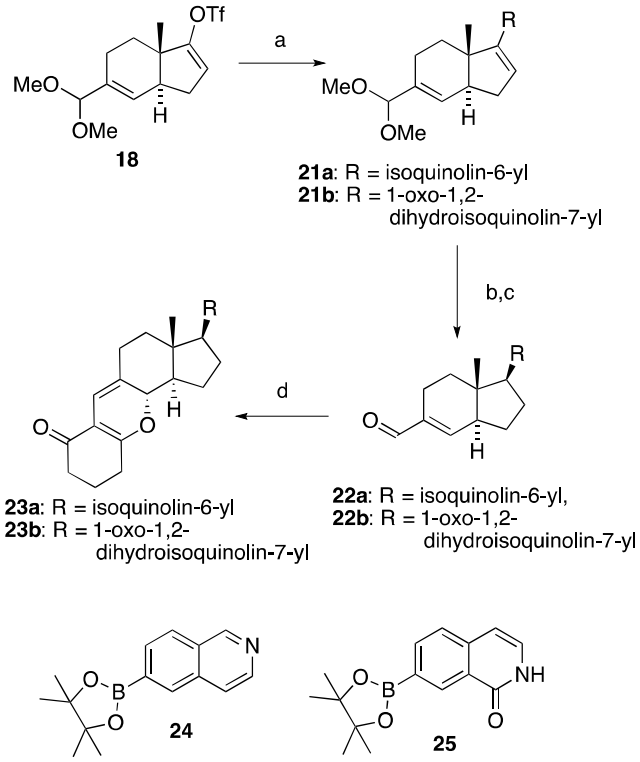


Scheme 4 (revised)

To analyze the detailed SAR of the cortistatin A (**1**), we then focused our attention on analog synthesis. From previous SAR analysis of the naturally occurring cortistatins, we determined that the isoquinoline side chain is crucial for the potent growth inhibitory activity against HUVEC, and that the functionalities on the core structure, such as the hydroxyl group on the A-ring portion, might be not essential but important.[5d] However, scope and limitation on the modification of those parts remained unclear. To acquire additional information, we synthesized several analogs with modified isoquinoline or A-ring moieties.

Firstly, compounds with a varied side chain were prepared from the enol triflate **18**. As shown in Scheme 5, Suzuki coupling with the corresponding boronate ester **24**[15] and **25**,[16] followed by hydrogenation, hydrolysis of the acetal, and Knoevenagel condensation, yielded analogs **23a** and **23b** with an isoquinolin-6-yl or an

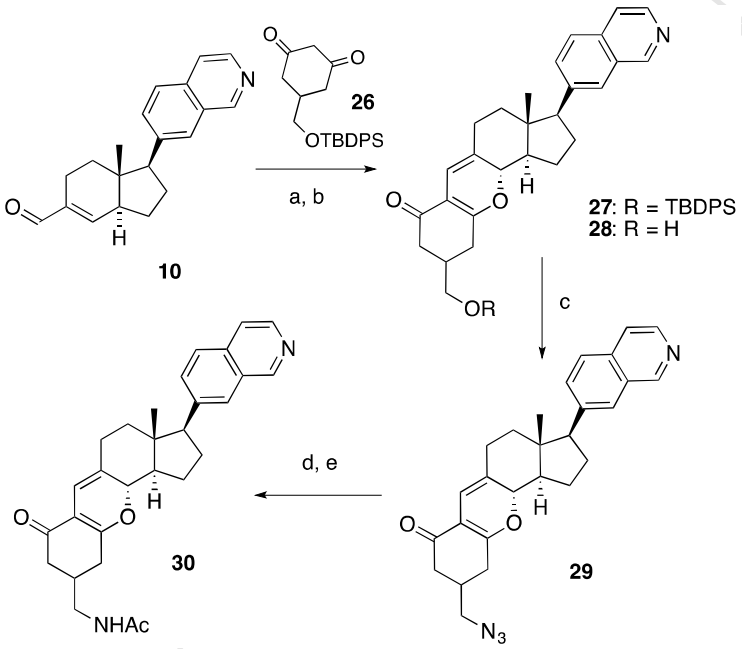
isoquinolin-1-one-7-yl side chain, respectively, in comparable yields with those obtained in the synthesis of **2**. In the hydrogenation of **21b**, an additional reduction on the α,β-unsaturated acetal moiety was observed at extended reaction times, whereas a complete chemoselectivity was obtained in those of **19** and **21a**. The basic nitrogen in the isoquinoline moieties of **19** and **21a** might coordinate with the Pd and weaken its reactivity in the hydrogenation reaction.



Scheme 5 (revised)

Secondly, functionalization of the A-ring portion was attempted. Considering the σ-symmetric property of 1,3-diketone, 5-hydroxymethyl-1,3-cyclohexanedione [17] was used to prepare analogs with a polar substituent. The Knoevenagel condensation

reaction between the *tert*-butyldiphenylsilyl (TBDPS)-protected dione (**26**) and the α,β-unsaturated aldehyde **10** proceeded smoothly, and the subsequent tetra-*n*-butylammonium fluoride (TBAF) treatment yielded an analog **28** with a hydroxymethyl moiety (Scheme 6). The hydroxyl group in **28** was further converted to an acetamide moiety in a three-step sequence involving a Mitsunobu reaction with diphenylphosphoryl azide (DPPA) [18], a Staudinger reaction, and acetylation, thereby providing analog **30**.



Scheme 6 (revised)

The antiproliferative activities of the synthetic analogs against HUVECs and KB3-1 cells were subsequently evaluated (Table 1). The results revealed that analogs **23a** and **23b** showed quite weak antiproliferative activities against HUVECs (half-maximal

inhibitory concentration (IC_{50}), 18 or 90 μM), which were even weaker than those against KB 3-1 cells (IC_{50}, 10.5 or 25 μM). These results clearly indicate that the target molecule (protein) strictly recognized the isoquinoline moiety; only the analog with the isoquinolin-7-yl moiety was able to interact with the target molecule.[9] In contrast, the introduction of the additional functionality on the A-ring portion enhanced the compound's potency against the HUVECs. Therefore, compounds **28** and **29** with a hydroxymethyl and an azidomethyl moiety, respectively, showed strong antiproliferative activities against the HUVECs (IC_{50}, 0.016 or 0.015 μM) with a high selectivity (> 500-fold) over the KB 3-1 cells (IC_{50}, 21.2 or 10.9 μM). Furthermore, the acetamide analog, **30**, surprisingly exhibited a highly potent activity against the HUVECs (IC_{50}, 0.0026 μM) as well as an excellent selectivity over the KB 3-1 cells (IC_{50}, 8.2 μM, > 3000-fold), which is comparable to that of cortistatin A (**1**). This evidence clearly indicates that the acetamide moiety dramatically enhanced the growth inhibitory activity against HUVECs.

Table 1. Antiproliferative activities of cortistatin analogs.

	1		**2**		**23a**		**23b**		**28**		**29**		**30**	
Cell line	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.	IC_{50}	S.I.
HUVEC	0.0018	1	0.035	1	18	1	90	1	0.016	1	0.015	1	0.0026	1
KB3-1	7.0	3900	10.5	300	10.5	0.58	25	0.28	21.2	1325	10.9	727	8.2	3125

IC_{50}= μM; S.I.= Selective index: IC_{50} against KB3-1 cells/IC_{50} against HUVECs.

3. Conclusion

In summary, we developed a second-generation synthetic method for producing

cortistatin A analogs. The high-yielding and short-step synthetic method also provided us with the opportunity to prepare various analog compounds, and we produced the acetamide analog **30** as a promising anti-angiogenic drug lead compound. Further lead optimization studies and *in vivo* evaluations of the active compounds are currently underway.

4. Experimental

4-1. General experimental

JEOL ECA-500 (^{1}H: 500 MHz, ^{13}C: 125 MHz), JEOL ECS-400 (^{1}H: 400 MHz, ^{13}C: 100 MHz), and Agilent NMR system (^{1}H: 600 MHz, ^{13}C: 150 MHz) spectrometer was used to obtain ^{1}H- and ^{13}C-NMR data using tetramethylsilane as an internal standard. A JASCO FT/IR-5300 infrared spectrometer was used for obtaining IR spectra. Mass spectra were obtained with a Waters Q-Tof Ultima API using MeOH as a solvent. Silica gel (Kanto, 40-100 μm) and pre-coated thin layer chromatography (TLC) plates (Merck, $60F_{254}$) were used for column chromatography and TLC. Spots on TLC plates were detected by spraying phosphomolybdic acid solution (5 g phosphomolybdic acid in 100 mL of EtOH) and acidic *p*-anisaldehyde solution (*p*-anisaldehyde: 25 mL, *c*-H_2SO_4: 25 mL, AcOH: 5 mL, EtOH: 425 mL) with subsequent heating. Unless otherwise noted, all the reactions were performed under N_2 atmosphere using purchased reagents and solvents without further purification. After workup, the organic phases were dried over Na_2SO_4.

4.2.

(3a*S*,7a*S*)-5-(Dimethoxymethyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-one (16)

Dimethyl (1-diazo-2-oxopropyl)phosphonate (0.44 mL, 2.9 mmol) and K_2CO_3 (537 mg, 3.9 mmol) were added to a solution of **11** [13b] (476 mg, 1.9 mmol) in MeOH (15.5 mL) at 0 °C, and the whole mixture was stirred for 1 h at rt. H_2O was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 4:1) to give **16** (350 mg, 80%) as colorless amorphous solid.

$[\alpha]^{22}_{D}$ +71.8° (*c* = 1.38 in $CHCl_3$). IR (KBr): 1742, 1071, 1051 cm^{-1}. ^{1}H-NMR (500 MHz, acetone-d_6) δ: 5.87 (1H, s), 4.53 (1H, s), 3.25 (6H, s), 2.53-2.47 (1H, m), 2.47 (1H, dd, *J* = 18.9, 8.6 Hz), 2.16-2.08 (3H, m), 2.03-1.99 (1H, m), 1.80-1.69 (2H, m), 1.48 (1H, dd, *J* = 18.9, 10.9 Hz), 0.83 (3H, s). ^{13}C-NMR (125 MHz, acetone-d_6) δ: 217.7, 136.7, 125.9, 106.3, 53.6, 53.4, 48.0, 44.3, 36.5, 29.1, 23.1, 22.6, 13.2. ESI MS: *m/z* 247 $(M+Na)^+$. HR-ESI MS: *m/z* 247.1310, calcd for $C_{13}H_{20}O_3Na$. Found: 247.1314.

4.3. (3a*S*,7a*S*)-6-(Dimethoxymethyl)-3a-methyl-3a,4,5,7a-tetrahydro-1*H*-inden-3-yl trifluoromethanesulfonate (18)

KHMDS (0.5 M in toluene, 1.3 mL, 0.65 mmol) was added dropwise to a solution of **16** (100 mg, 0.45 mmol) and *N*-phenyltriflimide (207 mg, 0.58 mmol) in THF (4.5 mL) at –78 °C, and the whole mixture was stirred at that temperature for 30 min. H_2O was

added to the mixture at 0 °C, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 5:1) to give **18** (148 mg, 93%) as a colorless oil.

$[\alpha]^{22}_D$ +51.2° (*c* = 1.61 in $CHCl_3$). IR (KBr): 1213, 1144, 1059 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 5.93 (1H, s), 5.59 (1H, s), 4.51 (1H, s), 3.33 (3H, s), 3.30 (3H, s), 2.76-2.71 (1H, m), 2.36 (1H, ddd, *J* = 14.9, 6.3, 3.5 Hz), 2.26-2.21 (1H, m), 2.19-2.14 (1H,m), 1.84-1.75 (2H, m), 0.94 (3H, s). ^{13}C-NMR (125 MHz, CD_3OD) δ: 160.3, 137.1, 125.7, 116.9, 107.4, 54.3, 54.0, 47.8, 46.4, 31.1, 30.0, 22.3, 15.3. ESI MS: *m/z* 379 $(M+Na)^+$. HR-ESI MS: *m/z* 379.0803, calcd for $C_{14}H_{19}O_5F_3SNa$. Found: 379.0836.

4.4.

7-((3a*S*,7a*S*)-6-(Dimethoxymethyl)-3a-methyl-3a,4,5,7a-tetrahydro-1*H*-inden-3-yl) isoquinoline (19)

7-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (147 mg, 0.65 mmol), $Pd(PPh_3)_4$ (72.1 mg, 0.062 mmol) and K_2CO_3 (172 mg, 1.25 mmol) were added to a solution of **18** (148 mg, 0.42 mmol) in DMF (4.2 mL), and the whole mixture was stirred at 50 °C for 90 min. H_2O was added to the mixture at rt, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column (toluene/acetone = 10:1) to give **19** (101 mg, 72%) as a colorless oil.

$[\alpha]^{22}_D$ +73.3° (*c* = 0.91 in $CHCl_3$). IR (KBr): 2932, 1103, 1073 cm^{-1}. ^{1}H-NMR (500

MHz, acetone-d_6) δ: 9.31 (1H, s), 8.46 (1H, d, *J* = 5.7 Hz), 8.13 (1H, s), 7.89 (2H, d, *J* = 1.1 Hz), 7.73 (1H, d, *J* = 5.7 Hz), 6.25 (1H, s), 6.03 (1H, s), 4.56 (1H, s), 3.29 (3H, s), 3.28 (3H, s), 2.79-2.71 (1H, m), 2.43 (1H, ddd, *J* = 14.9, 6.9, 3.4 Hz), 2.39-2.35 (1H, m), 2.28-2.21 (3H, m), 1.78 (1H, dd, *J* = 19.5, 8.5 Hz) 1.12 (3H, s). ^{13}C-NMR (125 MHz, acetone-d_6) δ: 153.7, 153.5, 143.8, 136.7, 136.2, 135.5, 130.6, 129.9, 129.8, 127.2, 126.2, 124.6, 120.7, 106.7, 53.5, 53.4, 50.3, 48.3, 32.9, 32.8, 22.9, 16.6. ESI MS: *m/z* 336 $(M+H)^+$. HR-ESI MS: *m/z* 336.1964, calcd for $C_{22}H_{26}NO_2$. Found: 336.1989.

4.5.

7-((1*S*,3a*S*,7a*S*)-5-(Dimethoxymethyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-yl)isoquinoline (20)

Pd/C (11.0 mg) was added to a solution of **19** (36.8 mg, 0.11 mmol) in AcOEt (2.0 mL), and the whole mixture was stirred under a H_2 atmosphere for 20 h. The reaction mixture was filtered through Celite pad, and the solvent was removed from the filtrate under reduced pressure to give **20** (37.1 mg, quant.) as a colorless oil.

$[\alpha]^{24}_D$ +17.3° (*c* = 1.98 in $CHCl_3$). IR (KBr): 2932, 2876, 1196, 1073, 849 cm^{-1}. ^{1}H-NMR (500 MHz, acetone-d_6) δ: 9.27 (1H, s), 8.45 (1H, d, *J* = 5.7 Hz), 7.98 (1H, s), 7.87 (1H, d, *J* = 8.6 Hz), 7.73 (1H, d, *J* = 5.7 Hz), 7.68 (1H, d, *J* = 8.6 Hz), 5.88 (1H, s), 4.49 (1H, s), 3.24 (1H, s), 3.23 (1H, s), 3.07 (1H, t, *J* = 9.7 Hz), 2.46-2.44 (2H, m), 2.15-2.13 (2H, m), 1.96-1.93 (2H, m), 1.65-1.63 (2H, m), 1.60-1.56 (1H, m), 0.50 (3H, s). ^{13}C-NMR (125 MHz, acetone-d_6) δ: 153.1, 143.3, 141.4, 135.7, 135.3, 133.0, 129.6, 127.3, 126.8, 126.5, 120.7, 106.7, 55.8, 53.4, 53.3, 49.0, 45.3, 34.7, 27.1, 25.6, 22.9,

12.4. ESI MS: *m/z* 338 $(M+H)^+$. HR-ESI MS: *m/z* 338.2120, calcd for $C_{22}H_{28}NO_2$. Found: 338.2100.

4.6.

(1*S*,3a*S*,7a*S*)-1-(Isoquinolin-7-yl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-indene-5-carbaldehyde (10)

Pyridinium *p*-toluenesulfonate (27.6 mg, 0.11 mmol) was added to a solution of **20** (37.1 mg, 0.11 mmol) in acetone/H_2O (20:1, 2.1 mL), and the whole mixture was stirred for 1 h. Sat. $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 1:1) to give **10** (31.3 mg, 98%) as a colorless oil.

$[\alpha]^{24}_D$ +55.8° (*c* = 2.10 in $CHCl_3$). IR (KBr): 2965, 2922, 1680, 850 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 9.43 (1H, s), 9.24 (1H, s), 8.49 (1H, s), 7.81 (1H, s), 7.76 (1H, d, *J* = 8.6 Hz), 7.63 (1H, d, *J* = 5.2 Hz), 7.59 (1H, dd, *J* = 8.6, 1.7 Hz), 6.87 (1H, d, *J* = 1.7 Hz), 3.03 (1H, t, *J* = 9.7 Hz), 2.69-2.64 (1H, m), 2.45-2.38 (2H, m), 2.28-2.22 (1H, m), 2.17-2.09 (2H, m), 1.80-1.73 (2H, m), 1.67-1.61 (1H, m), 0.48 (3H, s). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 194.1, 152.3, 151.8, 142.5, 141.3, 139.6, 134.7, 132.0, 128.6, 126.1, 125.9, 120.1, 55.0, 50.0, 44.9, 33.1, 26.4, 24.0, 20.5, 12.3. ESI MS: *m/z* 292 $(M+H)^+$. HR-ESI MS: *m/z* 292.1701, calcd for $C_{20}H_{22}NO$. Found: 292.1695.

4.7.

(3*S*,3a*R*,11a*S*,11b*R*)-3-(Isoquinolin-7-yl)-3a-methyl-1,3,3a,4,5,8,9,10,11a,11b-decahydrocyclopenta[c]xanthen-7(*2H*)-one (2)

Cyclohexane-1,3-dione (16.2 mg, 0.15 mmol) and ethylenediamine (7.2 μL, 0.11 mmol) were added to a solution of **10** (21.0 mg, 0.072 mmol) in AcOEt (1.0 mL), and the whole mixture was stirred for 19 h. H_2O was added to the mixture, and the whole mixture was extracted with CH_2Cl_2. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 1:3) to give **2** (19.3 mg, 70%) as a colorless oil.

All the spectral data were identical to those of the previous report. [8]

4.8.

6-((3a*S*)-6-(Dimethoxymethyl)-3a-methyl-3a,4,5,7a-tetrahydro-1*H*-inden-3-yl)isoquinoline (21a)

6-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinoline (**24**, 214.3 mg, 0.84 mmol), $Pd(PPh_3)_4$ (97 mg, 0.084 mmol) and K_2CO_3 (232 mg, 1.70 mmol) were added to a solution of **18** (200 mg, 0.56 mmol) in DMF (5.6 mL), and the whole mixture was stirred at 50 °C for 1 h. H_2O was added to the mixture, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/acetone = 2:1) to give **21a** (177.8 mg, 75%) as a colorless oil.

$[\alpha]^{27}_{D}$ +9.5° (*c* = 0.60 in MeOH). IR (KBr): 2929, 2844, 1626, 1102, 1073, 1053 cm^{-1}. ^{1}H-NMR (600 MHz, acetone-d_6) δ: 9.22 (1H, s), 8.47 (1H, d, *J* = 5.4 Hz), 8.03 (1H, d, *J*

= 8.4 Hz), 7.98 (1H, s), 7.79 (1H, m), 7.78 (1H, s), 6.30 (1H, s), 6.03 (1H, s), 4.56 (1H, s), 3.29 (3H, s), 3.28 (3H, s), 2.75 (1H, t, *J* = 7.8 Hz), 2.44 (1H, ddd, *J* = 15.6, 7.2, 3.0 Hz), 2.35 (1H, dd, *J* = 12.0, 7.2 Hz), 2.28-2.21 (3H, m), 1.79-1.74 (1H, m), 1.12 (3H, s). ^{13}C-NMR (125 MHz, acetone-d_6) δ: 153.7, 152.6, 144.1, 139.4, 136.6, 136.1, 131.0, 128.4, 128.0, 127.5, 126.0, 123.4, 121.1, 106.5, 53.4, 53.3, 50.1, 48.2, 32.7, 32.7, 22.7, 16.5. ESI MS: *m/z* 336 $(M+H)^+$. HR-ESI MS: *m/z* 336.1964, calcd for $C_{22}H_{26}NO_2$. Found: 336.1955.

4.9.

(1*S*,7a*S*)-1-(isoquinolin-6-yl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-indene-5-carb aldehyde (22a)

Pd/C (10%, 14.2 mg) was added to a solution of **21a** (42.5 mg, 0.13 mmol) in AcOEt (2.2 mL), and the whole mixture was stirred under a H_2 atmosphere for 22 h. The reaction mixture was filtered through Celite pad, and the solvent was removed from the filtrate under reduced pressure to give a hydrogenation product, 6-((1*S*,7a*S*)-5-(dimethoxymethyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-yl)iso quinoline (40.9 mg, 96%) as a colorless oil.

$[\alpha]^{26}_D$ +17.7° (*c* = 1.19 in MeOH). IR (KBr): 2927, 2876, 1630, 1103, 1072 cm^{-1}. ^{1}H-NMR (600 MHz, acetone-d_6) δ: 9.20 (1H, s), 8.43 (1H, d, *J* = 5.4 Hz), 7.96 (1H, d, *J* = 8.7 Hz), 7.76 (1H, s), 7.69 (1H, d, *J* = 5.4 Hz), 7.54 (1H, t, *J* = 8.7 Hz), 5.84 (1H, s), 4.45 (1H, s), 3.20 (3H, s), 3.18 (3H, s), 2.94 (1H, t, *J* = 9.6 Hz), 2.39-2.34 (2H, m), 2.11-2.04 (2H, m), 1.93-1.85 (2H, m), 1.60-1.58 (2H, m), 1.56-1.48 (1H, m), 0.44 (3H,

s). ^{13}C-NMR (150 MHz, acetone-d_6) δ: 152.6, 144.5, 143.7, 136.3, 135.5, 129.9, 128.4, 127.3, 127.0, 125.7, 120.8, 106.5, 55.8, 53.2, 53.1, 48.9, 45.2, 34.5, 26.8, 25.4, 22.7, 12.3. ESI MS: *m/z* 338 $(M+H)^+$. HR-ESI MS: *m/z* 338.2120, calcd for $C_{22}H_{28}NO_2$. Found: 338.2104.

Pyridinium *p*-toluenesulfonate (20.4 mg, 0.081 mmol) was added to a solution of an aliquot of the above product (27.4 mg, 0.081 mmol) in acetone/H_2O (10:1, 1.65 mL), and the whole mixture was stirred for 12 h. Sat. $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 1:1) to give **22a** (22.0 mg, 93%) as a colorless oil. $[\alpha]^{26}_D$ +64.1° (*c* = 0.62 in MeOH). IR (KBr): 2964, 2924, 2878, 1679, 1628 cm^{-1}. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.43 (1H, s), 9.22 (1H, s), 8.50 (1H, d, *J* = 6.0 Hz), 7.91 (1H, d, *J* = 8.4 Hz), 7.66 (1H, s), 7.61 (1H, d, *J* = 6.0 Hz), 7.51 (1H, d, *J* = 8.4 Hz), 6.87 (1H, s), 3.03 (1H, t, *J* = 9.6 Hz), 2.67-2.58 (1H, m), 2.47-2.39 (2H, m), 2.25-2.22 (1H, m), 2.17-2.09 (2H, m), 1.80-1.74 (2H, m), 1.67-1.62 (1H, m), 0.48 (3H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 194.1, 151.9, 151.7, 143.2, 142.8, 141.3, 135.7, 129.0, 127.7, 127.0, 125.2, 120.4, 55.3, 50.1, 45.1, 33.1, 26.3, 24.0, 20.5, 12.4. ESI MS: *m/z* 292 $(M+H)^+$. HR-ESI MS: *m/z* 292.1701, calcd for $C_{20}H_{22}NO$. Found: 292.1690.

4.10.

(3*S*,3a*R*,11a*S*)-3-(isoquinolin-6-yl)-3a-methyl-2,3,3a,4,5,8,9,10,11a,11b-decahydrocy

clopenta[c]xanthen-7(1*H*)-one (23a)

Cyclohexane-1,3-dione (4.0 mg, 0.034 mmol) and ethylenediamine (5 μL, 0.08 mmol) were added to a solution of **22a** (5.0 mg, 0.017 mmol) in AcOEt (0.2 mL), and the whole mixture was stirred for 2 h. H_2O was added to the mixture, and the whole mixture was extracted with CH_2Cl_2. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 1:2) to give **23a** (4.6 mg, 69%) as a colorless oil.

$[\alpha]^{26}_{D}$ +1.7° (*c* = 0.57 in MeOH). IR (KBr): 2928, 2880, 2854, 1645, 1631, 1604, 1406, 1381, 1218, 1168,754 cm^{-1}. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.21 (1H, s), 8.49 (1H, d, *J* = 5.7 Hz), 7.90 (1H, d, *J* = 8.4 Hz), 7.63 (1H, s), 7.60 (1H, d, *J* = 5.7 Hz), 7.47 (1H, d, *J* = 8.4 Hz), 6.19 (1H, s), 4.92 (1H, d, *J* = 10.2 Hz), 3.01 (1H, t, *J* = 9.6 Hz), 2.42-2.27 (7H, m), 2.16-2.12 (3H, m), 1.99-1.93 (2H, m), 1.74-1.72 (1H, m), 1.61-1.58 (1H, m), 1.50-1.47 (1H, m), 0.60 (3H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 194.9, 171.4, 152.0, 143.0, 142.6, 135.6, 129.1, 128.8, 127.7, 126.9, 125.3, 120.3, 110.9, 110.7, 80.7, 56.9, 55.4, 46.8, 36.8, 36.4, 29.1, 28.0, 26.3, 23.9, 20.6, 12.4. ESI MS: *m/z* 386 $(M+H)^+$. HR-ESI MS: *m/z* 386.2120, calcd for $C_{26}H_{28}NO_2$. Found: 386.2102.

4.11.

7-((3a*S*)-6-(Dimethoxymethyl)-3a-methyl-3a,4,5,7a-tetrahydro-1*H*-inden-3-yl)isoquinolin-1(2*H*)-one (21b)

7-(4,4,5,5-Tetramethyl-1,3,2-dioxaborolan-2-yl)isoquinolin-1(2*H*)-one (**25**, 93.3 mg, 0.34 mmol), $Pd(PPh_3)_4$ (78.6 mg, 0.06 mmol) and K_2CO_3 (158.8 mg, 1.15 mmol) were

added to a solution of **18** (82 mg, 0.23 mmol) in DMF (2.3 mL), and the whole mixture was stirred at 50 °C for 6 h. H_2O was added to the mixture, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/acetone = 4:1) to give **21b** (39.7 mg, 49%) as a colorless oil.

$[\alpha]^{27}_D$ +25.3° (*c* = 1.11 in MeOH). IR (KBr): 3165, 2926, 2848, 1652, 1635, 1613, 1101, 1073 cm^{-1}. ^{1}H-NMR (600 MHz, CD_3OD) δ: 8.31 (1H, s), 7.74 (1H, d, *J* = 8.1 Hz), 7.55 (1H, d, *J* = 8.1 Hz), 7.13 (1H, d, *J* = 7.2 Hz), 6.62 (1H, d, *J* = 7.2 Hz), 6.09 (1H, s), 5.98 (1H, s), 4.52 (1H, s), 3.31 (3H, s), 3.30 (3H, s), 2.68-2.66 (1H, m), 2.37-2.33 (1H, ddd, *J* = 15.0, 7.2, 3.0 Hz), 2.27-2.16 (3H, m), 2.15 (2H, s), 1.71-1.65 (1H, m), 1.05 (3H, s). ^{13}C-NMR (150 MHz, CD_3OD) δ: 165.0, 154.1, 138.5, 136.9, 136.3, 132.7, 129.7, 128.5, 127.5, 127.2, 126.8, 124.7, 107.8, 107.7, 54.1, 54.0, 50.7, 48.5, 33.2, 33.1, 23.0, 16.7. MALDI MS: *m/z* 352 $(M+H)^+$. HR-MALDI MS: *m/z* 352.1907, calcd for $C_{22}H_{26}NO_3$. Found: 352.1917.

4.12.

(1*R*,7a*S*)-7a-Methyl-1-(1-oxo-1,2-dihydroisoquinolin-7-yl)-2,3,3a,6,7,7a-hexahydro-1*H*-indene-5-carbaldehyde (22b)

Pd/C (10%, 6.3 mg) was added to a solution of **21b** (18.9 mg, 0.054 mmol) in AcOEt (1.0 mL), and the whole mixture was stirred under a H_2 atmosphere for 5 h. The reaction mixture was filtered through Celite pad, and the solvent was removed from the filtrate under reduced pressure to give a hydrogenation product,

7-((1*S*,7a*S*)-5-(dimethoxymethyl)-7a-methyl-2,3,3a,6,7,7a-hexahydro-1*H*-inden-1-yl)iso quinolin-1(2*H*)-one (17.6 mg, quant.) as a colorless oil.

$[\alpha]^{27}_{D}$ +18.8° (*c* = 0.83 in MeOH). IR (KBr): 3164, 2926, 2874, 2830, 1652, 1636, 1614, 1103, 1070 cm^{-1}. ^{1}H-NMR (600 MHz, CD_3OD) δ: 8.18 (1H, s), 7.58 (1H, d, *J* = 8.1 Hz), 7.55 (1H, d, *J* = 8.1 Hz), 7.11 (1H, d, *J* = 7.2 Hz), 6.63 (1H, d, *J* = 7.2 Hz), 5.85 (1H, s), 4.45 (1H, s), 3.26 (3H, s), 3.25 (3H, s), 2.88 (1H, t, *J* = 9.6 Hz), 2.38-2.36 (1H, m), 2.33-2.31 (1H, m), 2.12-2.06 (2H, m), 1.94-1.88 (2H, m), 1.58-1.51 (3H, m), 0.44 (3H, s). ^{13}C-NMR (150 MHz, CD_3OD) δ: 165.1, 141.5, 138.18, 138.17, 135.8, 135.2, 128.4, 128.1, 127.0, 126.6, 107.9, 107.8, 56.2, 54.02, 54.00, 49.5, 45.5, 35.1, 27.6, 27.4, 26.0, 23.0, 12.5. ESI MS: *m/z* 354 $(M+H)^+$. HR-ESI MS: *m/z* 354.2069, calcd for $C_{22}H_{28}NO_3$. Found: 354.2083.

Pyridinium *p*-toluenesulfonate (11.0 mg, 0.044 mmol) was added to a solution of an aliquot of the above product (15.4 mg, 0.044 mmol) in acetone/H_2O (10:1, 1.1 mL), and the whole mixture was stirred for 1 h. Sat. $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with AcOEt. Removal of the solvent from the AcOEt extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/acetone = 2:1) to give **22b** (16.3 mg, quant.) as a colorless oil.

$[\alpha]^{26}_{D}$ +36.8° (*c* = 1.44 in MeOH). IR (KBr): 3163, 2968, 2923, 2877, 1680, 1651, 1635, 754 cm^{-1}. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 11.80 (1H, s), 9.41 (1H, s), 8.28 (1H, s), 7.55 (1H, d, *J* = 7.8 Hz), 7.51 (1H, d, *J* = 7.8 Hz), 7.18 (1H, d, *J* = 6.9 Hz), 6.85 (1H, d, *J* = 1.2 Hz), 6.56 (1H, d, *J* = 6.9 Hz), 2.98 (1H, d, *J* = 9.6 Hz), 2.62 (1H, m), 2.44-2.40 (2H,

m), 2.20-2.15 (1H, m), 2.06-2.05 (1H, m), 1.76-1.71 (2H, m), 1.61-1.60 (1H, m), 0.46 (3H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 194.1, 164.5, 152.1, 141.3, 139.2, 136.6, 133.6, 127.2, 126.2, 125.7, 125.6, 106.5, 54.9, 50.0, 44.7, 33.0, 29.2, 26.4, 23.9, 20.5, 12.9. ESI MS: *m/z* 308 $(M+H)^+$. HR-ESI MS: *m/z* 308.1651, calcd for $C_{20}H_{22}NO_2$. Found: 308.1664.

4.13.

7-((3*S*,3a*R*,11a*S*)-3a-Methyl-7-oxo-1,2,3,3a,4,5,7,8,9,10,11a,11b-dodecahydrocyclopenta[c]xanthen-3-yl)isoquinolin-1(2*H*)-one (23b)

Cyclohexane-1,3-dione (8.0 mg, 0.071 mmol) and piperidine (10 μL, 0.16 mmol) were added to a solution of **22b** (10.9 mg, 0.035 mmol) in 1,4-dioxane (0.7 mL), and the whole mixture was stirred for 13 h. H_2O was added to the mixture, and the whole mixture was extracted with $CHCl_3$. Removal of the solvent from the $CHCl_3$ extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 1 : 2) to give **23b** (7.3 mg, 51%) as a colorless oil.

$[\alpha]^{27}_D$ –8.6° (*c* = 0.64 in MeOH). IR (KBr): 3184, 2961, 2925, 2855, 1651, 1613, 1598, 1261, 800 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 10.78 (1H, brs), 8.26 (1H, s), 7.54-7.49 (2H, m), 7.14 (1H, brs), 6.57 (1H, d, *J* = 6.8 Hz), 6.18 (1H, s), 4.91 (1H, d, *J* = 10.0 Hz), 2.96 (1H, t, *J* = 9.4 Hz), 2.43-2.35 (7H, m), 2.12-2.11 (3H, m), 1.98-1.92 (2H, m), 1.72-1.69 (1H, m), 1.59-1.55 (1H, m), 1.47-1.40 (1H, m), 0.59 (3H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 194.0, 170.4, 163.3, 137.9, 135.6, 132.9, 128.0, 126.1, 125.4, 124.7, 124.6, 109.9, 109.7, 105.6, 79.8, 55.6, 54.4, 45.5, 35.8, 35.4, 28.1, 27.1, 25.4, 22.9, 19.6,

11.3. ESI MS: *m/z* 424 $(M+Na)^+$. HR-ESI MS: *m/z* 424.1889, calcd for $C_{26}H_{27}NO_3Na$. Found: 424.1886.

4.14. 5-(((*tert*-Butyldiphenylsilyl)oxy)methyl)cyclohexane-1,3-dione (26)

Imidazole (40 mg, 0.59 mmol) and *tert*-butylchlorodiphenylsilane (0.08 mL, 0.32 mmol) were added to a solution of (3,5-dimethoxycyclohexa-2,5-dien-1-yl)methanol (50 mg, 0.29 mmol) in DMF (2.9 mL), and the whole mixture was stirred for 1 h. Sat. NH_4Cl aq. was added to the mixture, and the whole mixture was extracted with Et_2O. Removal of the solvent from the Et_2O extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 20:1) to give *tert*-butyl((3,5-dimethoxycyclohexa-2,5-dien-1-yl)methoxy)diphenylsilane (50 mg, 53%) as a colorless oil.

IR (KBr): 2953, 2933, 2901, 2857, 1694, 1206, 1148, 1111, 703 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.62-7.60 (4H, m), 7.35-7.28 (6H, m), 4.68-4.66 (2H, m), 3.46 (2H, d, *J* = 6.9 Hz), 3.41 (6H, s), 3.13-3.08 (1H, m), 2.59-2.57 (2H, m), 0.95 (9H, s). ^{13}C-NMR (125 MHz, acetone-d_6) δ: 153.7, 136.3, 134.6, 130.5, 128.5, 93.3, 70.5, 54.3, 40.0, 32.1, 27.2, 19.8. MALDI MS: *m/z* 409 $(M+H)^+$. HR-MALDI MS: *m/z* 409.2194, calcd for $C_{22}H_{26}NO_3$. Found: 409.2200.

1N HCl (0.19 mL) was added to a solution of an aliquot of the above product (39.5 mg, 0.097 mmol) in THF (0.48 mL), and the whole mixture was stirred for 3 h. Removal of the solvent from the reaction mixture under reduced pressure gave **26** (43.9 mg, quant.)

as a colorless oil.

Mixture of keto-form and enol-form. IR (KBr): 2931, 2896, 2858, 1588, 1224, 1112, 703 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.66-7.64 (4H, m), 7.44-7.35 (6H, m), 5.38 (0.5H, s), 3.70 (1.5H, s), 3.64-3.59 (2H, m), 3.57-3.34 (0.5H, m), 2.73-2.60 (0.5H, m), 2.47-2.26 (4H, m), 1.07 (9H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 203.6, 199.8, 178.5, 135.5, 135.4, 134.8, 133.2, 133.12, 133.09, 132.4, 129.8, 129.7, 129.67, 129.4, 127.7, 127.6, 127.5, 101.9, 67.1, 66.5, 66.3, 60.3, 57.6, 55.8, 43.4, 39.5, 36.3, 36.2, 33.4, 31.7, 26.7, 26.6, 26.5, 21.0, 19.2, 19.0, 14.1. MALDI MS: *m/z* 403 $(M+Na)^+$. HR-MALDI MS: *m/z* 403.1700, calcd for $C_{22}H_{26}NO_3$. Found: 403.1697.

4.15.

(3*S*,3a*R*,11a*S*)-9-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-3-(isoquinolin-7-yl)-3a-methyl-2,3,3a,4,5,8,9,10,11a,11b-decahydrocyclopenta[c]xanthen-7(1*H*)-one (27)

5-(((*tert*-Butyldiphenylsilyl)oxy)methyl)cyclohexane-1,3-dione (**26**, 23.0 mg, 0.06 mmol) and ethylenediamine (5 μL, 0.08 mmol) were added to a solution of **10** (11.7 mg, 0.04 mmol) in AcOEt (0.8 mL), and the whole mixture was stirred for 19 h. H_2O was added to the mixture, and the whole mixture was extracted with $CHCl_3$. Removal of the solvent from the $CHCl_3$ extract under reduced pressure gave a crude product, which was purified by SiO_2 column (*n*-hexane/AcOEt = 1:2) to give **27** (14.0 mg, 53%) as a colorless oil.

IR (KBr): 2957, 2929, 2856, 1648, 1607, 1111, 703 cm^{-1}. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.24 (1H, s), 8.49 (1H, d, *J* = 4.8 Hz), 7.80 (1H, s), 7.77 (1H, d, *J* = 8.4 Hz), 7.65 (4H,

d, *J* = 6.6 Hz), 7.58 (1H, d, *J* = 8.4 Hz), 7.45-7.38 (6H, m), 6.19 (1/2H, s), 6.17 (1/2H, s), 4.97 (1/2H, d, *J* = 10.2 Hz) , 4.93 (1/2H, d, *J* = 10.2 Hz), 3.63-3.57 (2H, m), 3.02 (1H, t, *J* = 9.0 Hz), 2.48-2.28 (7H, m), 2.19-2.12 (3H, m), 1.77-1.70 (1H, m), 1.61-1.58 (1H, m), 1.51-1.46 (1H, m), 1.06 (9H, s), 0.62 (3/2H, s), 0.61(3/2H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 194.5, 194.2, 170.8, 170.7, 151.9, 141.9, 139.4, 139.4, 135.5, 135.5, 134.8, 133.3, 133.2, 133.2, 132.4, 129.7, 129.0, 128.6, 128.5, 127.7, 126.4, 126.4, 125.8, 120.3, 111.1, 110.6, 110.6, 110.0, 81.0, 80.8, 66.6, 66.4, 56.7, 55.6, 55.2, 46.8, 46.6, 39.4, 39.1, 37.0, 36.6, 35.9, 35.3, 31.0, 30.7, 29.2, 29.0, 26.8, 26.3, 26.3, 24.0, 23.8, 19.3, 12.4, 12.3. ESI MS: *m/z* 676 $(M+Na)^+$. HR-ESI MS: *m/z* 676.3223, calcd for $C_{43}H_{47}NO_3NaSi$. Found: 676.3249.

4.16.

(3*S*,3a*R*,11a*S*)-9-(Hydroxymethyl)-3-(isoquinolin-7-yl)-3a-methyl-2,3,3a,4,5,8,9,10,11a,11b-decahydrocyclopenta[c]xanthen-7(1*H*)-one (28)

Acetic acid (1.8 μL, 0.032 mmol) and tetra-*n*-butylammonium fluoride (1.0 M in THF, 32 μL, 0.032 mmol) were added to a solution of **27** (14.0 mg, 0.021 mmol) in THF (0.2 mL), and the whole mixture was stirred for 16 h. Sat. $NaHCO_3$ aq. was added to the mixture, and the whole mixture was extracted with $CHCl_3$. Removal of the solvent from the $CHCl_3$ extract under reduced pressure gave a crude product, which was purified by SiO_2 column ($CHCl_3$/MeOH/H_2O = 60:3:1, lower phase) to give **28** (9.7 mg, 100%) as a colorless oil.

IR (KBr): 3367, 2925, 2880, 1639, 1601, 1420, 1407, 1381, 1208, 754 cm^{-1}. ^{1}H-NMR

(600 MHz, $CDCl_3$) δ: 9.22 (1H, s), 8.47 (1H, s), 7.79 (1H, s), 7.76 (1H, d, *J* = 8.4 Hz), 7.65 (1H, s), 7.57 (1H, d, *J* = 7.8 Hz), 6.17 (1/2H, s), 6.16 (1/2H, s), 4.95 (1/2H, d, *J* = 10.2 Hz), 4.91 (1/2H, d, *J* = 10.2 Hz), 3.67-3.58 (2H, m), 3.03-2.99 (1H, m), 2.51-2.10 (11H, m), 1.73-1.69 (1H, m), 1.59-1.58 (1H, m), 1.50-1.45 (1H, m), 0.61 (3/2H, s), 0.60 (3/2H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 194.2, 194.0, 170.7, 170.5, 152.0, 142.0, 139.3, 134.8, 132.4, 129.1, 128.7, 126.4, 125.8, 120.3, 111.0, 110.6, 110.5, 110.1, 81.0, 80.9, 65.8, 65.5, 56.7, 55.5, 55.3, 46.7, 46.6, 39.2, 38.9, 37.0, 36.6, 35.8, 35.2, 31.0, 30.6, 29.2, 29.0, 26.3, 26.3, 23.9, 23.7, 12.3, 12.3. ESI MS: *m/z* 416 $(M+H)^+$. HR-ESI MS: *m/z* 416.2226, calcd for $C_{27}H_{30}NO_3$. Found: 416.2231.

4.17.

(3*S*,3a*R*,11a*S*)-9-(Azidomethyl)-3-(isoquinolin-7-yl)-3a-methyl-2,3,3a,4,5,8,9,10,11a, 11b-decahydrocyclopenta[c]xanthen-7(1*H*)-one (29)

Diphenylphosphoryl azide (45 μL) was added to a solution of **28** (66.1 mg, 0.16 mmol), diethyl azodicarboxylate (2.2 M in toluene, 94 μL, 0.21 mmol) and triphenylphosphine (54.3 mg, 0.21 mmol) in THF (1.6 mL), and the whole mixture was stirred for 40 min. MeOH was added to the mixture, and the solvent from the whole mixture was removed under reduced pressure to give a crude product. Purification by SiO_2 column ($CHCl_3$/MeOH/H_2O = 100:3:1, lower phase) to give **29** (70.1 mg, quant.) as a colorless oil.

IR (KBr): 2961, 2925, 2882, 2854, 2100, 1646, 1605, 1407, 1382, 1209, 755 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.22 (1H, s), 8.49 (1H, d, *J* = 5.8 Hz), 7.79 (1H, s), 7.76

(1H, d, *J* = 8.2 Hz), 7.63 (1H, d, *J* = 5.8 Hz), 7.56 (1H, d, *J* = 8.2 Hz), 6.17 (1/2H, s), 6.16 (1/2H, s), 4.97 (1/2H, d, *J* = 10.6 Hz), 4.93 (1/2H, d, *J* = 10.6 Hz), 3.41-3.31 (2H, m), 3.02 (1H, t, *J* = 9.4 Hz), 2.53-2.10 (11H, m), 1.75-1.71 (1H, m), 1.62-1.57 (1H, m), 1.52-1.47 (1H, m), 0.62 (3/2H, s), 0.61 (3/2H, s). ^{13}C-NMR (125 MHz, $CDCl_3$) δ: 193.0, 192.7, 169.7, 169.5, 152.2, 142.4, 139.1, 134.7, 132.2, 129.5, 129.1, 128.5, 126.3, 125.8, 120.1, 110.8, 110.7, 110.3, 110.2, 81.2, 81.1, 77.2, 77.0, 76.8, 56.7, 56.6, 55.5, 55.3, 55.2, 46.8, 46.6, 40.0, 39.7, 37.0, 36.6, 33.5, 33.0, 31.7, 31.4, 29.7, 29.3, 29.0, 26.3, 26.3, 23.9, 23.7, 22.7, 12.4, 12.3. ESI MS: *m/z* 441 $(M+H)^+$. HR-ESI MS: *m/z* 441.2291, calcd for $C_{27}H_{29}N_4O_2$. Found: 441.2292.

4.18.

***N*-(((3*S*,3a*R*,11a*S*)-3-(Isoquinolin-7-yl)-3a-methyl-7-oxo-1,2,3,3a,4,5,7,8,9,10,11a,11b-dodecahydrocyclopenta[c]xanthen-9-yl)methyl)acetamide (30)**

Triphenylphosphine (125 mg, 0.47 mmol) was added to a solution of **29** (70 mg, 0.16 mmol) in THF/H_2O (4:1, 2.0 mL), and the whole mixture was stirred for 60 h. AcOH aq. was added to the mixture, and the whole mixture was successively extracted with Et_2O and CH_2Cl_2. The aqueous phase was basicified with Sat. $NaHCO_3$ aq., and was extracted with CH_2Cl_2. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a corresponding amine (48.2 mg, 73%) as a colorless oil.

N,*N*-Diisopropylethylamine (0.04 mL, 0.22 mmol) and acetic anhydride (0.01 mL, 0.11 mmol) were added to a solution of an aliquot of the above amine (9.1 mg, 0.022 mmol) in CH_2Cl_2 (0.4 mL), and the whole mixture was stirred for 90 min. H_2O was added to

the mixture, and the whole mixture was extracted with CH_2Cl_2. Removal of the solvent from the CH_2Cl_2 extract under reduced pressure gave a crude product, which was purified by SiO_2 column ($CHCl_3$/MeOH/H_2O = 60:3:1, lower phase) to give **30** (5.6 mg, 56%) as a colorless oil.

IR (KBr): 3292, 2962, 2926, 2879, 2855, 1644, 1603, 1554, 1420, 1407, 1381, 1211, 756 cm^{-1}. ^{1}H-NMR (600 MHz, $CDCl_3$) δ: 9.25 (1H, s), 8.49 (1H, s), 7.81 (1H, s), 7.77 (1H, d, *J* = 8.7 Hz), 7.67 (1H, s), 7.58 (1H, d, *J* = 8.7 Hz), 6.15 (1/2H, s), 6.13 (1/2H, s), 5.83 (1H, d, *J* = 7.2 Hz), 4.95 (1/2H, d, *J* = 10.2 Hz), 4.91 (1/2H, d, *J* = 10.2 Hz), 3.36-3.34 (1H, m), 3.24-3.19 (1H, m), 3.02 (1H, t, *J* = 9.6 Hz), 2.50-2.10 (11H, m), 2.01 (3H, s), 1.74-1.71 (1H, m), 1.59-1.57 (1H, m), 1.49-1.46 (1H, m), 0.61 (3/2H, s), 0.60 (3/2H, s). ^{13}C-NMR (150 MHz, $CDCl_3$) δ: 193.7, 193.5, 170.5, 170.5, 170.3, 151.8, 141.6, 139.4, 134.9, 132.5, 129.3, 128.9, 126.5, 125.9, 120.4, 110.8, 110.7, 110.4, 110.1, 81.1, 81.0, 77.2, 77.0, 76.8, 56.6, 56.6, 55.5, 55.2, 46.8, 46.6, 43.7, 43.5, 40.4, 40.1, 36.9, 36.6, 33.8, 33.1, 32.1, 31.9, 29.6, 29.3, 29.2, 29.0, 26.3, 26.3, 23.9, 23.7, 23.2, 12.3, 12.3. ESI MS: *m/z* 457 $(M+H)^+$. HR-ESI MS: *m/z* 457.2491, calcd for $C_{29}H_{33}N_2O_3$. Found: 457.2506.

4.19. Cell culture

HUVECs ($5x10^5$ cells/vial) was obtained from Kurabo Inc. and grown in the HuMedia-EG2 medium with growth supplements (Kurabo Inc.). Human KB epidermoid carcinoma cells (KB3-1) was cultured in the RPMI 1640 medium supplemented with

heat-inactivated 10% fetal bovine serum (FBS) and kanamycin (50 μg/mL) in a humidified atmosphere of 5% CO_2 at 37 ºC.

4.20. Growth inhibition assay

A suspension of HUVECs in the proliferation medium (HuMedia-EG2) with growth supplements was plated into well of 96-well plate ($2x10^3$ cells/well/100 μL). After 24 h, the culture medium was removed and replaced with fresh essential minimal medium (HuMedia-EB2) with growth factor [bFGF (30 ng/mL) or VEGF (30 ng/mL)] of endothelial cells and various concentrations of testing compound. Plates were incubated for an additional 72 h in a humidified atmosphere of 5% CO_2 at 37 ºC, and cell proliferation was detected by WST-8 colorimetric reagent. KB 3-1 cells in RPMI 1640 medium ($2x10^3$ cells/well/100 μL) were also inoculated into well of 96-well plate and treated as same as in the case of HUVECs for the evaluation of anti-proliferative effect. The IC_{50} value was determined by linear interpolation from the growth inhibition curve. We selectively assessed of anti-proliferative activity [selective index (S.I.)] from the differences of IC_{50} values against HUVECs and KB 3-1 cells.

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Supplementary data

Supplementary data related to this article can be found at …

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Legends

Figure 1. Chemical structures of cortistatin A (**1**), a simplified analog (**2**), and a potent analog in this work (**30**).

Scheme 1. First-Generation synthetic method for cortistatin analog (**2**).

Scheme 2. Retrosynthetic analysis of second-generation synthesis of cortistatin analogs.

Scheme 3. Synthesis of α,β-unsaturated acetal (**16)** and plausible reaction mechanism.

Scheme 4. Reagents and conditions: (a) $PhNTf_2$, KHMDS, THF, –78°C, 93%; (b) (isoquinolin-7-yl)pinacolborane, $Pd(PPh_3)_4$, K_2CO_3, DMF, 50°C, 72%; (c) H_2, Pd-C, AcOEt, rt, quant.; (d) PPTS, acetone/H_2O, rt, 98%; (e) 1,3-cyclohexanedione, ethylenediamine, AcOEt, rt, 70%.

Scheme 5. Reagents and conditions (a) **24** or **25**, $Pd(PPh_3)_4$, K_2CO_3, DMF, 50 °C, 75 and 49% for **21a** and **21b**, respectively; (b) H_2, Pd-C, AcOEt, rt; (c) PPTS, acetone/H_2O, rt, 89% and quant. for **22a** and **22b**, respectively; (d) 1,3-cyclohexanedione, ethylenediamine, AcOEt, rt, 69 and 51% for **23a** and **23b**, respectively.

Scheme 6. Reagents and conditions (a) **26**, ethylenediamine, AcOEt, rt, 53%; (b) TBAF, AcOH, THF, rt, quant.; (c) DPPA, DEAD, PPh_3, THF, rt, quant.; (d) PPh_3, THF/H_2O, rt, 73%; (e) Ac_2O, *i*PrNEt$_2$, CH_2Cl_2, rt, 56%.