Regular Article

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Discoveries and Syntheses of Highly Potent Antimalarial Troponoids

Ryo Saito,^a Goh Sennari,^a Asuka Nakajima,^a Aoi Kimishima,^{a,b} Masato Iwatsuki,^{a,b}
 Aki Ishiyama,^{a,b} Rei Hokari,^b Tomoyasu Hirose,^{a,b} and Toshiaki Sunazuka^{*a,b}

^aGraduate School of Infection Control Sciences, Kitasato University; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan; and ^bOmura Satoshi Memorial Institute, Kitasato University; 5–9–1 Shirokane, Minato-ku, Tokyo 108–8641, Japan.
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Novel derivatives of puberulic acid were synthesized and their antimalarial properties were evaluated *in vitro* against the *Plasmodium falciparum* K1 parasite strain, cytotoxicity against a human diploid embryonic cell line MRC-5, and *in vivo* efficacy using a *Plasmodium berghei*-infected mouse model. From previous information that three hydroxy groups on the troponone framework were essential for antimalarial activity, we converted the carboxylic acid moiety into the corresponding esters, amides, and ketones. These derivatives showed antimalarial activity against chloroquine-resistant *Plasmodium in vitro* equivalent to puberulic acid. We identified that the pentane-3-yl ester, cyclohexyl ester, *iso*-butyl ketone, cyclohexyl methyl ketone all show an especially potent antiparasitic effect *in vivo* at an oral dose of 15 mg/kg without any apparent toxicity. These esters were more effective than the existing commonly used antimalarial drug, artesunate.

Key words puberulic acid; viticolin; tropolone; troponone; structure–activity relationship; natural product

Introduction

Malaria is a life-threatening disease caused by parasites that are transmitted to people through the bites of infected *Anopheles* mosquitoes. According to WHO¹⁾ in 2019, there were 229 million cases of malaria in 87 malaria endemic countries and the disease claimed 409000 lives. To combat malaria, the WHO recommends a combination therapy whereby two or more drugs, each with different modes of action, are used, predominantly an artemisinin-based combination therapy (ACT).²⁾ Although this provides adequate cure rate as well as delaying the development of drug resistance, challenges to eliminate and eradicate malaria, one of which is persistently emerging drug-resistant parasites, still remain. In sub-Saharan Africa where health systems are relatively inadequate, understaffed and under-resourced and conditions of poverty persist, drug-resistant parasites are known to evolve quickly.¹⁾ Therefore, therapeutic medications possessing new modes of action, which are appropriately affordable, and easily and safely used in vulnerable population, are in high and urgent demand. In addition, oral drugs are preferable to parenteral drugs in terms of administration³⁾ in that situation.

Through our screening program, chemical synthesis and biological evaluation, we have identified that highly-oxygen-

ated troponoids show promising antimalarial activity *in vitro* against *Plasmodium falciparum* K1 (chloroquine-resistant) (PfK1) Plasmodium (Fig. 1). An initial discovery that the natural products, puberulic acid (**1**),^{4,5)} viticolin A (**2**) and B (**3**)⁶⁾ show antimalarial properties *in vitro*⁷⁾ encouraged us to pursue structure–activity relationship (SAR) studies of this class of compounds. Total synthesis of **1**⁸⁾ and derivatizations, utilizing the synthetic route, revealed preliminary results of the SAR.⁹⁾ It is suggested that the carboxylic acid moiety at the C4 position of **1** could be convertible, while four contiguous oxygen atoms, including three hydroxy groups on the aromatic ring, might be better untouched. Overcoming the previously revealed toxicity issues as well as improving efficacy through oral administration (*p.o.*), we sought to derivatize the C4 position, to provide more detailed SAR information. Herein, we report synthesis of novel derivatives bearing a carbonyl group at C4 and their biological evaluation both *in vitro* and *in vivo*, which discovered first orally-active antimalarial troponoids.

Results and Discussion

Syntheses of Esters and Amides For large scale supply of **1**, because of low metabolite productivity through fungal cultivation, we prepared it by chemical synthesis. Ester deriva-

Fig. 1. Selected Antimalarial Troponoids and Their Activities

* To whom correspondence should be addressed. e-mail: sunazuka@lisci.kitasato-u.ac.jp

Chart 1. Syntheses of Ester and Amide Derivatives

tives (**6a–6d**) were synthesized by acidic alcoholysis of **1** with MeOH, EtOH, *i*-PrOH, or *n*-PrOH in good yield (Chart 1(a)). Our failures to convert **1** directly to the corresponding *t*-Bu ester and amides necessitated a novel approach (Chart 1(b)). As reported in our previous work,^{8,9)} there is a challenge that compounds possessing an α -hydroxy carbonyl on the aromatic ring cannot be monitored in a reaction mixture and purified using silica gel due to the ease of chelation with metals. Therefore, hydroxy groups on the aromatic ring were protected by Bn groups to make manipulation easy. Treatment of the Me ester **6a** with BnBr, followed by saponification afforded the protected carboxylic acid **7** along with its constitutional isomer **7'**, which were readily separable by column chromatography. Esterification of the major isomer **7** using *iso*-urea reagent **8**¹⁰⁾ afforded the desired *t*-Bu ester **9**. After hydrogenolysis of the Bn groups using Pd/C, we initially struggled to purify the desired tropolone **9** due to the aforementioned chelation problem. After an extensive survey of catalysts, it was found that the membrane-support Pd catalyst¹¹⁾ effected global cleavage of the Bn groups to provide **10** as a pure material after a concise working-up operation. Similarly, condensation of **7** with secondary amines, and deprotection using the same Pd catalyst gave the desired amides **12a** and **12b**, respectively. Although, we were able to effect condensation with primary amines, these amides decomposed under hydrogenolysis conditions. It is generally known that the N–H bond of aromatic amides serve as a directing group for *ortho*-selective C–H functionalization,¹²⁾ and we assumed this property cause side

reaction observed in this case.

In Vitro and in Vivo Antimalarial Evaluation of Ester and Amide Derivatives Antimalarial properties of the synthesized ester and amide derivatives were evaluated *in vitro* against the *PfK1* malaria parasite strain and for cytotoxicity against a human diploid embryonic cell line (MRC-5)¹³⁾ (Table 1). Those derivatives (except for **6a**) showed good antimalarial activity *in vitro* similar to **1**, but also demonstrated unwanted cytotoxicity. We also conducted *in vivo* tests (at a dose of 15 mg/kg $\times$ 4) in a 4-d suppressive test using a *P. berghei*-infected mouse model through intraperitoneal (i.p.) or oral (*p.o.*) administration.¹⁴⁾ These evaluations revealed that the *i*-Pr ester **6c** and *t*-Bu ester **10** produced 99% inhibition (i.p.) with no accompanying toxicity. In comparison, other esters (**6b** and **6d**) and amides (**12a** and **12b**) showed moderate inhibition and lethal toxicity. Remarkably, **6c** also showed 64% inhibition when given orally (*p.o.*) with no toxicity, which represents the first report that a troponoid have antimalarial activity following oral administration. Comparing esters in terms of structural properties, the bulky esters **6c** and **10** (which consist of a secondary or tertiary alcohol) are more effective than **6a** and **6b** (which were synthesized from a primary alcohol). From these results, we hypothesized that the antimalarial activity *in vivo* of these troponoids might be affected by the potential to undertake hydrolysis. Owing to potent antimalarial properties of ester derivatives, we turned our attention to bulky esters for investigation of further SAR, aiming to make more potent compounds than **6c** for use *via*

Table 1. Antimalarial Properties of **6a–6d**, **10**, and **12a** and **12b**

Compound	<i>In vitro</i> , IC ₅₀ (ng/mL)		<i>In vivo</i> , inhibition (method of administration)
	Antimalarial activity (<i>Pf</i> K1) ^{a)}	Cytotoxicity (MRC-5) ^{b)}	
1	8.70	4180.0	0% (<i>p.o.</i> , 100 mg/kg)
6a	186.0	0.087	n.d.
6b	6.38	0.024	43.3% (<i>i.p.</i> , 15 mg/kg) ^{c)}
6c	5.86	0.087	99.0% (<i>i.p.</i>, 15 mg/kg)
6d	6.67	0.73	64.2% (<i>p.o.</i>, 15 mg/kg)
10	9.68	15.48	71.5% (<i>i.p.</i> , 15 mg/kg) ^{c)}
12a	19.43	682.42	99.7% (<i>i.p.</i>, 15 mg/kg)
12b	23.88	227.48	66.5% (<i>i.p.</i> , 15 mg/kg)
Chloroquine	235.28	>9610.53	49.5% (<i>i.p.</i> , 15 mg/kg)
			n.d.

n.d.: no data, a) Chloroquine-resistant *P. falciparum* strain, b) Human diploid embryonic cell line, c) Quit administration after 3 doses due to weight loss, and 3/5 of mice died by day 5.

Table 2. *In Vitro* and *in Vivo* Antimalarial Evaluation of Bulky Esters **6d–6q**

Compound	R₅ (yield)	<i>In vitro</i> , IC ₅₀ (ng/mL)		<i>In vivo</i> , inhibition (<i>p.o.</i> , 15 mg/kg)
		Antimalarial activity (<i>Pf</i> K1) ^{a)}	Cytotoxicity (MRC-5) ^{b)}	
6e	(94%)	35.21	54.09	92.9%
6f	(68%)	57.08	<12.21	n.d.
6g	(64%)	21.21	2.27	n.d.
6h	(75%)	23.96	16.85	97.6%
6i	(59%)	19.81	19.62	79.3%
6j	(21%)	317.40	6025.26	n.d.
6k	(95%)	45.90	16.70	82.0%
6l	(48%)	96.91	<12.21	n.d.
6m	(45%)	3552.00	2238.01	n.d.
6n	(30%)	222.80	142.23	n.d.
6o	(27%)	110.80	65.65	n.d.
6p	(62%)	1676.00	1093.03	n.d.
6q	(72%)	187.80	119.40	n.d.
Artesunate		3.56	>9610.53	90.7%
Chloroquine		235.28	>9610.53	n.d.

n.d.: no data, a) Chloroquine-resistant *P. falciparum* strain, b) Human diploid embryonic cell line.

(a) Attempts for ketone synthesis

(b) Nucleophilic addition before aromatization

Chart 2. Synthesis of Ketone Derivatives

p.o. administration.

Syntheses and Antimalarial Evaluation of Bulky Esters Fourteen bulky esters, **6e–q**, were synthesized using the same procedure shown in Chart 1(a), and evaluated for *in vitro* and *in vivo* antimalarial activity using the same method as mentioned above (Table 2). Both acyclic (**6e–f**) and cycloalkyl esters (**6g–i**) showed strong antimalarial activity *in vitro*, but **6j** (which includes a sulfur atom) showed weak activity. Among the cycloalkyl methyl esters **6k–m**, the antimalarial activity tended to be weaker as the rings became larger. Esters **6n–q**, all bearing a stereo center, showed weak activity. We selected several derivatives which possessed potent antimalarial activity *in vitro* and *in vivo* (i.p.) (see Supplementary Materials) and evaluated them *in vivo* (*p.o.*). All of them inhibited multiplication of parasites without any noticeable toxicity. In particular, esters **6e** and **6h** displayed greater inhibition than the commonly used therapeutic antimalarial, artesunate.

Syntheses of Ketone Derivatives and Their Antimalarial Evaluation To investigate SAR for tolerance for hydrolysis, we attempted to synthesize ketone derivatives. Initially, we thought that the same intermediate **7**, utilized for *t*-Bu ester and amide syntheses, would be a good starting material in terms of ease of monitoring and purification (Chart 2(a)). Condensation of **7**, with Ethanethiol using dicyclohexylcarbodiimide (DCC) afforded the thioester **13** in 96% yield, which was used to investigate Fukuyama ketone synthesis.¹⁵⁾ Unfortunately, the desired coupling reaction did not proceed

under any conditions. In our next strategy, **6** was converted to the Weinreb amide **15** by condensation using 1*H*-Benzotriazol-1-yloxytripyrrolidinophosphonium Hexafluorophosphate (PyBop), envisioning the use of a practical method to synthesize ketones. Interestingly, Grignard reagent did not proceed nucleophilic substitution to the amide moiety, instead, nucleophilic aromatic substitution preferentially.

To overcome this problem, we decided to introduce the alkyl chain before construction of the tropolone framework (Chart 2(b)). Using an intermediate for the total synthesis of **1**,⁷⁾ transient chemoselective diol protection of **16**, followed by oxidation of the primary alcohol and deprotection gave the conjugated aldehyde **17**. Subsequent nucleophilic addition of the Li reagents afforded secondary alcohols the **18a–e**. Finally, aromatization by multi-tandem oxidation produced tropolones (**19a–e**) and subsequent cleavage of the acetone group gave the ketone derivatives (**20a–e**) in reasonable yields over three steps.

In Vitro and in Vivo Antimalarial Evaluation of Ketone Derivatives Ketones **20a–e** were assayed for antimalarial activity using the same method as above (Table 3). Because all ketones (**20a–e**) showed strong antimalarial activity *in vitro*, we evaluated their *in vivo* efficacy (*p.o.*). As expected, all the ketones inhibited malaria parasite multiplication without toxicity *via* oral administration, in particular **20c** and **20d** showed nearly 90% inhibition. Of note, while most of the synthesized derivatives showed strong cytotoxicity *in vitro*, **20d** did not

Table 3. *In Vitro* and *in Vivo* Antimalarial Evaluation of Ketone Derivatives

Compound	R ₇	<i>In vitro</i> , IC ₅₀ (ng/mL)		<i>In vivo</i> , inhibition (<i>p.o.</i> , 15 mg/kg)
		Antimalarial activity (<i>Pf</i> K1) ^{a)}	Cytotoxicity (MRC-5) ^{b)}	
20a	Me	19.25	11.32	58.1%
20b	<i>n</i> -Bu	42.41	17.61	75.0%
20c	<i>i</i> -Bu	19.90	28.38	88.3%
20d		17.34	>1562.50	87.7%
20e		20.74	28.91	34.8%
Artesunate		3.56	>9610.53	90.7%

a) Chloroquine-resistant *P. falciparum* strain, b) Human diploid embryonic cell line.

possess any strong toxicity (IC₅₀ = >1562.50 ng/mL).

Conclusion

We have synthesized novel derivatives of puberulic acid, converting the carboxylic moiety to corresponding esters, amides and ketones, and evaluated their ability to inhibit malaria parasite replication. These derivatives were produced by exploiting the previously devised total synthetic route. Acidic alcoholysis with a primary or secondly alcohol was an effective method to synthesize ester derivatives. Syntheses of *t*-Bu ester and amides were accomplished using Bn-protected tropones by condensation and subsequent hydrogenolysis using a membraned-supported Pd catalyst. Ketone derivatives were synthesized utilizing an intermediate for the total synthesis. Installation of side chains before aromatization was found to be a key step in the process. Antimalarial evaluations *in vitro* and *in vivo* of 25 novel derivatives were carried out and revealed detailed SAR information. A lipophilic substituent at the C4 position tends to bestow a potent anti-plasmodial property. It was apparent that several ester and ketone derivatives possess potent anti-plasmodial efficacy without any measurable toxicity *in vivo* at an oral dose of 15 mg/kg. We believe that our SAR investigation provides invaluable information toward improving properties, bioavailability and expedition of new antimalarial drug candidates.

Experimental

Synthesis of Intermediates and Products

Instruments, Materials

Unless otherwise noted, reagents and solvents were purchased at highest commercial quality and used without further purification. Dry acetone, PhMe, *N,N*-dimethylformamide (DMF), tetrahydrofuran (THF), CH₂Cl₂, MeOH, PhH were purchased from Kanto Chemical Co., Inc. (Tokyo, Japan). Pre-coated silica gel plates with a fluorescent indicator (Merck 60 F254) were used for analytical (0.25 mm) and preparative (0.25 or 0.50 mm) thin layer chromatography. Flash chromatography was carried out with Kanto Chemical silica gel (Kanto Chemical, silica gel 60N, spherical neutral, 0.040–0.050 mm, Cat.-No. 37563-84) or Merck silica gel 230–400 mesh ASTM (60N, 0.040–0.063 mm, Cat.-No. 109385). Octadecyl silica (ODS) column chromatography was carried out with Sep-

Pak® Plus C18 Short Cartridge (Waters Co., Ltd., MA, U.S.A.) or CHROMATOREX® (Fuji Silysia Chemical Ltd., Aichi, Japan). ¹H-NMR spectra were recorded on JEOL JNM-ECA-500 (500 MHz) and ¹³C-NMR spectra were recorded on JEOL JNM-ECA-500 (125 MHz). Chemical shifts are expressed in ppm downfield from the internal solvent peaks for CDCl₃ (¹H; δ = 7.26 ppm, ¹³C; δ = 77.0 ppm), CD₃OD (¹H; δ = 3.31 ppm, ¹³C; δ = 49.0 ppm), CD₂Cl₂ (¹H; δ = 5.32 ppm, ¹³C; δ = 53.84 ppm), (CD₃)₂CO (¹H; δ = 2.05 ppm, ¹³C; δ = 29.8, 206.3 ppm) and *J* values are given in Hertz. The following abbreviations were used to explain the multiplicities: s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, m = multiplet, br = broad. Infrared spectra for all compounds were measured on a Horiba FT-210 spectrometer. High- and Low-resolution (HR and LR) MS were measured on a JEOL JMS-AX505 HA, JEOL JMS-700 MStation and JEOL JMS-T100LP. Optical rotations were measured by using JASCO P-1010 polarimeter. Melting points were measured with a Optimelt, automated melting point system (Stanford Research Systems, CA, U.S.A.).

Chemistry

Methyl ester (**6a**): To puberulic acid **1** (31.1 mg, 0.16 mmol) in a sealed tube was added Methanol (1.57 mL) and TsOH·H₂O (2.99 mg, 0.02 mmol) at room temperature. After being stirred at 120 °C for 16 h, the reaction mixture was cooled to room temperature and H₂O (8 mL) was added. The resulting mixture was extracted with CHCl₃ (10 mL × 3). After that, the organic layer was dried over sodium sulfate, and concentrated under reduced pressure to yield desired product **6a** as a yellow solid (30.5 mg, 92%), mp 108.2 °C; ¹H-NMR (500 MHz, CD₃OD) δ: 7.85 (s, 2H), 3.93 (s, 3H); ¹³C-NMR (125 MHz, CD₃OD) δ: 168.2, 160.4, 156.5, 128.1, 119.5, 53.7; HRMS-electrospray ionization (ESI) (*m/z*) [M – H][–] Calcd for C₉H₇O₆ 211.0243. Found 211.0240.

Compound **6b–6q** were prepared from **1** according to similar procedure of **6a**.

Ethyl ester **6b**: yellow solid, yield 91%, mp 221.6 °C; ¹H-NMR (500 MHz, CD₃OD) δ: 7.86 (s, 2H), 4.38 (q, 2H), 1.40 (t, 3H); ¹³C-NMR (125 MHz, CD₃OD) δ: 167.7, 160.3, 156.6, 128.5, 119.4, 63.4, 14.5; HRMS-ESI (*m/z*) [M – H][–] Calcd for C₁₀H₉O₆ 225.0399. Found 225.0388.

i-Propyl ester **6c**: yellow solid, yield quant., mp 175.8 °C; ¹H

(500 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 7.88 (s, 2H), 5.22–5.18 (complex m, 1H), 1.39 (s, 3H), 1.38 (s, 3H); ^{13}C -NMR (125 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 166.2, 159.5, 155.6, 128.9, 119.2, 70.9, 22.0; HRMS-electron ionization (EI) (m/z) [M] Calcd for $\text{C}_{11}\text{H}_{12}\text{O}_6$ 240.0634. Found 240.0638.

n-Propyl ester **6d**: yellow solid, yield 95%, mp 180.6 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.87 (s, 2H), 4.30 (t, 2H), 1.82–1.81 (complex m, 2H), 1.06 (t, 3H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.6, 160.2, 156.5, 128.6, 119.4, 68.9, 23.1, 10.7; HRMS-ESI (m/z) [$\text{M} - \text{H}$] $^-$ Calcd for $\text{C}_{11}\text{H}_{11}\text{O}_6$ 239.0556. Found 239.0553.

Pentan-3-yl ester **6e**: yellow solid, yield 94%, mp 238.2 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.86 (s, 2H), 4.99–4.97 (complex m, 1H), 1.75–1.72 (complex m, 4H), 0.97–0.94 (complex m, 6H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.6, 160.2, 156.5, 128.6, 119.4, 68.9, 23.1, 10.7; HRMS-ESI (m/z) [$\text{M} - \text{H}$] $^-$ Calcd for $\text{C}_{13}\text{H}_{15}\text{O}_6$ 267.0869. Found 267.0874.

Pentan-3-yl methyl ester **6f**: yellow solid, yield 68%, mp 238.2 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.84 (s, 2H), 4.28 (d, $J = 5.2$ Hz, 2H), 1.72–1.65 (complex m, 1H), 1.51–1.45 (complex m, 4H), 0.99–0.96 (dd, $J = 7.5$, 7.5 Hz, 6H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.6, 160.2, 156.5, 128.4, 119.4, 69.1, 42.1, 24.5, 11.4; HRMS-EI (m/z) [M] Calcd for $\text{C}_{14}\text{H}_{18}\text{O}_6$ 282.1103. Found 282.3027.

Cyclopentyl ester **6g**: yellow solid, yield 64%, mp 151.3 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.80 (s, 2H), 5.36–5.36 (complex m, 1H), 2.02–1.99 (complex m, 2H), 1.87–1.85 (complex m, 4H), 1.72–1.70 (complex m, 2H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.4, 160.2, 156.5, 128.8, 119.4, 80.5, 27.6, 10.0; HRMS-EI (m/z) [M] Calcd for $\text{C}_{13}\text{H}_{14}\text{O}_6$ 266.0790. Found 266.0795.

Cyclohexyl ester **6h**: white solid, yield 75%, mp 181.6 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.85 (s, 2H), 5.00–4.95 (complex m, 1H), 2.01–1.92 (complex m, 2H), 1.85–1.76 (complex m, 2H), 1.67–1.56 (complex m, 3H), 1.54–1.34 (complex m, 3H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 170.0, 160.3, 156.6, 128.9, 119.4, 76.0, 32.5, 26.5, 24.7; HRMS-EI (m/z) [M] Calcd for $\text{C}_{14}\text{H}_{16}\text{O}_6$ 280.0947. Found 280.0943.

Cycloheptyl ester **6i**: yellow solid, yield 59%, mp 182.7 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.84 (s, 2H), 5.17–5.13 (complex m, 1H), 2.02–2.01 (complex m, 2H), 1.87–1.85 (complex m, 4H), 1.80–1.75 (complex m, 2H), 1.66–1.65 (complex m, 2H), 1.64–1.57 (complex m, 2H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 166.9, 160.4, 156.6, 128.8, 119.3, 78.6, 34.7, 29.4, 24.0; HRMS-EI (m/z) [M] Calcd for $\text{C}_{15}\text{H}_{18}\text{O}_6$ 294.1103. Found 294.1097.

4-Thiopyran ester **6j**: white yellow solid, yield 21%, mp 201.3 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.87 (s, 2H), 5.08–5.05 (complex m, 1H), 2.88–2.84 (complex m, 1H), 2.72–2.67 (complex m, 2H), 2.26–2.22 (complex m, 4H), 2.04–1.97 (complex m, 2H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 166.7, 160.1, 156.8, 128.3, 119.2, 74.2, 33.7, 29.5, 26.6; HRMS-EI (m/z) [M] Calcd for $\text{C}_{13}\text{H}_{14}\text{O}_6\text{S}$ 298.0511. Found 298.1256.

Cyclobutanyl methyl ester **6k**: yellow solid, yield 95%, mp 139.4 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.85 (s, 2H), 4.30 (d, $J = 11.5$, 2H), 2.81–2.78 (complex m, 1H), 2.17–2.14 (complex m, 2H), 2.00–1.90 (complex m, 4H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.8, 160.4, 156.6, 128.3, 119.3, 71.0, 35.5, 25.7, 19.3; HRMS-EI (m/z) [M] $^-$ Calcd for $\text{C}_{13}\text{H}_{13}\text{O}_6$ 266.0790. Found 266.0705.

Cyclopentyl methyl ester **6l**: white yellow solid, yield 48%,

mp 188.1 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.86 (s, 2H), 4.23 (d, $J = 7.5$, 2H), 2.40–2.37 (complex m, 1H), 1.90–1.86 (complex m, 2H), 1.70–1.63 (complex m, 4H), 1.41–1.38 (complex, 2H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.1, 160.5, 156.7, 129.1, 119.6, 76.1, 32.7, 26.6, 24.8; HRMS-EI (m/z) [M] $^+$ Calcd for $\text{C}_{14}\text{H}_{16}\text{O}_6$ 280.0947. Found 281.0071.

Cyclohexyl methyl ester **6m**: white yellow solid, yield 45%, mp 182.7 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.85 (s, 2H), 4.14 (d, $J = 6.5$, 2H), 1.83–1.78 (complex m, 4H), 1.77–1.68 (complex m, 1H), 1.38–1.21 (complex m, 4H), 1.15–1.10 (complex m, 2H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.7, 160.4, 156.8, 128.3, 119.2, 72.3, 38.5, 30.8, 27.5, 26.8; HRMS-EI (m/z) [M] $^+$ Calcd for $\text{C}_{15}\text{H}_{18}\text{O}_6$ 294.1103. Found 294.1097.

(*R*)-*sec*-Butyl ester **6n**: yellow solid, yield 30%, mp 192.5 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.86 (s, 2H), 5.08–5.04 (complex m, 1H), 1.79–1.70 (complex m, 2H), 1.36 (d, $J = 6.3$ Hz, 3H), 0.99 (dd, $J = 7.5$, 7.4 Hz, 3H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.2, 160.3, 156.6, 128.8, 119.4, 75.9, 29.9, 19.7, 10.5; HRMS-EI (m/z) [M] $^+$ Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_6$ 254.0790. Found 254.1337; $[\alpha]_{\text{D}}^{24} + 10.4$ ($c = 0.1$ MeOH).

(*S*)-*sec*-Butyl ester **6o**: yellow solid, yield 27%, mp 190.7 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.86 (s, 2H), 5.08–5.04 (complex m, 1H), 1.79–1.673 (complex m, 2H), 1.36 (d, $J = 6.3$ Hz, 3H), 0.99 (dd, $J = 7.4$, 7.5 Hz, 3H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.3, 160.1, 157.0, 128.1, 128.1, 75.7, 29.9, 19.7, 10.1; HRMS-EI (m/z) [M] $^+$ Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_6$ 254.0790. Found 254.1764; $[\alpha]_{\text{D}}^{24} - 7.3$ ($c = 0.1$ MeOH).

(*R*)-2-Pentyl ester **6p**: yellow solid, yield 62%, mp 191.2 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.85 (s, 2H), 5.16–5.12 (complex m, 1H), 1.81–1.73 (complex m, 1H), 1.69–1.58 (complex m, 1H), 1.46–1.38 (complex m, 2H), 1.36 (d, $J = 6.3$ Hz, 3H), 0.98 (dd, $J = 7.5$, 7.5 Hz, 3H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.2, 160.3, 156.5, 128.8, 119.4, 74.4, 39.1, 20.2, 19.8, 14.2; HRMS-EI (m/z) [M] Calcd for $\text{C}_{13}\text{H}_{16}\text{O}_6$ 268.0947. Found 268.4522; $[\alpha]_{\text{D}}^{24} + 8.9$ ($c = 0.1$ MeOH).

(*S*)-2-Pentyl ester **6q**: yellow solid, yield 72%, mp 196.8 °C; ^1H -NMR (500 MHz, CD_3OD) δ : 7.84 (s, 2H), 5.15–5.11 (complex m, 1H), 1.75–1.70 (complex m, 1H), 1.67–1.59 (complex m, 1H), 1.52–1.37 (complex m, 2H), 1.35 (d, $J = 6.3$ Hz, 3H), 0.97 (dd, $J = 6.9$, 7.5 Hz, 3H); ^{13}C -NMR (125 MHz, CD_3OD) δ : 167.2, 160.3, 156.6, 128.8, 119.3, 74.4, 39.1, 20.2, 19.8, 14.2; HRMS-EI (m/z) [M] $^+$ Calcd for $\text{C}_{13}\text{H}_{16}\text{O}_6$ 268.0947. Found 268.0092; $[\alpha]_{\text{D}}^{24} - 12.1$ ($c = 0.1$ MeOH).

2,6,7-Tri-benzyl-puberulic acid (**7**, **7'**): To a solution of methyl ester **6a** (93.1 mg, 0.44 mmol) in MeCN (4.39 mL) was added K_2CO_3 (606.49 mg, 4.39 mmol) and BnBr (0.52 mL, 4.39 mmol) at room temperature. After being stirred at 90 °C for 16 h, the reaction mixture was cooled to room temperature and quenched with H_2O . The resulting mixture was extracted with EtOAc (30 mL). The organic layer was dried over sodium sulfate, and concentrated under reduced pressure at 80 °C to remove BnBr. The residue was purified by silica gel flash column chromatography (Hexane/EtOAc = 6:1) to afford mixture of corresponding tri-Bn ether and its regio isomer (161.35 mg, 76%). This mixture was used in the next reaction without further purification. To a solution of the mixture in THF/ H_2O , MeOH (1.10 mL $\times$ 3) was added K_2CO_3 (91.43 mg, 0.66 mmol) at room temperature. After being stirred at 50 °C for 2 h, the reaction mixture was cooled to room temperature, and quenched with 1N aq. HCl (10 mL) at 0 °C. The resulting solution was extracted with CHCl_3 (30 mL $\times$ 3) and the

organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography ($\text{CHCl}_3/\text{MeOH} = 10:1$) to afford **7** (85.6 mg, 83%) and its regioisomer **7'** (8.6 mg, 8%).

7: yellow solid, mp 165.3 °C; $^1\text{H-NMR}$ (500 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 7.99 (s, 1H), 7.60–7.30 (complex m, 16H), 5.25 (s, 4H), 5.19 (s, 2H); $^{13}\text{C-NMR}$ (125 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 174.9, 163.1, 157.6, 156.1, 138.7, 137.8, 137.3, 129.4, 129.1, 128.8, 125.1, 111.6, 74.1, 71.6; HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{29}\text{H}_{24}\text{Na}_4\text{O}_6$ 491.1471. Found 491.1462.

7': yellow solid, mp 139.4 °C; $^1\text{H-NMR}$ (500 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 7.60–7.21 (complex m, 17H), 5.24 (s, 2H), 5.19 (s, 2H), 4.96 (s, 2H); $^{13}\text{C-NMR}$ (125 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 181.2, 168.5, 160.1, 154.1, 138.4, 138.0, 136.7, 133.4, 129.6, 129.4, 129.2, 129.0, 106.7, 76.1, 74.0, 72.1; HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{29}\text{H}_{24}\text{Na}_4\text{O}_6$ 491.1471. Found 491.1460.

2,6,7-Tri-benzyl-4-*t*-butyl ester (9): To a solution of **7** (10.0 mg, 21.34 μmol) in *t*-BuOH/THF (1:1, 0.43 mL) under N_2 atmosphere was added *O*-*tert*-butyl-*N,N'*-diisopropylisourea (25.45 μL , 0.60 mmol) at room temperature. After being stirred for 2 h, the reaction mixture was quenched with sat. aq. NaHCO_3 (5 mL). The resulting solution was extracted with EtOAc (10 mL $\times$ 3), and the organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (Hexane/EtOAc = 2:1) to afford **9** (10.3 mg, 92%) as a yellow oil; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.89 (s, 1H), 7.57 (s, 1H), 7.47–7.26 (complex m, 15H), 5.31 (s, 2H), 5.24 (s, 2H), 5.14 (s, 2H), 1.53 (s, 9H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 176.0, 166.1, 163.3, 159.9, 155.7, 139.3, 138.8, 138.5, 130.0, 129.7, 129.4, 129.3, 129.2, 129.1, 128.7, 121.8, 112.6, 84.4, 75.0, 73.8, 72.1, 28.2; HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{33}\text{H}_{32}\text{Na}_4\text{O}_6$ 547.2097. Found 747.2092.

***t*-Butyl ester (10)**: To a solution of **9** (32.7 mg, 62.40 μmol) in MeOH (1.25 mL) under N_2 atmosphere was added Pd/*i*O-brane[®] (1 cm^2 , 4.70 μmol) at room temperature. After being stirred for 2 h under H_2 atmosphere, the reaction mixture was filtrated through cotton, washed with MeOH. The resulting solution was concentrated under reduced pressure to afford **9** (15.2 mg, 96%) as a yellow solid, mp 172.0 °C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.81 (s, 2H), 1.60 (s, 9H); $^{13}\text{C-NMR}$ (125 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 165.8, 159.5, 155.6, 129.8, 119.0, 83.2, 28.2; HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_6$ 254.0790. Found 254.0795.

2,6,7-Tri-benzyl-4-*N,N*-dimethyl amide (11a): To a solution of common intermediate **7** (21.7 mg, 43.82 μmol) in THF (0.44 mL) under N_2 atmosphere was added PyBop (34.2 mg, 65.73 μmol) and DIPEA (19.21 μL , 0.11 mmol) and Dimethylamine (65.73 μL , 0.13 mmol) at room temperature. After being stirred for 30 m, the reaction solution was quenched with 1N aq. HCl. The resulting solution was extracted with CH_2Cl_2 , and the organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (hexane/EtOAc = 1:5) to afford **11a** (18.4 mg, 85%) as yellow oil; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.47–7.25 (complex m, 15H), 7.08 (s, 1H), 6.87 (s, 1H), 5.30 (s, 2H), 5.23 (s, 2H), 5.09 (s, 2H), 3.02 (s, 3H), 2.66 (s, 3H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 175.6, 172.5, 164.6, 161.1, 154.1, 139.2, 139.0, 138.9, 138.7, 130.0, 129.8, 129.7, 129.5, 129.4, 129.2, 128.8, 119.9, 112.7, 74.8, 73.9, 72.3, 39.4, 35.6, 30.8; HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{31}\text{H}_{29}\text{N}_1\text{Na}_4\text{O}_5$

254.0790. Found 254.0795.

4-*N,N*-Dimethyl amide (12a): To a solution of **11a** (15.3 mg, 30.90 μmol) in MeOH (0.62 mL) under N_2 atmosphere was added Pd/*i*O-brane[®] (1 cm^2 , 4.70 μmol) at room temperature. After being stirred for 4 h under H_2 atmosphere, the reaction mixture was filtrated through cotton, washed with MeOH. The resulting solution was concentrated under reduced pressure to afford **12a** (6.8 mg, quant.) as a yellow solid, mp 224.4 °C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.00 (s, 2H), 3.08 (s, 3H), 2.97 (s, 3H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 173.3, 159.0, 158.4, 136.8, 116.9, 39.7, 35.6; HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{10}\text{H}_{11}\text{NO}_5$ 225.0637. Found 225.0647.

Compound **11b** was prepared from **7** according to similar procedure of **11a**.

2,6,7-Tri-benzyl-4-*N*-isopropyl-*N*-methyl amide (11b): yellow solid, yield 84%, mp 83.5 °C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.45–7.24 (complex m, 15H), 7.00 and 6.98 (rotamer s, 1H), 6.79 and 6.73 (rotamer s, 1H), 5.33 and 5.30 (rotamer s, 2H), 5.23 (s, 2H), 5.10 (s, 2H), 4.68 and 3.62 (rotamer s, 1H), 2.87 and 2.30 (rotamer s, 3H), 1.16 (s, 3H), 1.00 (s, 3H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 175.6, 172.1, 164.8, 164.5, 161.2, 153.9, 138.3, 137.5, 136.8, 130.0, 129.7, 129.2, 119.5, 118.8, 112.8, 112.1, 74.8, 74.0, 72.3, 51.9, 46.8, 30.8, 26.5, 20.1, 19.2; HRMS-ESI (m/z) $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{33}\text{H}_{33}\text{N}_1\text{Na}_4\text{O}_5$ 546.2256. Found 546.2246.

Compound **12b** was prepared from **11b** according to similar procedure of **12a**.

4-*N*-Isopropyl-*N*-methyl amide puberulic acid (12b): yellow solid, yield quant., mp 128.7 °C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 6.95 (s, 2H), 4.82–4.77 and 3.91–3.88 (rotamer complex m, 1H), 2.93 and 2.80 (rotamer s, 3H), 1.25–1.19 (rotamer, 6H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 172.9, 158.5, 158.4, 137.4, 116.9, 116.3, 49.3, 46.8, 31.3, 26.6, 20.1, 19.2; HRMS-ESI (m/z) $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{33}\text{H}_{33}\text{N}_1\text{Na}_4\text{O}_5$ 546.2256. Found 546.2246.

2,6,7-Tri-benzyl-4-thioethyl ester (13): To a solution of **7** (8.6 mg, 18.36 μmol) in CH_2Cl_2 (1.84 mL) under N_2 atmosphere was added DCC (14.7 mg, 55.07 μmol) and *N,N*-dimethyl-4-aminopyridine (DMAP) (21.99 μg , 0.18 μmol) and ethanethiol (2.65 μL , 36.71 mmol) at 0 °C. After being stirred for 30 min, warm to room temperature. After being stirred for 11 h, the reaction solution was quenched with 1N aq. HCl (10 mL). The resulting solution was extracted with CH_2Cl_2 (10 mL $\times$ 3), and the organic layer was dried over sodium sulfate, and concentrated under reduced pressure. The residue was purified by preparative TLC (hexane/EtOAc = 2:1) to afford **13** (9.0 mg, 96%) as yellow oil; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.76 (s, 1H), 7.49–7.25 (complex m, 16H), 5.28 (s, 2H), 5.22 (s, 2H), 5.12 (s, 2H), 3.03 (ddd, $J = 7.5, 7.5, 3\text{H}$), 1.31 (dd, $J = 7.5, 7.5, 3\text{H}$); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 192.5, 174.5, 162.3, 158.3, 154.5, 138.5, 137.9, 137.8, 128.6, 128.4, 128.2, 128.1, 127.9, 127.8, 127.6, 120.6, 109.2, 73.7, 72.8, 71.0, 24.3, 13.5; HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{31}\text{H}_{28}\text{O}_5\text{S}$ 512.0230. Found 512.0795.

2,6,7-Tri-benzyl-4-*N*-methoxy-*N*-methyl amide (15): To a solution of **7** (50.0 mg, 0.11 mmol) in THF (11.0 mL) under N_2 atmosphere was added PyBop (114.49 mg, 0.22 mmol) and DIPEA (115.27 μL , 0.66 mmol) and *N,O*-dimethylhydroxylamine·HCl (31.21 mg, 0.32 mmol) at room temperature. After being stirred for 2.5 h, the reaction solution was quenched with 1N aq. HCl (5 mL). The resulting solution was extracted with CH_2Cl_2 (10 mL $\times$ 3), and the organic layer was dried

over sodium sulfate and concentrated under reduced pressure. The residue was purified by preparative TLC (Hexane/EtOAc = 1:5) to afford **15** (44.5 mg, 79%) as yellow oil; $^1\text{H-NMR}$ (500 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 7.54–7.29 (complex m, 15H), 7.21 (s, 1H), 6.98 (s, 1H), 5.22 (s, 4H), 5.13 (s, 2H), 3.52 (s, 3H), 3.23 (s, 3H); $^{13}\text{C-NMR}$ (125 MHz, $(\text{CD}_3)_2\text{CO}$) δ : 174.6, 170.2, 163.5, 158.4, 154.5, 139.5, 137.8, 137.5, 134.9, 129.4, 129.0, 128.9, 128.6, 121.5, 112.5, 73.9, 71.7, 61.7; HRMS-ESI (m/z) $[\text{M} + \text{H}]^+$ Calcd for $\text{C}_{31}\text{H}_{29}\text{N}_1\text{Na}_1\text{O}_6$ 534.3261. Found 534.3146.

(3*S*,4*S*,5*S*,6*S*)-3,4-*O*-Isopropylidene-5,6-dihydroxy-1-carbaldehyde-cyclohept-1-ene (**17**): To a stirred solution of **16** (110.3 mg, 0.48 mmol) in THF (4.79 mL) under N_2 atmosphere was added trimethylboroxine (23, 43 μL , 0.17 mmol) at room temperature. After being stirred for 40 min, the reaction mixture was concentrated under reduced pressure to afford the crude product. To a solution of the crude product in CH_2Cl_2 (4.79 mL) under N_2 atmosphere was added PIDA (170.07 mg, 0.53 mmol) and 2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPO) (3.74 mg, 23.95 μmol) at 0°C. After being stirred for 5 min., the reaction mixture was warmed to room temperature, after being stirred further 20 h, quenched with sat. aq. NaHCO_3 (10 mL), the resulting solution was extracted with CHCl_3 (10 mL $\times$ 3). The organic layer was concentrated under reduced atmosphere. The residue was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}$ = 20/1) to afford **17** (104.5 mg, 95%) as a yellow oil, $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 9.45 (s, 1H), 6.66 (d, J = 1.7, 1H), 5.22 (d, J = 7.45, 1H), 4.46 (dd, J = 8.05, 8.04, 1H), 4.06–4.05 (complex m, 1H), 3.51 (dd, J = 4.6, 5.8), 3.28 (q, J = 3.8, 1H), 2.85 (s, 1H), 2.69 (s, 1H), 1.92–1.90 (complex m, 1H), 1.52 (s, 3H), 1.43 (s, 3H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 191.6, 150.4, 136.7, 108.7, 75.3, 74.1, 71.8, 65.7, 26.9, 26.3, 24.1; HRMS-ESI (m/z) $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{11}\text{H}_{16}\text{O}_5$ 228.0998. Found 228.0935.

Methyl ketone **20a**: To a solution aldehyde **17** (26.4 mg, 115.74 μmol) in Et_2O (1.16 mL) under N_2 atmosphere was added MeLi (1.86 mL, 3.00 mmol) at -78°C . After being stirred for 20 min, the reaction mixture was purified by silica gel column chromatography ($\text{CHCl}_3/\text{MeOH}$ = 10/1) to afford diastereomixture of **18a** as white solid. To a solution of the diastereomixture of **18a** in CH_2Cl_2 (1.16 mL) under N_2 atmosphere was added DMSO (0.12 mL, 1.74 mmol), and triethylamine (0.48 mL, 3.47 mmol), and $\text{SO}_3 \cdot \text{pyridine}$ (0.18 g, 1.16 mmol) at 0°C. The mixture was stirred at room temperature for 12 h, monitored by LC-UV analysis, then quenched with 1*N* aq. HCl (3.0 mL), and extracted with CHCl_3 (5.0 mL $\times$ 3). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to yield crude product **19a** as a dark brown amorphous. This crude product was used in subsequent reaction without further purification.

To a stirred solution of the crude product in a Pyrex tube was added 80% trifluoroacetic acid (TFA) (2.31 mL) at room temperature. The reaction mixture was warmed to 120°C, and after being stirred for 8 h, cooled to room temperature, and concentrated under reduced pressure. The resulting residue was purified by Sep-pak® Plus C18 Short Cartridge to afford **20a** (5.7 mg, 25% in 3 steps) as a yellow solid, mp 160.9°C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.77 (s, 2H), 2.63 (s, 3H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 199.4, 160.2, 156.6, 135.2, 118.7, 26.7; HRMS-ESI (m/z) $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_9\text{H}_8\text{O}_5$ 196.0372. Found 195.0294.

Compounds **20b–e** were prepared from **17** according to similar procedure of **20a**.

n-Butyl ketone **20b**: yellow solid, yield 25% (3 steps), mp 188.8°C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.74 (s, 2H), 3.03 (dd, J = 7.0, 7.5, 2H), 1.69–1.66 (complex m, 2H), 1.44–1.39 (complex m, 2H), 0.97 (dd, J = 7.5, 7.0, 2H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 201.8, 160.2, 156.7, 135.3, 118.4, 39.1, 30.8, 27.9, 23.4, 14.3; HRMS-ESI (m/z) $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_5$ 238.0841. Found 238.0757.

i-Butyl ketone **20c**: pale brown solid, yield 31% (3 steps), mp 158.5°C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.73 (s, 2H), 2.90 (d, J = 6.9, 2H), 2.24–2.19 (complex m, 1H), 1.00 (d, J = 6.3, 2H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 201.5, 160.1, 156.7, 135.7, 118.4, 48.9, 26.6, 22.9; HRMS-ESI (m/z) $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{12}\text{H}_{14}\text{O}_5$ 238.0841. Found 238.0763.

Pentane-3-yl methyl ketone **20d**: brown solid, yield 27% (3 steps), mp 186.9°C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.71 (s, 2H), 2.92 (d, J = 7.0, 2H), 1.92–1.89 (complex m, 1H), 1.43–1.34 (complex m, 4H), 0.90 (dd, J = 7.5, 7.5, 6H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 202.1, 160.2, 156.7, 135.5, 118.1, 43.3, 38.9, 26.8, 11.1; HRMS-ESI (m/z) $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{14}\text{H}_{18}\text{O}_5$ 266.1154. Found 266.1070.

Cyclohexyl methyl ketone **20e**: brown solid, yield 25% (3 steps), mp 180.4°C; $^1\text{H-NMR}$ (500 MHz, CD_3OD) δ : 7.74 (s, 2H), 3.04–3.01 (complex m, 2H), 1.72–1.64 (complex m, 2H), 1.43–1.39 (complex m, 3H), 1.32–1.27 (complex m, 2H), 0.96 (dd, J = 7.5, 7.5, 4H); $^{13}\text{C-NMR}$ (125 MHz, CD_3OD) δ : 202.0, 160.9, 156.9, 123.0, 118.0, 39.1, 27.9, 23.4, 14.3; HRMS-ESI (m/z) $[\text{M} - \text{H}]^-$ Calcd for $\text{C}_{15}\text{H}_{18}\text{O}_5$ 278.1154. Found 278.1276.

In Vitro Antimalarial Evaluation *In vitro* antimalarial activities against *P. falciparum* chloroquine-resistant K1 and chloroquine-sensitive FCR3 strains were measured using a modified Malstat reagent method.¹²⁾

Cytotoxicity against MRC-5 cells was also measured as described previously.¹²⁾

This study was approved by “Kitasato Institute Hospital Research Ethics Committee (No. 12102)” on the donation of human erythrocytes from volunteers to cultivate malaria parasites.

In Vivo Antimalarial Evaluation *In vivo* antimalarial activities were assessed according to Peters 4-d suppressive test¹³⁾ using mice infected with rodent malaria *Plasmodium berghei* N strain.

Male CD-1 (ICR) mice were purchased from Charles River Japan Inc., Japan. Five mice were used as each treated and untreated (vehicle) control.

The statistical analysis (Dunnett’s test and Tukey–Kramer’s honestly significant difference test) was using JMP statistical software (JMP® 8 SAS Institute Inc., Cary, NC, U.S.A.), and *p* values <0.05 were considered statistically significant.

All animal experiments were performed under the ethical guidelines of The Kitasato Institute.

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Conflict of Interest The authors declare no conflict of

interest.

Supplementary Materials The online version of this article contains supplementary materials, which include ^1H -NMR, ^{13}C -NMR spectra of synthetic intermediates and derivatives, along with results of detailed biological evaluations.

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