Note

Synthesis and Evaluation of Novel Cyclopropane Nucleoside as Potential Tube Formation Agents

Norikazu Sakakibara,^{*,a} Junsuke Igarashi,^b Maki Takata,^c Ryoji Konishi,^c Yoshihisa Kato,^a and Ikuko Tsukamoto^c

^aFaculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri University; 1314–1 Shido, Sanuki, Kagawa 769–2193, Japan; ^bDepartment of Cardiovascular Physiology, Faculty of Medicine, Kagawa University; 1750–1 Ikenobe, Miki, Kita, Kagawa 761–0793, Japan; and ^cDepartment of Pharmaco-Bio-Informatics, Faculty of Medicine, Kagawa University; 1750–1 Ikenobe, Miki, Kita, Kagawa 761–0793, Japan.

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Five novel nucleoside analogs with mono or bis-hydroxymethylated cyclopropane rings at the *N*⁹-position of the 2-chloroadenine moiety (2-chloro-carbocyclic oxetanocin A [COA-Cl] analog) were synthesized and evaluated using human umbilical vein endothelial cells. All the prepared compounds (2a–e) showed good to moderate activity with angiogenic potency. *cis*-2'-(Hydroxymethyl)cycloprop-1'-yl derivative (2b) at 100 μ M had greater angiogenic activity than the other compounds did, with relative tube areas of 2.71 ± 0.45 (mean $\pm$ standard deviation (S.D.)), which was superior to the potency of COA-Cl (2.30 ± 0.59).

Key words medicinal chemistry; cyclopropane nucleoside; angiogenic activity

Angiogenesis, the process by which new capillary vessels are generated from preexisting ones, plays an important role in various physiological and pathological processes including wound healing, tumor growth, and metastasis. Angiogenesis promoters play an important role in the therapy of various symptoms caused by a lack of blood flow, such as Buerger's disease or chronic arteriosclerosis obliterans. However, the clinical applications of these promoters as medicinal agents remain minimal compared with those of angiogenesis inhibitors, which are used as antitumor therapies. The lack of use of angiogenesis promoters as therapeutic agents may be owing to the limited knowledge of their effects, except for growth factors derived from living systems. For example, vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF) are macromolecular glycoproteins that are chemically and biologically unstable. Hence, they are used only in treatments administered *via* injection or gene transfection approaches.^{1–3} Therefore, we synthesized the first low-molecular-weight angiogenesis promoter, 2-chloro-carbocyclic oxetanocin A (2-Chloro-C.OXT-A; COA-Cl, **1a**, racemate, Fig. 1),^{4–8} with a molecular weight (MW) of 284 that is suitable for pharmaceutical use *via* percutaneous and transmucosal absorption. Furthermore, it is expected to have practical applications as a therapeutic agent for wound healing, Buerger's disease, or peripheral arterial obliterative disease, and could be developed as an adhesive skin patch, lotion, or oral agent. In addition to its angiogenic effect, we found that COA-Cl protects against oxygen-glucose deprivation in primary cortical neurons in a

suramin-sensitive manner.⁹ Because of its utility, COA-Cl is commercially available (chemical name: 2-Cl-C.OXT-A, Wako Pure Chemical Industries, Ltd., Osaka, Japan, order number: 032-21541, quantity: 1 mg, price: 15000 yen). Mechanistic studies revealed that COA-Cl is a novel and potent angiogenic agent that may behave as a partial agonist of the sphingosine-1-phosphate type 1 (S1P₁) receptor.^{4,10,11} The need to understand the utility and unique mechanism of action of COA-Cl **1a** has triggered intensive efforts to discover novel COA-Cl analogs with enhanced angiogenic activity and reduced cytotoxicity.^{4,6–8} However, our previous work resulted in analogs that were almost similar as or less potent than COA-Cl **1a**. For example, Fig. 1 shows the structure of COA-Cl derivative, COA-OMe (**1b**), in which the 2-chloro group at the 2-position of the purine system is substituted with a methoxy group and, the compound consequently exhibited half the vascularization activity of COA-Cl **1a**.⁶ Furthermore, substitution of an H in place of the Cl (**1c**: C.OXT-A) or replacement of a cyclobutane moiety with a pentose ring (**1d**: cladribine) showed no angiogenic potency.⁴ In a single exceptional case, a COA-Cl analog (**1e**), regioisomer of the two hydroxymethyl groups at the cyclobutane ring of **1a**, exhibited angiogenic activity that was comparable to that of COA-Cl **1a**.⁷ Collectively, these studies indicated that the structure of not only the cyclobutane ring moiety but also the 2-substituted purine skeleton in COA-Cl **1a** is essential for angiogenic activity.

As part of an ongoing effort to discover potential COA-Cl analogs with higher angiogenic activity, we studied the

Fig. 1. Structures of COA-Cl (**1a**), COA-OMe (**1b**), C.OXT-A (**1c**), COA-Cl Analog (**1d**), and Cladribine (**1e**)

* To whom correspondence should be addressed. e-mail: bara@kph.bunri-u.ac.jp

Fig. 2. Structures of COA-Cl (**1**) and Cyclopropane Nucleoside Analogs (**2a–e**)

Reagents and conditions: i, 2,6-Dichloropurine, PPh_3 , DIAD, THF, 50°C; ii, TBAF, THF, r.t.; iii, NH_3 , MeOH, 100°C.

Chart 1. Synthesis of Compounds **2a–d**

Reagents and conditions: i, Ac_2O , DMAP, r.t., 64%; ii, N,N -dimethylaniline, $POCl_3$, Et_4NCl , CH_3CN , 100°C, 37%; iii, $NaNO_2$, $BnNEt_3Cl$, $SbCl_5$, Cl_2CHCO_2H , CH_2Cl_2 , r.t., 76%; iv, NH_3 , MeOH, 100°C, 66%.

Chart 2. Synthesis of Compound **2e**

structure–activity relationship of the two hydroxymethyl groups bound to the cyclobutane ring at the 2' and 3' position, or around the functional group at the 2-position of the purine skeleton of COA-Cl. In this study, we designed five structurally relevant novel COA-Cl analogs by replacing the 2',3'-bis-hydroxymethylated cyclobutane ring of COA-Cl with a hydroxymethylated cyclopropane ring. Specifically, three novel COA-Cl derivative racemates (**2a** and **b**) and an achiral compound (**2c**) with a mono-hydroxymethylated cyclopropane ring at the N^9 -position in the 2-chloroadenine moiety, and two novel COA-Cl analog racemates (**2d**) and a chiral compound (**2e**) with a bis-hydroxymethylated cyclopropane ring at the N^9 -position in the 2-chloroadenine skeleton, were synthesized and evaluated using human umbilical vein endothelial cells

(HUVECs) as shown in Fig. 2.

Results and Discussion

Chemistry The cyclopropane nucleoside analogs **2a–d** shown in Chart 1 were prepared using known cyclopropane alcohols (**3a**,¹² **b**,¹² **c**,¹³ and **d**,¹⁴) as starting materials in two- or three-step reactions, according to a modified method from a previous report.^{6–8,15} Secondary alcohols **3a–d** were treated with 2,6-dichloropurine and underwent the Mitsunobu reaction^{15–18} in the presence of PPh_3 and diisopropyl azodicarboxylate (DIAD) to give 9-cyclopropylmethyl-2,6-dichloropurine congeners **4a–d** in 29–49% yield. Then, **4c** was subsequently treated with methanolic ammonia to obtain the desired product **2c** in 67% yield. Compound **4a** or **b** was

Fig. 3. Pictures of Stimulated Tube Formation by **1a**, **2a–e**

Additives are shown under the pictures.

Fig. 4. Tube Formation Assay Using Compounds **1a**, **2a–e**

The area of the formed tube is represented as a value relative to that in control wells with no additives. The effects of the solvent (10% saline) and positive control (VEGF; 10 ng/mL) are shown together. Results are expressed as mean ± standard error (S.E.) of 4–11 individual experiments.

treated with tetrabutylammonium fluoride (TBAF) to obtain the desilylated product **5a** or **b** respectively, and the next step involving the ammonolysis reaction in a sealed tube in the presence of methanol produced the target material **2a** or **b** in 36 or 63% yield in two steps, respectively. Because compound **4d** was treated with TBAF to obtain not only the desilylated 2,6-dichloropurine analog, but also a small amount of the desilylated 2-chloro-6-fluoropurine derivative as by-product, **4d** was first treated with methanolic ammonia, followed by desilylation with TBAF to generate **2d** in 48% yield in two steps.

The synthesis of the cyclopropane nucleoside analog **2e** is described in Chart 2. We started from (1'S,2'R)-9-[[1',2'-bis(hydroxymethyl)cycloprop-1'-yl]methyl]guanine (**6**) (A-5021), which had previously been obtained from Ajinomoto Co., Ltd., Japan in the course of cooperative research and showed potent antiherpetic activity against herpes simplex

virus and varicella zoster virus.¹⁹⁾ First, we prepared a diacetyl ester (**7**) in 64% yield, followed by chlorination at the C-6 position of the purine skeleton with tetraethylammonium chloride and phosphoryl chloride²⁰⁾ to afford the 2-amino-6-chloropurine analog (**8**) in 37% yield. The 2-amino group of the purine skeleton of the resulting compound **8** was then replaced with an amino group using SbCl₃, benzyltriethylammonium chloride, NaNO₂, and Cl₂CHCO₂H²¹⁾ to afford the 2,6-dichloropurine analog **9** in 76% yield, which was subsequently treated with methanolic ammonia to give the desired product **2e** in 66% yield.

Biological Activity The effects of the five synthesized cyclopropane nucleosides (**2a–e**) on angiogenesis were examined. We evaluated the analogs using a well-established tube formation assay.^{8,22–24)} Typical images are shown in Fig. 3. VEGF was used as a positive control. The area of

Fig. 5. Effects of Compounds **2a–e** on HUVEC Proliferation

Forty-eight hours after the addition of additives, cell viability was measured with a Cell Counting Kit-8. Cell viability is represented as a value relative to that in control wells with no additive. The effect of the solvent (10% saline) as well as the positive control (10 ng/mL VEGF) are shown together. Results were expressed as mean $\pm$ S.E. of 4–10 individual experiments.

the formed tube is presented as a value relative to that of tubes formed in wells without additives (saline). Figure 4 shows the dose-dependent tube formation responses, as well as the solvent (10% saline) and positive (VEGF, 10 ng/mL) controls. As shown in Figs. 3 and 4, angiogenic potencies were observed for each prepared compound, including the *trans*-2'-(hydroxymethyl)cycloprop-1'-yl derivative, **2a** (racemate), *cis*-2'-(hydroxymethyl)cycloprop-1'-yl derivative, **2b** (racemate), 1'-(hydroxymethyl)cycloprop-1'-yl derivative, **2c** (achiral compound), 2',2'-bis(hydroxymethyl)-cycloprop-1'-yl derivative, **2d** (racemate), and (1'*S*,2'*R*)-1',2'-bis(hydroxymethyl)cycloprop-1'-yl derivative **2e** (chiral compound, Fig. 2) at concentrations of 10 and 100 μ M. Among these compounds, **2a**, **2c**, **2d**, and **2e** exerted greater angiogenic activities (2.10 ± 0.17 , 1.82 ± 0.38 , 1.90 ± 0.07 and 2.02 ± 0.36 [mean $\pm$ standard deviation (S.D.)], respectively) at a concentration of 100 μ M. Furthermore, their effects were comparable to those of 10 ng/mL VEGF (1.81 ± 0.24 , Fig. 4). Compound **2b** showed the highest angiogenic activity at 100 μ M (2.71 ± 0.45), which was superior to the potency of COA-Cl **1a** (2.30 ± 0.59 at 100 μ M) and VEGF (1.81 ± 0.24 , Fig. 4). Moreover, as shown in Fig. 5, we performed a proliferation assay because angiogenesis is closely associated with complex cellular processes including the proliferation of endothelial cells,^{4,25,26} which showed that all the examined compounds (**2a–e**) did not stimulate the proliferation of HUVECs.

Conclusion

In conclusion, we designed five structurally relevant COA-Cl analogs, which were nucleosides with mono or bis-hydroxymethylated cyclopropane rings at the *N*⁹-position in the 2-chloroadenine moiety (**2a–e**). Furthermore, compound **2b** exhibited a greater angiogenic activity than the other compounds did at 100 μ M, which was also superior to the potency of COA-Cl (**1a**). Overall, our data may serve as the basis for further modification experiments to identify additional potent candidates that induce tube formation.

Experimental

Chemistry ¹H- and ¹³C-NMR spectra were taken with a Ultrashield™ 400 Plus FT NMR System (BRUKER). Chemical shifts and coupling constants (*J*) were given in δ and Hz, respectively. Melting points were determined on a Yanco MP-500D. High-resolution (HR)-MS was performed on a APEX IV mass spectrometer (BRUKER) with electrospray ionization mass spectroscopy (ESI-MS).

General Procedure for the Synthesis of 4a–d A solution of compounds **3a–d** (0.80 mmol), triphenylphosphine (220.3 mg, 0.84 mmol), 2,6-dichloropurine (158.8 mg, 0.84 mmol) and DIAD (165.6 μ L, 0.84 mmol) in tetrahydrofuran (THF) (6.7 mL) was stirred at 50°C. After 2 d stirring, the residual solution was purified by silica gel column chromatography (60% AcOEt in hexane) to give **4a–d**.

2,6-Dichloro-9-[[*trans*-2'-(*tert*-butyldimethylsilyloxymethyl)cycloprop-1'-yl]methyl]purine (4a) Yield 44%; colorless oil; ¹H-NMR (400 MHz, CDCl₃) δ : 8.44 (1H, s, H-8), 4.35 (1H, dd, *J*=14.4, 6.0 Hz, N-CHH-Cl'), 3.90 (1H, dd, *J*=14.4, 8.4 Hz, N-CHH-Cl'), 3.82 (1H, dd, *J*=10.8, 4.8 Hz, C2'-CHH-OTBS), 3.26 (1H, dd, *J*=10.8, 7.2 Hz, C2'-CHH-OTBS), 1.16–1.30 (2H, m, H-1' and H-2'), 0.84 (9 H, s, *tert*-butyldimethylsilyl (TBS)), 0.64–0.72 (2H, m, H-3'), 0.01 (3 H, s, TBS), 0.01 (3 H, s, TBS); ¹³C-NMR (100 MHz, CDCl₃) δ : 153.2, 152.8, 151.7, 145.5, 130.9, 65.1, 48.5, 25.9, 22.0, 18.3, 15.8, 8.7, −5.3; HR-MS (ESI) Calcd for C₁₆H₂₄Cl₂N₄NaOSi [M+Na]⁺: 409.09886. Found 409.09904.

2,6-Dichloro-9-[[*cis*-2'-(*tert*-butyldimethylsilyloxymethyl)cycloprop-1'-yl]methyl]purine (4b) Yield 29%; colorless oil; ¹H-NMR (400 MHz, CDCl₃) δ : 8.47 (1H, s, H-8), 4.52 (1H, dd, *J*=14.4, 6.0 Hz, N-CHH-Cl'), 4.13 (1H, dd, *J*=14.4, 8.8 Hz, N-CHH-Cl'), 4.10 (1H, dd, *J*=11.6, 4.4 Hz, C2'-CHH-OTBS), 3.52 (1H, dd, *J*=11.6, 8.8 Hz, C2'-CHH-OTBS), 1.29–1.47 (2H, m, H-1' and H-2'), 0.90 (9 H, s, TBS), 0.86–0.91 (1H, m, H-3'), 0.39–0.44 (1H, m, H-3'), 0.07 (3 H, s, TBS), 0.06 (3 H, s, TBS); ¹³C-NMR (100 MHz, CDCl₃) δ : 153.3, 152.7, 151.5, 146.5, 130.8, 62.0, 43.8, 26.0, 18.7, 18.4, 15.5, 7.3, −5.2, −5.4; HR-MS (ESI) Calcd for C₁₆H₂₄Cl₂N₄NaOSi [M+Na]⁺:

409.09886. Found 409.09897.

2,6-Dichloro-9-[[1'-(benzoyloxymethyl)cycloprop-1'-yl]methyl]purine (4c) Yield 49%; colorless oil; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 8.30 (1H, s, H-8), 7.83–7.88 (2H, m, Bz), 7.55–7.61 (1H, m, Bz), 7.40–7.47 (2H, m, Bz), 4.32 (2H, s, $\text{N-CH}_2\text{-Cl}'$), 4.15 (2H, s, $\text{C}2'\text{-CH}_2\text{-OBz}$), 0.99–1.06 (2H, m, H-2' and H-3'), 0.85–0.92 (2H, m, H-2' and H-3'); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 166.2, 153.6, 153.1, 151.9, 145.8, 133.4, 130.6, 129.5, 129.4, 128.5, 68.5, 49.6, 20.9, 14.2, 11.0; HR-MS (ESI) Calcd for $\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{N}_4\text{NaO}_2$ $[\text{M}+\text{Na}]^+$: 399.03860. Found 399.03859.

2,6-Dichloro-9-[[2',2'-bis(*tert*-butyldimethylsilyloxymethyl)cycloprop-1'-yl]methyl]purine (4d) Yield 38%; white solid; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 8.47 (1H, s, H-8), 4.54 (1H, dd, $J=14.4$, 6.4 Hz, $\text{N-CHH-Cl}'$), 4.16 (1H, dd, $J=14.4$, 9.2 Hz, $\text{N-CHH-Cl}'$), 4.04 (1H, d, $J=11.2$ Hz, $\text{C}2'\text{-CHH-OTBS}$), 3.66 (1H, d, $J=10.0$ Hz, $\text{C}2'\text{-CHH-OTBS}$), 3.60 (1H, d, $J=11.2$ Hz, $\text{C}2'\text{-CHH-OTBS}$), 3.41 (1H, d, $J=10.0$ Hz, $\text{C}2'\text{-CHH-OTBS}$), 1.31–1.40 (1H, m, H-1'), 0.88 (9 H, s, TBS), 0.85 (9 H, s, TBS), 0.80–0.85 (1H, m, H-3'), 0.57–0.62 (1H, m, H-3'), 0.06 (3 H, s, TBS), 0.04 (3 H, s, TBS), 0.01 (3 H, s, TBS), 0.00 (3 H, s, TBS); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 153.2, 152.7, 151.5, 146.2, 130.8, 66.0, 62.4, 44.0, 29.4, 25.8, 25.6, 18.3, 12.6, -0.02 ; HR-MS (ESI) Calcd for $\text{C}_{23}\text{H}_{40}\text{Cl}_2\text{N}_4\text{NaO}_2\text{Si}_2$ $[\text{M}+\text{Na}]^+$: 553.19591. Found 553.19602; mp: 67.0–68.2°C.

General Procedure for the Synthesis of 5a–b and 2d Compounds **4a–b** and **5d** (0.32 mmol) were dissolved in THF (2.0 mL), and 1.0 M tetrabutylammonium fluoride–THF solution (0.6 mL) was added to the solution, and then stirred for 20 min at room temperature. The mixture was evaporated, and was purified by silica gel column chromatography (10% MeOH in AcOEt) to give **5a–b** and **2d**.

General Procedure for the Synthesis of 5d and 2a–c Compounds **4c–d** and **5a–b** (0.12 mmol) were dissolved in NH_3 (14.0 mL)/MeOH (3.0 mL), and then sealed and stirred for 1–3 d at 100°C. The mixture was evaporated, and the residue was purified by silica gel column chromatography (20–25% MeOH in CH_2Cl_2) to afford **5d** and **2a–c**.

2,6-Dichloro-9-[[*trans*-2'-(hydroxymethyl)cycloprop-1'-yl]methyl]purine (5a) Yield 60%; colorless oil; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 8.58 (1H, s, H-8), 4.17 (1H, dd, $J=14.0$, 6.8 Hz, $\text{N-CHH-Cl}'$), 4.05 (1H, dd, $J=14.0$, 8.0 Hz, $\text{N-CHH-Cl}'$), 3.48 (1H, dd, $J=11.2$, 5.6 Hz, $\text{C}2'\text{-CHH-OH}$), 3.13 (1H, dd, $J=11.2$, 7.2 Hz, $\text{C}2'\text{-CHH-OH}$), 1.12–1.24 (2H, m, H-1' and H-2'), 0.61–0.69 (1H, m, H-3'), 0.48–0.56 (1H, m, H-3'); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 154.8, 153.7, 151.7, 148.8, 131.6, 73.6, 65.6, 21.7, 17.6, 9.6; HR-MS (ESI) Calcd for $\text{C}_{10}\text{H}_{10}\text{Cl}_2\text{N}_4\text{NaO}$ $[\text{M}+\text{Na}]^+$: 295.01239. Found 295.01260.

2,6-Dichloro-9-[[*cis*-2'-(hydroxymethyl)cycloprop-1'-yl]methyl]purine (5b) Yield 73%; colorless oil; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 8.69 (1H, s, H-8), 4.37 (2H, d, $J=7.6$ Hz, $\text{N-CH}_2\text{-Cl}'$), 3.93 (1H, dd, $J=11.6$, 5.6 Hz, $\text{C}2'\text{-CHH-OH}$), 3.48 (1H, dd, $J=11.6$, 8.8 Hz, $\text{C}2'\text{-CHH-OH}$), 1.49–1.61 (1H, m, H-1'), 1.28–1.39 (1H, m, H-2'), 0.83–0.92 (1H, m, H-3'), 0.41–0.48 (1H, m, H-3'); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 154.8, 153.6, 151.6, 149.2, 131.5, 62.2, 45.5, 19.7, 16.5, 8.9; HR-MS (ESI) Calcd for $\text{C}_{10}\text{H}_{10}\text{Cl}_2\text{N}_4\text{NaO}$ $[\text{M}+\text{Na}]^+$: 295.01239. Found 295.01244.

6-Amino-2-chloro-9-[[2',2'-bis(*tert*-butyldimethylsilyloxymethyl)cycloprop-1'-yl]methyl]purine (5d) Yield 71%;

white solid; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ : 8.15 (1H, s, H-8), 6.31 (2H, brs, NH_2), 4.43 (1H, dd, $J=14.4$, 6.0 Hz, $\text{N-CHH-Cl}'$), 4.05 (1H, dd, $J=14.4$, 8.8 Hz, $\text{N-CHH-Cl}'$), 3.97 (1H, d, $J=11.2$ Hz, $\text{C}2'\text{-CHH-OTBS}$), 3.66 (1H, d, $J=10.4$ Hz, $\text{C}2'\text{-CHH-OTBS}$), 3.60 (1H, d, $J=11.2$ Hz, $\text{C}2'\text{-CHH-OTBS}$), 3.41 (1H, d, $J=10.4$ Hz, $\text{C}2'\text{-CHH-OTBS}$), 1.27–1.36 (1H, m, H-1'), 0.90 (9 H, s, TBS), 0.86 (9 H, s, TBS), 0.74–0.82 (1H, m, H-3'), 0.52–0.58 (1H, m, H-3'), 0.05 (3 H, s, TBS), 0.03 (3 H, s, TBS), 0.01 (3 H, s, TBS), 0.01 (3 H, s, TBS); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ : 156.1, 153.9, 151.2, 141.1, 118.2, 66.2, 62.3, 43.4, 29.2, 28.8, 25.9, 18.3, 12.7, -5.3 ; HR-MS (ESI) Calcd for $\text{C}_{23}\text{H}_{42}\text{ClN}_5\text{NaO}_2\text{Si}_2$ $[\text{M}+\text{Na}]^+$: 534.24578. Found 534.24643; mp: 120.9–121.7°C.

2-Chloro-9-[[*trans*-2'-(hydroxymethyl)cycloprop-1'-yl]methyl]adenine (2a) Yield 60%; white solid; $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ : 8.09 (1H, s, H-8), 4.00 (1H, dd, $J=10.4$, 3.2 Hz, $\text{N-CHH-Cl}'$), 3.95 (1H, dd, $J=10.4$, 3.2 Hz, $\text{N-CHH-Cl}'$), 3.41 (1H, dd, $J=11.6$, 6.0 Hz, $\text{C}2'\text{-CHH-OH}$), 3.19 (1H, dd, $J=11.6$, 6.8 Hz, $\text{C}2'\text{-CHH-OH}$), 1.06–1.20 (2H, m, H-1' and H-2'), 0.57–0.64 (1H, m, H-3'), 0.45–0.52 (1H, m, H-3'); $^{13}\text{C-NMR}$ (400 MHz, CD_3OD) δ : 158.0, 155.2, 152.1, 142.7, 118.8, 65.7, 30.7, 21.5, 17.8, 9.5; HR-MS (ESI) Calcd for $\text{C}_{10}\text{H}_{12}\text{ClN}_5\text{NaO}$ $[\text{M}+\text{Na}]^+$: 276.06226. Found 276.06240; mp: 174.2–175.8°C.

2-Chloro-9-[[*cis*-2'-(hydroxymethyl)cycloprop-1'-yl]methyl]adenine (2b) Yield 87%; white solid; $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ : 8.12 (1H, s, H-8), 4.14 (1H, d, $J=2.0$ Hz, $\text{N-CH}_2\text{-Cl}'$), 4.12 (1H, d, $J=2.4$ Hz, $\text{N-CH}_2\text{-Cl}'$), 3.81 (1H, dd, $J=11.6$, 5.6 Hz, $\text{C}2'\text{-CHH-OH}$), 3.43 (1H, dd, $J=11.6$, 8.8 Hz, $\text{C}2'\text{-CHH-OH}$), 1.30–1.41 (1H, m, H-1'), 1.18–1.28 (1H, m, H-2'), 0.71–0.80 (1H, m, H-3'), 0.27–0.34 (1H, m, H-3'); $^{13}\text{C-NMR}$ (400 MHz, CD_3OD) δ : 158.0, 155.1, 152.0, 143.1, 118.8, 62.2, 44.5, 19.8, 17.0, 8.6; HR-MS (ESI) Calcd for $\text{C}_{10}\text{H}_{12}\text{ClN}_5\text{NaO}$ $[\text{M}+\text{Na}]^+$: 276.06226. Found 276.06226; mp: 197.0–198.2°C.

2-Chloro-9-[[1'-(hydroxymethyl)cycloprop-1'-yl]methyl]adenine (2c) Yield 67%; brownish solid; $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ : 8.13 (1H, s, H-8), 4.23 (2H, s, $\text{N-CH}_2\text{-Cl}'$), 3.37 (2H, s, $\text{C}2'\text{-CH}_2\text{-OH}$), 0.80–0.85 (2H, m, H-2' and H-3'), 0.57–0.61 (2H, m, H-2' and H-3'); $^{13}\text{C-NMR}$ (400 MHz, CD_3OD) δ : 158.1, 155.3, 152.5, 143.4, 118.7, 66.6, 49.9, 23.9, 10.1; HR-MS (ESI) Calcd for $\text{C}_{10}\text{H}_{12}\text{ClN}_5\text{NaO}$ $[\text{M}+\text{Na}]^+$: 276.06226. Found 276.06225; mp: 193.8–194.3°C.

2-Chloro-9-[[2',2'-bis(hydroxymethyl)cycloprop-1'-yl]methyl]adenine (2d) Yield 68%; white solid; $^1\text{H-NMR}$ (400 MHz, CD_3OD) δ : 8.14 (1H, s, H-8), 4.19 (1H, d, $J=2.0$ Hz, $\text{N-CHH-Cl}'$), 4.17 (1H, d, $J=2.0$ Hz, $\text{N-CHH-Cl}'$), 3.90 (1H, d, $J=11.6$ Hz, $\text{C}2'\text{-CHH-OH}$), 3.50 (1H, d, $J=11.6$ Hz, $\text{C}2'\text{-CHH-OH}$), 3.51 (1H, d, $J=11.2$ Hz, $\text{C}2'\text{-CHH-OH}$), 3.33 (1H, d, $J=11.2$ Hz, $\text{C}2'\text{-CHH-OH}$), 1.25–1.35 (1H, m, H-1'), 0.66–0.72 (1H, m, H-3'), 0.48–0.54 (1H, m, H-3'); $^{13}\text{C-NMR}$ (400 MHz, CD_3OD) δ : 158.0, 155.1, 152.0, 143.0, 118.9, 67.1, 62.6, 44.7, 30.6, 22.2, 13.7; HR-MS (ESI) Calcd for $\text{C}_{11}\text{H}_{14}\text{ClN}_5\text{NaO}_2$ $[\text{M}+\text{Na}]^+$: 306.07282. Found 306.07277; mp: 169.8–171.7°C.

(1'S,2'R)-9-[[1',2'-Bis(acetoxymethyl)cycloprop-1'-yl]methyl]guanine (7) A solution of compound **6** (1037.2 mg, 3.91 mmol) and *N,N*-dimethyl-4-aminopyridine (DMAP) (200.0 mg, 1.63 mmol) in Ac_2O (20 mL) was stirred for 12 h at room temperature. The mixture was then evaporated *in vacuo* and the residue was recrystallized with MeOH to give known

compound **7** (873.7 mg, 2.50 mmol, 64%). ¹H-NMR (400 MHz, dimethyl sulfoxide (DMSO)-*d*₆) δ : 10.56 (1H, brs, NH), 7.68 (1H, s, H-8), 6.34 (1H, brs, NH₂), 4.17 (1H, dd, *J*=12.0, 6.4 Hz, C2'-CHH-OAc), 3.94 (1H, dd, *J*=12.0, 9.2 Hz, C2'-CHH-OAc), 3.82–3.95 (4H, m, C1'-CH₂-OAc and N-CH₂-C1'), 1.56–1.65 (1H, m, H-2'), 1.95 (3H, s, Ac), 1.93 (3H, s, Ac), 1.09–1.15 (1H, m, H-3'), 0.68–0.74 (1H, m, H-3'); ¹³C-NMR (400 MHz, DMSO-*d*₆) δ : 170.2, 170.1, 156.8, 153.4, 151.5, 137.4, 116.2, 63.9, 63.2, 47.4, 24.5, 21.3, 20.6, 20.5, 14.0.

(1'S,2'R)-2-Amino-6-chloro-9-[[1',2'-bis(acetoxymethyl)-cycloprop-1'-yl]methyl]purine (8) Compound **7** (689.0 mg, 1.97 mmol), *N,N*-dimethylaniline (0.26 mL, 2.07 mmol), tetraethylammonium chloride (668.8 mg, 3.96 mmol) and phosphoryl chloride (0.72 mL, 4.71 mmol) in CH₃CN (7.0 mL) was stirred at 120°C. After 10 min stirring, the residual solution was extracted with AcOEt. The organic phase was washed 3 times with 5% NaHCO₃ solution and saturated sodium chloride solution, dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (AcOEt) to give a colorless oil **8** (267.6 mg, 0.72 mmol, 37%). ¹H-NMR (400 MHz, CDCl₃) δ : 7.88 (1H, s, H-8), 5.23 (1H, brs, NH₂), 4.34 (1H, dd, *J*=12.4, 6.4 Hz, C2'-CHH-OAc), 4.21 (1H, d, *J*=14.8 Hz, N-CHH-C1'), 4.17 (1H, d, *J*=12.8 Hz, C1'-CHH-OAc), 3.96 (1H, d, *J*=12.8 Hz, C1'-CHH-OAc), 3.92 (1H, d, *J*=14.8 Hz, N-CHH-C1'), 3.88 (1H, dd, *J*=12.4, 8.8 Hz, C2'-CHH-OAc), 1.99 (3H, s, Ac), 1.97 (3H, s, Ac), 1.72–1.83 (1H, m, H-2'), 1.12–1.18 (1H, m, H-3'), 0.70–0.76 (1H, m, H-3'); ¹³C-NMR (400 MHz, CDCl₃) δ : 170.8, 170.6, 159.2, 154.2, 151.3, 142.5, 125.1, 64.3, 63.6, 49.6, 24.5, 22.7, 20.8, 20.7, 15.1; HR-MS (ESI) Calcd for C₁₅H₁₈ClN₅NaO₄ [M+Na]⁺: 390.09395. Found 390.09409.

(1'S,2'R)-2,6-Dichloro-9-[[1',2'-bis(acetoxymethyl)-cycloprop-1'-yl]methyl]purine (9) SbCl₃ (312.1 mg 1.37 mmol) in CH₂Cl₂ (5.0 mL) was added to a mixture of compound **8** (251.5 mg, 0.68 mmol), benzyltriethylammonium chloride (155.3 mg 0.68 mmol), and NaNO₂ (0.94 g, 13.7 mmol) in CH₂Cl₂ (25 mL). Cl₂CHCO₂H (111.3 μ L, 2.74 mmol) was added, and the flask was flushed with dried N₂ and sealed, and the mixture was stirred at room temperature. The reaction was completed after 18 h, and celite (3 g) and CHCl₃ were added, and filtered. The filtrate was then extracted with CHCl₃, and the organic extracts were washed with saturated aqueous sodium chloride solution, and dried with sodium sulfate, and then evaporated. The residue was purified by silica gel column chromatography (AcOEt) to give a white solid **9** (200.8 mg, 0.52 mmol, 76%). ¹H-NMR (400 MHz, CDCl₃) δ : 8.29 (1H, s, H-8), 4.37 (1H, dd, *J*=12.4, 6.4 Hz, C2'-CHH-OAc), 4.34 (1H, d, *J*=14.4 Hz, N-CHH-C1'), 4.16 (1H, d, *J*=14.4 Hz, N-CHH-C1'), 4.12 (1H, d, *J*=12.8 Hz, C1'-CHH-OAc), 3.93 (1H, d, *J*=12.8 Hz, C1'-CHH-OAc), 3.88 (1H, dd, *J*=12.4, 8.8 Hz, C2'-CHH-OAc), 2.01 (3H, s, Ac), 2.00 (3H, s, Ac), 1.69–1.78 (1H, m, H-2'), 1.23–1.29 (1H, m, H-3'), 0.76–0.81 (1H, m, H-3'); ¹³C-NMR (400 MHz, CDCl₃) δ : 170.6, 170.5, 153.5, 153.2, 151.9, 145.8, 130.6, 63.8, 63.4, 53.4, 50.2, 24.7, 22.6, 20.8, 20.7, 15.3; HR-MS (ESI) Calcd for C₁₅H₁₆Cl₂N₄NaO₄ [M+Na]⁺: 409.04408. Found 409.04425; mp: 76.9–77.3°C.

(1'S,2'R)-2-Chloro-9-[[1',2'-bis(hydroxymethyl)-cycloprop-1'-yl]methyl]adenine (2e) Compound **9** (176.3 mg, 0.46 mmol) was dissolved in NH₃ (14.0 mL)/MeOH (9.0 mL), and then sealed and stirred for 15 h at 100°C. The mixture was evaporated, and the residue was recrystallized with

MeOH to give a white solid **2e** (85.2 mg, 0.21 mmol, 66%). ¹H-NMR (400 MHz, DMSO-*d*₆) δ : 8.18 (1H, s, H-8), 7.72 (1H, brs, NH₂), 4.61–4.68 (1H, m, OH), 4.54–4.61 (1H, m, OH), 4.12 (1H, d, *J*=14.4 Hz, N-CHH-C1'), 4.00 (1H, d, *J*=14.4 Hz, N-CHH-C1'), 3.55–3.66 (1H, m, C2'-CHH-OH), 3.38–3.46 (1H, m, C2'-CHH-OH), 3.25–3.38 (1H, m, C1'-CH₂-OH), 1.27–1.38 (1H, m, H-2'), 0.88–0.96 (1H, m, H-3'), 0.40–0.48 (1H, m, H-3'); ¹³C-NMR (400 MHz, DMSO-*d*₆) δ : 156.7, 152.8, 150.9, 141.5, 117.5, 60.7, 60.6, 48.3, 26.5, 24.6, 14.1; HR-MS (ESI) Calcd for C₁₁H₁₄ClN₅NaO₂ [M+Na]⁺: 306.07282. Found 306.06956; mp: 203.2–204.8°C; enantiomeric purity: 100% enantiomeric excess (ee) determined from HPLC analysis (CHIRALPAC IA column, 100% MeOH, 6.0 mL/min, 254 nm) Retention time: *t*=11.8 min.

Materials^{22–24)} HUVECs, normal human dermal fibroblasts (NHDF), endothelial cell growth medium (EGM-2), endothelial cell basal medium (EBM-2) and fibroblast growth medium (FGM-2) were purchased from Lonza Japan (Tokyo, Japan). VEGF-A was from Kurabo Co. (Osaka, Japan). Cell Counting Kit 8 was supplied by Dojindo Molecular Technologies (Kumamoto, Japan). Anti-CD31 monoclonal antibody Human, Mouse from Dako Japan (Tokyo, Japan) and anti-Mouse immunoglobulin G (IgG) antibody conjugated with Alexa Fluor 488, Gout, from Life Technologies (Carlsbad, CA, U.S.A.) were used for the immuno staining.

Cell Culture HUVEC and NHDF were cultured in EGM-2 and FGM-2, respectively.

Tube Formation Assay^{22–24)} HUVEC and NHDF were trypsinized and suspended separately, and were then mixed at 20000 of HUVEC and 140000 of NHDF in 500 μ L of culture medium and seeded on gelatin-coated 24-well plates. After seeding, the plates were incubated for 24 h to permit anchorage, and then, the medium was changed with the assay medium, consisting of 450 μ L of EBM-2 with 2% heat-inactivated fetal bovine serum (FBS) and 50 μ L of saline containing additives **2a–e**. The contents of EBM-2 are almost identical to those of EGM-2, except for the fact that the former does not include FBS, growth factors, or antibiotics. After 96 h, HUVEC was stained with first antibody against CD31 and second antibody conjugated with Alexa fluor 488. The area of the formed tube was measured by the ImageJ program. Two pictures from each well were provided for the estimation. VEGF (10 ng/mL) was used as a positive control.

Proliferation Assay^{4,25,26)} HUVECs were seeded on gelatin-coated 96-well plates, typically at 3000 cells/well in 100 μ L of EGM-2. After seeding, the plates were incubated for 24 h to permit anchorage, and then, the culture medium was changed with the assay medium, consisting of 90 μ L of EBM-2 with 2% heat-inactivated FBS and 10 μ L of saline containing additives. The proliferation assay was performed using a Cell Counting Kit-8 48 h after the addition of compounds **2a–e**.

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Conflict of Interest The authors declare no conflict of interest.

References

- 1) Bobek V., Taltynov O., Pinterova D., Kolostova K., *Vascul. Pharmacol.*, **44**, 395–405 (2006).
- 2) Collinson D. J., Donnelly R., *Eur. J. Vasc. Endovasc. Surg.*, **28**, 9–23 (2004).
- 3) O'Toole G., MacKenzie D., Buckley M. F., Lindeman R., Poole M., *Br. J. Plast. Surg.*, **54**, 1–7 (2001).
- 4) Tsukamoto I., Sakakibara N., Maruyama T., Igarashi J., Kosaka H., Kubota Y., Tokuda M., Ashino H., Hattori K., Tanaka S., Kawata M., Konishi R., *Biochem. Biophys. Res. Commun.*, **399**, 699–704 (2010).
- 5) Tsukamoto I., Konishi R., Tokuda M., Kubota Y., Maruyama T., Kosaka H., Igarashi J., PCT Int. Appl., WO 2010061931 A1 20100603 (2010).
- 6) Sakakibara N., Tsukamoto I., Tsurura T., Takata M., Konishi R., Maruyama T., *Heterocycles*, **85**, 1105–1116 (2012).
- 7) Sakakibara N., Igarashi J., Takata M., Demizu Y., Misawa T., Kurihara M., Konishi R., Kato Y., Maruyama T., Tsukamoto I., *Chem. Pharm. Bull.*, **63**, 701–709 (2015).
- 8) Sakakibara N., Igarashi J., Takata M., Konishi R., Suzue N., Kato Y., Maruyama T., Tsukamoto I., *Heterocycles*, **91**, 1823–1832 (2015).
- 9) Okabe N., Nakamura E., Himi N., Narita K., Tsukamoto I., Maruyama T., Sakakibara N., Nakamura T., Itano T., Miyamoto O., *Brain Res.*, **1506**, 115–131 (2013).
- 10) Igarashi J., Hashimoto T., Kubota Y., Shoji K., Maruyama T., Sakakibara N., Takuwa Y., Ujihara Y., Katanosaka Y., Mohri S., Naruse K., Yamashita T., Okamoto R., Hirano K., Kosaka H., Takata M., Konishi R., Tsukamoto I., *Pharmacology Research & Perspectives*, **2**, e00068 (2014).
- 11) Igarashi J., Okamoto R., Yamashita T., Hashimoto T., Karita S., Nakai K., Kubota Y., Takata M., Yamaguchi F., Tokuda M., Sakakibara N., Tsukamoto I., Konishi R., Hirano K., *Physiological reports*, **4**, e12742 (2016).
- 12) Osler J. D., Unsworth W. P., Taylor R. J. K., *Org. Biomol. Chem.*, **11**, 7587–7594 (2013).
- 13) Wakita N., Hara S., *Tetrahedron*, **66**, 7939–7945 (2010).
- 14) Kim H. S., Barak D., Harden T. K., Boyer J. L., Jacobson K. A., *J. Med. Chem.*, **44**, 3092–3108 (2001).
- 15) Bisacchi G. S., Braitman A., Ciani C. W., Clark J. M., Field A. K., Hagen M. E., Hockstein D. R., Malley M. F., Mitt T., Slusarchyk W. A., Sundeen J. E., Terry B. J., Tuomari A. V., Weaver E. R., Young M. G., Zahler R., *J. Med. Chem.*, **34**, 1415–1421 (1991).
- 16) Sakakibara N., Hamasaki T., Baba M., Demizu Y., Kurihara M., Irie K., Iwai M., Asada E., Kato Y., Maruyama T., *Bioorg. Med. Chem.*, **21**, 5900–5906 (2013).
- 17) Sakakibara N., Baba M., Okamoto M., Toyama M., Demizu Y., Misawa T., Kurihara M., Irie K., Kato Y., Maruyama T., *Antivir. Chem. Chemother.*, **24**, 3–18 (2015).
- 18) Sakakibara N., Balboni G., Congiu C., Onnis V., Demizu Y., Misawa T., Kurihara M., Kato Y., Maruyama T., Toyama M., Okamoto M., Baba M., *Antivir. Chem. Chemother.*, **24**, 62–71 (2015).
- 19) Sekiyama T., Hatsuya S., Tanaka Y., Uchiyama M., Ono N., Iwayama S., Oikawa M., Suzuki K., Okunishi M., Tsuji T., *J. Med. Chem.*, **41**, 1284–1298 (1998).
- 20) Nair V., Young D., DeSilvia R. Jr., *J. Org. Chem.*, **52**, 1344–1347 (1987).
- 21) Janeba Z., Francom P., Robins M. J., *J. Org. Chem.*, **68**, 989–992 (2003).
- 22) Sekiya S., Shimizu T., Yamato M., Kikuchi A., Okano T., *Biochem. Biophys. Res. Commun.*, **341**, 573–582 (2006).
- 23) Asakawa N., Shimizu T., Tsuda Y., Sekiya S., Sasagawa T., Yamato M., Fukai F., Okano T., *Biomaterials*, **31**, 3903–3909 (2010).
- 24) Sasagawa T., Shimizu T., Yamato M., Okano T., *Biomaterials*, **35**, 206–213 (2014).
- 25) Yonesu K., Kawase Y., Inoue T., Takagi N., Tsuchida J., Takuwa Y., Kumakura S., Nara F., *Biochem. Pharmacol.*, **77**, 1011–1020 (2009).
- 26) Wong M. L. H., Prawira A., Kaye A. H., Hovens C. M., *J. Clin. Neurosci.*, **16**, 1119–1130 (2009).