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# Structure–activity relationship of anti-malarial spongean peroxides having a 3-methoxy-1,2-dioxane structure

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Abstract—In order to study the structure-activity relationship of anti-malarial spongean peroxides, several analogues concerning with the 6-methoxyacetyl moiety and the 3-pentyl residue in methyl 2-(3-methoxy-3-pentyl-1,2-dioxan-6-yl)acetate were synthesized and evaluated for anti-malarial activity. The *tert*-butyl ester analogue 14 showed stability in mouse serum and a high selectivity index against the malaria parasite, *Plasmodium falciparum*, and the citronellyl analogue 31 exhibited the strongest in vitro anti-malarial activity against *P. berghei* infected mice.

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#### 1. Introduction

Malaria is one of the most deadly diseases for humans worldwide and more than 2.5 million people die from it each year.<sup>1</sup> Due to the emergence and ongoing spread of the chloroquine-resistant strains of *Plasmodium falciparum*,<sup>2</sup> including multi-drug resistant strains to conventional anti-malarial drugs, cyclic peroxides like artemisinin (1) and its derivatives (2–3) are regarded as new anti-malarial principals (Chart 1).<sup>3</sup> In this context, we studied the structure–activity relationship (SAR) of the cyclic peroxide, methyl 2-(3-methoxy-3-pentyl-1,2dioxan-6-yl)acetate (7), as a scaffold.

In our previous report, we disclosed that the spongean peroxides, methyl esters (5, 6) of peroxyplakoric acid  $A_3$  and  $B_3^4$ , showed potent in vitro anti-malarial activity and established a facile synthetic method for construction of their core skeleton, a 3-methoxy-1,2-dioxane moiety, by Sc(OTf)<sub>3</sub>-mediated peroxyhemiacetalization and intramolecular Michael addition.<sup>5</sup> Furthermore, the synthetic peroxide 7 was found to exhibit higher





selectivity index against *P. falciparum* than those of **5** and **6**.<sup>6</sup> However, compound **7** did not exhibit in vivo anti-malarial activity, since the methyl ester moiety in **7** was hydrolyzed in mouse serum to afford the corresponding carboxylic acid **8**, which showed significantly reduced anti-malarial activity. The ester moiety in **7** was converted to an amide moiety, and the ethyl and propyl amide analogues (**9**, **10**) were found to exhibit in vivo anti-malarial potency.<sup>7</sup> This paper deals with a more detailed SAR study of the ester function and the 3-alkyl side chain in **7**.

*Keywords*: Anti-malarial; Cyclic peroxide; Structure-activity relationship; Marine sponge.

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#### 2. Chemistry

The amide analogues (9, 10) showed in vivo anti-malarial activity in a four-day suppressive test using *Plasmodium berghei* infected mice; however, a tendency to reduced in vitro activity (9: IC<sub>50</sub> 0.54  $\mu$ M, 10: 0.31  $\mu$ M) in comparison with that of 7 (7: IC<sub>50</sub> 0.12  $\mu$ M) was seen. In order to study SAR of the 6-methoxyacetyl portion in the synthetic peroxide 7, we further designed and synthesized several analogues (13–26) as depicted in Chart 2.

In consideration of metabolism in the serum,<sup>7</sup> three ester analogues:  $\alpha$ -methyl analogue **13**, *tert*-butyl ester analogue **14**, and phenyl ester analogue **15**, with steric hin-

drance circumjacent to the ester portion in 7 and the two ketone analogues (16, 17) were designed.

Preparation of the ester analogues (13–15) and the methyl ketone analogue 16 was conducted as illustrated in Scheme 1. Treatment of the ketoaldehyde,<sup>6</sup> which was derived from ketoalcohol 32 by Swern oxidation, with methyl 2-(triphenyl- $\lambda^5$ -phosphanylidene)propionate<sup>8</sup> or commercially available *tert*-butyl (triphenyl- $\lambda^5$ -phosphanylidene)acetate furnished keto- $\alpha$ , $\beta$ -unsaturated esters (33: 88%, 34: 92% for two steps), respectively. Conversion from the keto- $\alpha$ , $\beta$ -unsaturated esters (33, 34) to 3-methoxy-1,2-dioxanes (13, 14) was carried out via peroxyhemiacetal. Namely, the peroxyhemiacetalization of 33 and 34 in the presence of Sc(OTf)<sub>3</sub> and subsequent



Chart 2. Design for structure-activity relationship study of spongean peroxides.



Scheme 1. Reagents and conditions: (a) DMSO, (COCl)<sub>2</sub>, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>; (b) Ph<sub>3</sub>P=C(R<sup>2</sup>)COR<sup>1</sup>, CH<sub>2</sub>Cl<sub>2</sub>, two steps 88% for 33, two steps 92% for 34, two steps 89% for 35; (c) H<sub>2</sub>O<sub>2</sub>·H<sub>2</sub>NCONH<sub>2</sub>, Sc(OTf)<sub>3</sub>, MeOH, 8.3% for 16; (d) Et<sub>2</sub>NH, CF<sub>3</sub>CH<sub>2</sub>OH, two steps 38% for 13, two steps 20% for 14; (e) PhOH, EDCI·HCl, pyridine, 98% for 15; (f) NaHMDS, THF, -78 °C; (g) MsCl, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>, two steps 73%; (h) H<sub>2</sub>O<sub>2</sub>·H<sub>2</sub>NCONH<sub>2</sub>, Sc(OTf)<sub>3</sub>, MeOH, 17%.

intramolecular Michael addition of the peroxyhemiacetal provided two corresponding ester analogues (13: 38%, 14: 20% for two steps), respectively. Treatment of the ketoaldehyde with commercially available 1-(triphenyl- $\lambda^5$ -phosphanylidene)propan-2-one furnished a keto- $\alpha$ , $\beta$ -unsaturated ketone 35 in 89% yield. Then, 35 was further converted to a methyl ketone 16 by peroxyhemiacetalization in the presence of Sc(OTf)<sub>3</sub>. On the other hand, the carboxylic acid 8 was coupled with phenol in the presence of N-ethyl-N'-3-dimethylaminopropylcarbodiimide hydrochloride (EDCI·HCl) in pyridine to give a phenyl ester analogue 15 in 98% yield. Next, the analogue 17 was synthesized from the keto- $\alpha$ , $\beta$ unsaturated ketone 38, which was prepared from acetophenone (36) and ketoaldehyde 37 by aldol condensation, in the same fashion as for the preparation of 16.

Artemisinin (1), a typical cyclic peroxide with potent anti-malarial activity, is proved to be degradated by one-electron reduction with Fe(II) of heme in the food vacuole,<sup>9</sup> which is the characteristic acidic organelle (pH 5.0-5.4) in the malaria parasite.<sup>10,11</sup> The resulting radical species were presumed to form covalent adducts with proteins in the food vacuole to inhibit the proliferation of the malaria parasites. O'Neill and co-workers<sup>12</sup> noted this character of the heme-rich parasite food vacuole, and they synthesized an artemisinin derivative 4 with the N atom (Chart 1) and assessed the 'ion-trapping' effect in expecting the accumulation of 4 in the food vacuole. Watanabe and co-workers reported that the imidazole moiety facilitated oxidation of heme iron.<sup>13</sup> We designed amide analogues (18-26) having the N atom as depicted in Chart 2. Preparation of the eight amide analogues (18–25) having the N atom and the dimeric analogue 26 was carried out by treatment of the pentafluorophenyl ester  $39^7$  with commercially available amines having the N atom, in good yields (Scheme 2).

When the 3-pentyl analogue 7 was treated with FeSO<sub>4</sub> as a model experiment in the food vacuole, **11** and **12** were afforded as major products (Chart 2).<sup>14</sup> This result suggested that the alkyl radical **i**, which was a plausible active principal against the malaria parasite, was generated in this reaction. Previously, we reported that the antimalarial activity of the analogue with a methyl residue at C-3 in the 3-methoxy-1,2-dioxane moiety was weaker than those of the analogues with a pentyl or nonyl residue,<sup>6</sup> since the methyl radical is less stable than pentyl or nonyl radicals. For the purpose of production of a more stable radical, we designed 3-cyclohexyl analogue **27**, 3phenyl analogue **28**, 3-benzyl analogue **29**, and two analogues (**30**, **31**) with an olefin portion (Chart 2).



Scheme 2. Reagents and conditions: (a)  $R-NH_2$ , THF, 91% for 18; quant. for 19; quant. for 20; 85% for 21; quant. for 22; 95% for 23; 88% for 24; quant. for 25; quant. for 26.



Scheme 3. Reagents and conditions: (a) MeNHOMe·HCl, Me<sub>2</sub>AlCl, CH<sub>2</sub>Cl<sub>2</sub>; (b) BrMg-R, THF; (c) DMSO, (COCl)<sub>2</sub>, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>; (d) Ph<sub>3</sub>P=CHCOOCH<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, four steps 45% for 41, four steps 63% for 42, four steps 59% for 43, four steps 77% for 44, four steps 69% for 45; (e) H<sub>2</sub>O<sub>2</sub>·H<sub>2</sub>NCONH<sub>2</sub>, Sc(OTf)<sub>3</sub>, MeOH; (f) Et<sub>2</sub>NH, CF<sub>3</sub>CH<sub>2</sub>OH, two steps 1.1% for 27, two steps 42% for 28, two steps 28% for 29, two steps 58% for 30, two steps 51% for 31.

The syntheses of the analogues (27–31) having a different alkyl moiety at the C-3 position of the 3-methoxy-1,2-dioxane were conducted as illustrated in Scheme 3. Treatment of the Weinreb amide, which was prepared from  $\gamma$ -butyrolactone 40, with each Grignard reagent provided the corresponding ketoalcohols. The Swern oxidation of the ketoalcohols and subsequent Wittig reaction with methyl (triphenyl-25-phosphanylidene)acetate furnished the corresponding keto- $\alpha$ ,  $\beta$ -unsaturated esters (41: 45%, 42: 63%, 43: 59%, 44: 77%, 45: 69% for four steps). The peroxyhemiacetalization of the keto- $\alpha$ ,  $\beta$ -unsaturated esters (41–45) in the presence of Sc(OTf)<sub>3</sub> and subsequent intramolecular Michael addition provided the corresponding analogues possessing a different alkyl moiety at the C-3 position of the 3methoxy-1,2-dioxane (27: 1.1%, 28: 42%, 29: 28%, 30: 58%, **31**: 51% for two steps). Generally, the reaction of peroxyhemiacetalization takes two days, while the reaction for 41 proceeded very slowly to afford 27 in poor yield because of the steric hindrance.

#### 3. Biological properties and discussion

The in vitro anti-malarial activity against *P. falciparum* and cytotoxicity against human epidermoid carcinoma cells (KB 3-1) of the synthesized analogues (13-31) were evaluated.<sup>15</sup> The in vitro anti-malarial activity of the three ester analogues (13-15) and the related analogues (16, 17) was depicted in Table 1. Compounds 13 and 14 showed similar activity to that of the methyl ester analogue 7, while the phenyl ester analogue 15 and two related analogues (16, 17) showed only weak activity. Moreover, the selectivity index of 14 against P. falciparum was higher than that of 7. These results indicated that the ester function is related with anti-malarial activity. As a result of the assessment for stability in the mouse serum, tert-butyl ester analogue 14 was shown to be free from metabolism in comparison with the methyl ester analogue 7 and the phenyl ester analogue 15 (Fig. 1). Hence, 14 is expected to exhibit in vivo anti-malarial activity.

Table 1. In vitro anti-malarial activity of analogues (13-17)

Compd	$\mathbb{R}^1$	$\mathbb{R}^2$	IC <sub>50</sub> (µM)		Selectivity
			P. falciparum	KB 3-1	index
13	CH <sub>3</sub> O	Me	0.26	26	100
14	t-BuO	Н	0.12	70	583
15	PhO	Н	>3.1	N. T.	
16	Me	Н	>4.1	N. T.	
17	Ph	Н	>3.3	N. T.	
7	CH <sub>3</sub> O	Н	0.12	43	360
5			0.15	21	140
6			0.12	28	230

N.T.: not tested.



Figure 1. Stability of esters (7, 14, and 15).

Similarly, the analogues (18–26) having the N atom were evaluated for in vitro anti-malarial activity. Each analogue (18–26) showed similar activity, which was somewhat weaker than that of 7 (Table 2).

Among the analogues (13-17) with steric hindrance circumjacent to the ester portion and the analogues (18-26) with the N atom on the amide portion, *tert*-butyl ester analogue 14 and several amide analogues (22-25) were evaluated for in vivo anti-malarial activity against *P*. *berghei* infected mice. The results are summarized in Table 3. Although the methyl ester analogue 7 showed

Table 2. In vitro anti-malarial activity of analogues (18-26)

Compd	IC <sub>50</sub> (μΝ	Selectivity index	
	P. falciparum	KB 3-1	
18	0.48	12	26
19	0.42	12	28
20	0.63	16	26
21	0.85	17	20
22	0.38	18	47
23	0.36	6	16
24	0.36	29	79
25	0.26	12	47
26	0.46	5	10
10	0.31	12	39

Table 3. In vivo anti-malarial activity of analogues

Compd	ED <sub>50</sub> (mg/kg) P. berghei	$T/C^{\rm a}$
14	32	125
22	>10 (27%)	117
23	>10 (20%)	125
24	>10 (15%)	122
25	18	136
7	>30	81
10	9.3	138
Artemisinin	5	145

<sup>a</sup> Dose of **14**, **22–25**, **7**, and **10**: 10 mg/kg, artemisinin: 5 mg/kg T/C is the quotient of the survival days of the treated animals (*T*) and those of the control animals (*C*). *T/C* values of >120 are considered to be active.

little in vivo activity at 30 mg/kg by intraperitoneal administration, the *tert*-butyl ester analogue **14** showed in vivo potency (T/C 125). Among the amide analogues (**22–25**) having the N atom, the imidazole analogue **25** showed the highest in vivo potency ( $ED_{50} = 18 \text{ mg/kg}$ , T/C 136). In the case of the propyl amide analogue **10**, which showed potent in vivo anti-malarial activity, the mortality of mice was observed at the 100 mg/kg dose, while, no obvious sign of acute toxicity such as decrease of body weight and diarrhea was observed at the same dose of **25**. This result suggests that the toxicity of the 3-methoxy-1,2-dioxane for mice was decreased appreciably.

On the other hand, the analogues possessing a different 3-alkyl side chain (27–31) were evaluated for in vitro anti-malarial activity and cytotoxicity (Table 4). The anti-malarial activities of 27, 28, and 29 were reduced in comparison with that of 7, while the pentenyl analogue 30 showed similar activity to that of 7 and the citronellyl analogue 31 showed stronger activity. Moreover, the methyl ester analogue 7 showed no in vitro anti-malarial activity at the dose less than  $0.01 \,\mu\text{g/mL}$ , while the two analogues (30, 31) having an olefin function showed about 40% growth inhibitory activity against malaria parasites at the same dose (Fig. 2). On the other hand, this tendency was not observed against KB 3-1 cells (Fig. 3).

As shown in Chart 3, the radical species **ii** and **v** first derived from the olefin analogues (30, 31) could be easily converted to secondary stable radicals (**iii** or **iv**, **vi** or **vii**). From these evidences, we presumed that the pentyl

Table 4. In vitro anti-malarial activity of analogues (27-31)

Compd	IC <sub>50</sub> (μ)	Selectivity index	
	P. falciparum	KB 3-1	
27	1.2	N.T.	
28	1.2	N.T.	
29	>3.6	N.T.	
30	0.28	78	279
31	0.033	5.2	158
7	0.12	43	360

N.T.: not tested.



Figure 2. In vitro anti-malarial activity of analogues (7, 30, and 31).



Figure 3. In vitro cytotoxic activity of analogues (7, 30, and 31).



**Chart 3.** Plausible radical production from 3-methoxy-1,2-dioxane moiety.

radical i produced from the 3-pentyl analogue 7 is unstable and short-lived, while the radical species produced from the olefin analogues (30, 31) would be more long-lived to form covalent adducts with a parasite's protein.

#### 4. Experimental

The following instruments were used to obtain physical data: a Hitachi 330 spectrophotometer for UV spectra; a JASCO FT/IR-5300 infrared spectrometer for IR spectra; a JEOL JMS SX-102 mass spectrometer for FAB-MS; a JEOL JNM AL-500 (500 MHz) NMR spectrometer for <sup>1</sup>H NMR (CDCl<sub>3</sub> solution with tetramethysilane (TMS) as an internal standard unless otherwise specified). HPLC was performed using a Hitachi L-6000 pump equipped with Hitachi L-4000H UV detector. Silica gel (Merck 60-230 mesh) and pre-coated thin layer chromatography (TLC) plates (Merck, Kiesel gel, 60F<sub>254</sub>) were used for column chromatography and TLC. Spots on TLC plates were detected by spraying acidic *p*-anisaldehyde solution (*p*-anisaldehyde: 25 mL, c-H<sub>2</sub>SO<sub>4</sub>: 25mL, AcOH: 5mL, EtOH: 425mL) with subsequent heating.

### 4.1. General procedure A: preparation of Weinreb amide followed by Grignard reaction

Me<sub>2</sub>AlCl (1.0 M in *n*-hexane, 1.9 equiv) was treated with the anhydrous CH<sub>2</sub>Cl<sub>2</sub> solution of MeONHMe·HCl (1.5 M, 1.9 equiv) at 0 °C, and the whole was stirred for 1 h at room temperature. After adding an anhydrous CH<sub>2</sub>Cl<sub>2</sub> solution of  $\gamma$ -butyrolactone (**40**, 0.17 M), the whole was stirred for 1 h at room temperature. After quenching with phosphate buffer (pH 8.0) at 0 °C, the whole was stirred for 10 min. The reaction mixture was diluted with anhydrous CHCl<sub>3</sub>, and the resulting residue was removed by Celite column. The filtrate was extracted with CHCl<sub>3</sub>, dried over MgSO<sub>4</sub>, and concentrated under reduced pressure to afford a Weinreb amide.

A THF solution of the Weinreb amide (0.18 M) was treated with Grignard reagent (8.0 equiv), which was prepared from commercially available alkylbromide, at room temperature for 30min. The reaction mixture was quenched with 25% aqueous H<sub>2</sub>SO<sub>4</sub> at 0°C, and the whole was stirred for 10min at room temperature. The reaction mixture was poured into saturated aqueous NaHCO<sub>3</sub>, and the whole was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl and dried over MgSO<sub>4</sub>. Removal of solvent from the EtOAc extract under reduced pressure gave a crude product, which was further purified by SiO<sub>2</sub> column (*n*-hexane–EtOAc) to furnish a ketoalcohol.

#### 4.2. General procedure B: Swern oxidation

DMSO (6.0 equiv) was added to the anhydrous  $CH_2Cl_2$  solution of (COCl)<sub>2</sub> (0.06 M, 3.0 equiv) at -78 °C, and the whole was stirred for 20 min. After adding an anhydrous  $CH_2Cl_2$  solution of ketoalcohol (0.028 M) to the reaction mixture at -78 °C, the whole was stirred for 30 min. Then, the reaction mixture was treated with  $Et_3N$  (8.0 equiv) at -78 °C for 2h. After the reaction mixture was diluted with anhydrous  $Et_2O$ , the filtrate given through  $Na_2SO_4$  column was concentrated under reduced pressure to afford a ketoaldehyde.

### 4.3. General procedure C: Wittig reaction

An anhydrous  $CH_2Cl_2$  solution of ketoaldehyde (0.016M) was treated with phosphonylidene reagent (1.2–2.3 equiv) at room temperature over night. Removal of solvent under reduced pressure gave a crude product, which was further purified by SiO<sub>2</sub> column (*n*-hexane–EtOAc) to furnish a keto- $\alpha$ , $\beta$ -unsaturated ester.

### 4.4. General procedure D: preparation of peroxyhemiacetal

An anhydrous MeOH solution of keto- $\alpha$ , $\beta$ -unsaturated ester (0.025 M) was treated with Sc(OTf)<sub>3</sub> (0.003 M) and H<sub>2</sub>O<sub>2</sub>·H<sub>2</sub>NCONH<sub>2</sub> (7.5 equiv) at room temperature for 48 h. The reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub>, and the resulting residue was removed by column packed with aluminum oxide 90 (neutral, 70–230 mesh, Merck Co. Ltd). Removal of solvent from the filtrate under reduced pressure gave a crude product, which was further purified by SiO<sub>2</sub> column (*n*-hexane–EtOAc) rapidly to furnish a peroxyhemiacetal.

### 4.5. General procedure E: preparation of 3-methoxy-1,2dioxane

Et<sub>2</sub>NH (0.005 M) was added to a CF<sub>3</sub>CH<sub>2</sub>OH solution of peroxyhemiacetal (0.016 M) at 0 °C, and the whole mixture was stirred at room temperature for 8h. Removal of solvent from the whole mixture under reduced pressure gave a product, which was purified by SiO<sub>2</sub> column (*n*-hexane–EtOAc) to furnish a 3-methoxy-1,2-dioxane.

### 4.6. Methyl (E)-2-methyl-6-oxoundec-2-enoate (33)

As described in procedures B and C, **32** (98 mg, 0.63 mmol) was converted to **33** (152 mg, two steps 88%).

Compound **33**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1715, 1646. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.87 (3H, t, J = 7.3 Hz, H-11), 1.20–1.32 and 1.56 (total 6H, m), 1.83 (3H, d, J = 1.2 Hz, 2-CH<sub>3</sub>), 2.38 (2H, t, J = 7.3 Hz, 7-H), 2.41 (2H, td, J = 7.3, 7.3 Hz, 4-H), 2.52 (2H, t, J = 7.3 Hz, 5-H), 3.70 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 6.65 (1H, td, J = 7.3, 1.2 Hz, 3-H). FAB-MS *m*/*z*: 249 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>13</sub>H<sub>22</sub>NaO<sub>3</sub>: 249.1467, found: 249.1475.

# 4.7. Methyl 2-(3-methoxy-3-pentyl-1,2-dioxan-6-yl)propanoate (13)

As described in procedures D and E, **33** (80 mg, 0.35 mmol) was converted to **13** (36 mg, two steps 38%). The  $\alpha$ -methyl analogue **13** was obtained as a diastereo-mixture in a ratio of 1:1. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signals due to 3-OCH<sub>3</sub> and CO<sub>2</sub>CH<sub>3</sub> were definitely separated between the two isomers of 1'-CH<sub>3</sub>. The ratio of *syn* and *anti* isomers was difficult to determine.

Compound **13**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1744. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.87 (3H, t, J = 7.3 Hz, CH<sub>3</sub>-a), 0.88 (3H, t, J = 7.3 Hz, CH<sub>3</sub>-b), 1.22 (3H, d, J = 7.3 Hz, 1'-CH<sub>3</sub>-a), 1.29 (3H, d, J = 6.7 Hz, 1'-CH<sub>3</sub>-b), 1.13–1.44, 1.46–1.68, 1.74–1.89, and 2.15 (total 12H × 2, m), 2.50 (1H, dq, J = 12.2, 7.3 Hz, 1'-Ha), 3.06 (1H, dq, J = 9.8, 6.7 Hz, 1'-Hb), 3.24 (3H, s, 3-OCH<sub>3</sub>-a), 3.27 (3H, s, 3-OCH<sub>3</sub>-b), 3.67 (3H, s, CO<sub>2</sub>CH<sub>3</sub>-a), 3.68 (3H, s, CO<sub>2</sub>CH<sub>3</sub>-b), 4.00 (1H, m, 6-Ha), 4.17 (1H, m, 6-Hb). FAB-MS *m*/*z*: 297 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>14</sub>H<sub>26</sub>NaO<sub>5</sub>: 297.1678, found: 297.1694.

### 4.8. *tert*-Butyl (*E*)-6-oxoundec-2-enoate (34)

As described in procedures B and C, **32** (200mg, 1.3 mmol) was converted to **34** (303 mg, two steps 92%).

Compound **34**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1717, 1655. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.85 (3H, t, J = 6.7 Hz, 11-CH<sub>3</sub>), 1.17–1.31 (4H, m), 1.43 (9H, s, *tert*-Bu), 1.54 (2H, m), 2.36 (2H, t, J = 7.3 Hz, 7-H), 2.40 (2H, td, J = 7.3, 6.7 Hz, 4-H), 2.52 (2H, t, J = 7.3 Hz, 5-H), 5.70 (1H, d, J = 15.8 Hz, 2-H), 6.78 (1H, dt, J = 15.8, 6.7 Hz, 3-H). FAB-MS *m/z*: 277 (M + Na)<sup>+</sup>. FAB-HRMS *m/z*: calcd for C<sub>15</sub>H<sub>26</sub>NaO<sub>3</sub>: 277.1779, found: 277.1779.

# 4.9. *tert*-Butyl 2-(3-methoxy-3-pentyl-1,2-dioxan-6-yl)-acetate (14)

As described in procedures D and E, **34** (100 mg, 0.39 mmol) was converted to **14** (24 mg, two steps 20%).

The *tert*-butyl ester analogue **14** was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-OCH<sub>3</sub> was definitely separated between the two isomers.

Compound 14: colorless oil; *syn:anti* = 5:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1744. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.87 (3H, t, J = 6.7 Hz, CH<sub>3</sub>), 1.20–1.36 and 1.44–1.89 (total 12H, m), 1.43 (9H, s, *tert*-Bu), 2.24 (1H, dd, J = 15.3, 6.1 Hz, 1'-Ha), 2.40 (1H, dd, J = 15.3, 7.3 Hz, 1'-Hb), 3.24 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.26 (3H, s, 3-OCH<sub>3</sub>, *anti*), 4.42 (1H, m, 6-H). FAB-MS *m/z*: 325 (M + Na)<sup>+</sup>. FAB-HRMS *m/z*: calcd for C<sub>16</sub>H<sub>30</sub>NaO<sub>5</sub>: 325.1991, found: 325.1987.

### 4.10. Phenyl 2-(3-methoxy-3-pentyl-1,2-dioxan-6-yl)acetate (15)

A pyridine (0.5 mL) solution of **8** (5 mg, 0.02 mmol) was treated with phenol (2.3 mg, 0.024 mmol) in presence of EDCI·HCl (7.7 mg, 0.04 mmol) at room temperature for 4h. Removal of solvent under reduced pressure gave a crude product, which was further purified by SiO<sub>2</sub> column (*n*-hexane–EtOAc = 10:1) to furnish **15** (6.3 mg, 98%). The phenyl ester analogue **15** was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-OCH<sub>3</sub> was definitely separated between the two isomers.

Compound **15**: colorless oil; *syn:anti* = 4.8:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1763. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.83 (3H, t, *J* = 6.7 Hz, CH<sub>3</sub>), 1.17–1.31, 1.54–1.69, and 1.79–1.90 (total 12H, m), 2.56 (1H, dd, *J* = 15.9, 5.5 Hz, 1'-Ha), 2.68 (1H, dd, *J* = 15.9, 7.8 Hz, 1'-Hb), 3.22 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.25 (3H, s, 3-OCH<sub>3</sub>, *anti*), 4.54 (1H, m, 6-H), 7.03 (2H, d, *J* = 8.5 Hz, Ph), 7.16 (1H, d, *J* = 7.3 Hz, Ph), 7.30 (2H, dd, *J* = 8.5, 7.3 Hz, Ph). FAB-MS *m*/*z*: 345 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>18</sub>H<sub>26</sub>NaO<sub>5</sub>: 345.1678, found: 345.1662.

### 4.11. (E)-Dodec-3-ene-2,7-dione (35)

As described in procedures B and C, **32** (142 mg, 0.91 mmol) was converted to **35** (158 mg, two steps 89%).

Compound **35**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1715, 1676, 1628. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.86 (3H, t, J = 7.3 Hz, 12-CH<sub>3</sub>), 1.19–1.34 (total 4H, m), 1.56 (2H, tt, J = 7.9, 7.3 Hz, 9-H), 2.20 (3H, s, COCH<sub>3</sub>), 2.38 (2H, t, J = 7.3 Hz, 8-H), 2.46 (2H, td, J = 7.3, 6.7 Hz, 5-H), 2.56 (2H, t, J = 7.3 Hz, 6-H), 6.04 (1H, d, J = 15.9 Hz, 3-H), 6.76 (1H, dt, J = 15.9, 6.7 Hz, 4-H). FAB-MS *m*/*z*: 197 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>12</sub>H<sub>21</sub>O<sub>2</sub>: 197.1542, found: 197.1555.

### 4.12. 1-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)propan-2one (16)

As described in procedure D, **35** (10 mg, 0.05 mmol) was converted to **16** (1.0 mg, 8.3%). The methyl ketone analogue **16** was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-OCH<sub>3</sub> was definitely separated between the two isomers.

Compound **16**: colorless oil; *syn:anti* = 12.5:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1717. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.82 (3H, t, J = 6.7Hz, CH<sub>3</sub>), 1.13–1.30 and 1.40–1.63 (total 10H, m), 1.68 (1H, ddd, J = 15.9, 11.4, 4.3 Hz), 1.80 (1H, ddd, J = 12.2, 5.5, 1.8 Hz), 2.12 (3H, s, COCH<sub>3</sub>), 2.36 (1H, dd, J = 16.5, 5.5 Hz, 1'-Ha), 2.57 (1H, dd, J = 16.5, 6.7 Hz, 1'-Hb), 3.19 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.24 (3H, s, 3-OCH<sub>3</sub>, *anti*), 4.43 (1H, m, 6-H). FAB-MS *m*/*z*: 267 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>13</sub>H<sub>24</sub>NaO<sub>4</sub>: 267.1573, found: 267.1573.

### 4.13. (*E*)-1-Phenylundec-2-ene-1,6-dione (38)

NaHMDS (1.0M in THF, 1.54mL, 1.54mmol) was added to the THF solution (3mL) of 1-phenylethan-1one (**36**, 221  $\mu$ L, 1.9mmol) at -78 °C, and the whole was stirred for 30min. Ketoaldehyde (**37**, 297mg, 1.4mmol) in THF (100mL) was added to the reaction mixture at -78 °C, and the whole was stirred for 5h. The reaction mixture was poured into 5% aqueous HCl solution, extracted with EtOAc, washed with brine, and dried over MgSO<sub>4</sub>. Removal of solvent under reduced pressure gave a crude product. The crude product was dissolved in CH<sub>2</sub>Cl<sub>2</sub> (3mL) and treated with MsCl (164  $\mu$ L, 2.1 mmol) and Et<sub>3</sub>N (793  $\mu$ L, 5.7 mmol) at room temperature overnight. The reaction mixture was poured into saturated aqueous NaHCO<sub>3</sub>, extracted with CH<sub>2</sub>Cl<sub>2</sub>, dried over MgSO<sub>4</sub>. Removal of solvent under reduced pressure gave a crude product, which was further purified by SiO<sub>2</sub> column (*n*-hexane–EtOAc = 5:1) to furnish **38** (358 mg, two steps 73%).

Compound **38**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1715, 1671, 1622. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.87 (3H, t, J = 7.3 Hz, 11-CH<sub>3</sub>), 1.20–1.34, and 1.46–1.61 (total 6H, m), 2.41 (2H, t, J = 7.9 Hz, 7-H), 2.55–2.64 (4H, m, 4-H and 5-H), 6.88 (1H, d, J = 15.3 Hz, 2-H), 6.98 (1H, dt, J = 15.3, 6.1 Hz, 6-H), 7.45 (2H, dd, J = 7.3, 6.7 Hz, Ph), 7.54 (1H, d, J = 6.7 Hz, Ph), 7.89 (2H, d, J = 7.3 Hz, Ph). FAB-MS m/z: 259 (M + H)<sup>+</sup>. FAB-HRMS m/z: calcd for C<sub>17</sub>H<sub>23</sub>O<sub>2</sub>: 259.1698, found: 259.1705.

### 4.14. 2-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)-1-phenylethan-1-one (17)

As described in procedure D, 38 (18.3 mg, 0.07 mmol) was converted to 17 (3.6 mg, 17%).

The phenyl ketone analogue 17 was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were determined as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-OCH<sub>3</sub> was definitely separated between the two isomers.

Compound 17: colorless oil; *syn:anti* = 3.9:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1687. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.83 (3H, t, J = 6.7 Hz, CH<sub>3</sub>), 1.16–1.32 and 1.45–1.86 (total 12H, m), 2.87 (1H, dd, J = 16.5, 6.7 Hz, 1'-Ha), 3.17 (1H, dd, J = 16.5, 6.1 Hz, 1'-Hb), 3.21 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.23 (3H, s, 3-OCH<sub>3</sub>, *anti*), 4.64 (1H, m, 6-H), 7.39 (2H, t, J = 7.3 Hz, Ph), 7.50 (1H, t, J = 7.3 Hz, Ph), 7.87 (2H, d, J = 7.3 Hz, Ph). FAB-MS *m*/*z*: 329 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>18</sub>H<sub>26</sub>NaO<sub>4</sub>: 329.1728, found: 329.1732.

### 4.15. General procedure F: preparation of amide analogues having N atom

An amine having N atom (1.0 equiv) was added to the THF solution (0.2 mL) of **39** (0.09 M) at room temperature, and the whole was stirred for 10 min. Removal of solvent under reduced pressure gave a crude product, which was further purified by  $SiO_2$  column (*n*-hexane–EtOAc = 3:1, CHCl<sub>3</sub>–MeOH = 3:1) to furnish an amide analogue having N atom.

The amide analogues (18–26) were obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-OCH<sub>3</sub> was definitely separated between the two isomers. The ratio of *syn* and *anti* zisomers of 21 was difficult to determine.

### 4.16. *N*-[2-(Ethylamino)ethyl]-2-(3-methoxy-3-pentyl-1,2-dioxan-6-yl)acetamide (18)

As described in procedure F, **39** (7.6mg, 0.018 mmol) was converted to **18** (5.2 mg, 91%).

Compound 18: colorless solid; syn:anti = 4.1:1. IR  $v_{max}$ (KBr) cm<sup>-1</sup>: 1649, 1543. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.82 (3H, t, J = 7.3 Hz), 1.09 (3H, t, J = 7.3 Hz), 1.22–1.29, and 1.50–1.85 (total 12H, m), 2.26 (1H, dd, J = 15.3, 4.3 Hz, 1'-Ha), 2.65 (1H, dd, J = 15.3, 7.9 Hz)1'-Hb), 2.32 (2H, q, J = 7.3 Hz, NCH<sub>2</sub>CH<sub>3</sub>), 2.75 (2H, dt, J = 6.1, 3.1 Hz), 3.19 (3H, s, 3-OCH<sub>3</sub>, syn), 3.21  $(3H, s, 3-OCH_3, anti), 3.33$  (2H, dt, J = 5.5, 3.1 Hz),4.37 (1H, m, 6-H), 6.52 (1H, br s, NH). FAB-MS m/z:  $(M + H)^{+}$ . 317 FAB-HRMS m/z: calcd for C<sub>16</sub>H<sub>33</sub>N<sub>2</sub>O<sub>4</sub>: 317.2440, found: 317.2442.

### 4.17. *N*-[2-(Diethylamino)ethyl]-2-(3-methoxy-3-pentyl-1,2-dioxan-6-yl)acetamide (19)

As described in procedure F, **39** (7.6mg, 0.018mmol) was converted to **19** (6.2mg, quant.).

Compound **19**: colorless solid; *syn:anti* = 3.9:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1672, 1541. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.82 (3H, t, J = 7.3 Hz), 1.00–1.12, 1.15–1.29, and 1.49–1.85 (total 18H, m), 2.23 (1H, dd, J = 15.3, 4.9 Hz, 1'-Ha), 2.32 (1H, dd, J = 15.3, 8.5 Hz, 1'-Hb), 2.62 (6H, br s, NCH<sub>2</sub>), 3.19 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.21 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.33 (2H, br s, CONHCH<sub>2</sub>), 4.39 (1H, m, 6-H). FAB-MS *m*/*z*: 345 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>18</sub>H<sub>37</sub>N<sub>2</sub>O<sub>4</sub>: 345.2753, found: 345.2753.

### 4.18. 2-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)-*N*-[2-tetrahydropyridin-1(2*H*)-ylethyl]acetamide (20)

As described in procedure F, **39** (7.6 mg, 0.018 mmol) was converted to **20** (6.4 mg, quant.).

Compound **20**: colorless solid; *syn:anti* = 4.0:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1649, 1541. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.82 (3H, t, J = 6.7 Hz), 1.12–1.28 and 1.30–1.83 (total 18H, m), 2.24 (1H, dd, J = 15.3, 4.3 Hz, 1'-Ha), 2.32 (1H, dd, J = 15.3, 8.5 Hz, 1'-Hb), 2.61 (6H, br s, NCH<sub>2</sub>), 3.19 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.20 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.40 (2H, dt, J = 5.5, 5.5 Hz, CONHCH<sub>2</sub>), 4.40 (1H, m, 6-H). FAB-MS *m*/*z*: 357 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>19</sub>H<sub>37</sub>N<sub>2</sub>O<sub>4</sub>: 357.2753, found: 357.2759.

### 4.19. 2-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)-*N*-[2-tetrahydropyrazin-1(2*H*)-ylethyl]acetamide (21)

As described in procedure F, 39 (7.6mg, 0.018mmol) was converted to 21 (5.4mg, 85%).

Compound **21**: colorless solid. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1651, 1543. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.83 (3H, t, J = 7.1 Hz), 1.10–1.30, 1.46–1.65, and 1.72–1.86 (total 12H, m), 2.26 (1H, dd, J = 15.8, 3.5Hz, 1'-Ha), 2.32 (1H, dd, J = 15.8, 8.7Hz, 1'-Hb), 2.35–2.57 (2H, m, NCH<sub>2</sub>), 2.68 (4H, br s, NCH<sub>2</sub>), 3.11 (4H, br s, NCH<sub>2</sub>), 3.20 (3H, s, 3-OCH<sub>3</sub>), 3.29 (2H, dt, J = 6.3, 5.4Hz, CONHCH<sub>2</sub>), 4.32 (1H, m, 6-H). FAB-MS *m*/*z*: 358 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>18</sub>H<sub>36</sub>N<sub>3</sub>O<sub>4</sub>: 358.2706, found: 358.2713.

### 4.20. 2-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)-*N*-[2-(1,4-oxazinan-4-yl)ethyl]acetamide (22)

As described in procedure F, **39** (7.6mg, 0.018mmol) was converted to **22** (6.4mg, quant.).

Compound **22**: colorless solid; syn:anti = 4.3:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1649, 1541. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.83 (3H, t, J = 6.7 Hz), 1.15–1.40 and 1.47–1.85 (total 12H, m), 2.24 (1H, dd, J = 15.3, 3.7 Hz, 1'-Ha), 2.32 (1H, dd, J = 15.3, 8.5 Hz, 1'-Hb), 2.46 (6H, br s, NCH<sub>2</sub>), 3.20 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.22 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.32 (2H, dt, J = 6.1, 5.5 Hz, CONHCH<sub>2</sub>), 3.68 (4H, br s, CH<sub>2</sub>OCH<sub>2</sub>), 4.36 (1H, m, 6-H). FAB-MS m/z: 359 (M + H)<sup>+</sup>. FAB-HRMS m/z: calcd for C<sub>18</sub>H<sub>35</sub>N<sub>2</sub>O<sub>5</sub>: 359.2545, found: 359.2552.

# 4.21. 2-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)-*N*-(2-phenyl-ethyl)acetamide (23)

As described in procedure F, 39 (7.6 mg, 0.018 mmol) was converted to 23 (6.0 mg, 95%).

Compound **23**: colorless solid; *syn:anti* = 4.2:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1647, 1543. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.83 (3H, t, J = 7.3 Hz), 1.18–1.36 and 1.44–1.86 (total 12H, m), 2.22 (1H, dd, J = 15.9, 4.3 Hz, 1'-Ha), 2.26 (1H, dd, J = 15.9, 8.5 Hz, 1'-Hb), 2.75 (2H, J = 7.3 Hz, CH<sub>2</sub>-Ph), 3.17 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.22 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.44 (2H, m, CONHCH<sub>2</sub>), 4.30 (1H, m, 6-H), 7.10–7.27 (5H, m, Ph). FAB-MS *m*/*z*: 350 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>20</sub>H<sub>31</sub>NNaO<sub>4</sub>: 372.2151, found: 372.2130.

# 4.22. 2-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)-*N*-(2-pyridin-4-ylethyl)acetamide (24)

As described in procedure F, 39 (7.6mg, 0.018mmol) was converted to 24 (5.5mg, 88%).

Compound **24**: colorless solid; *syn:anti* = 3.7:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1651, 1555. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.83 (3H, t, J = 7.3 Hz), 1.16–1.34 and 1.43–1.86 (total 12H, m), 2.25 (2H, d, J = 6.1 Hz, 1'-H), 2.78 (1H, dd, J = 13.4, 6.7 Hz, CH<sub>2</sub>-Py), 2.83 (1H, dd, J = 13.4, 6.7 Hz, CH<sub>2</sub>-Py), 2.83 (1H, dd, J = 13.4, 6.7 Hz, CH<sub>2</sub>-Py), 3.18 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.22 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.42 (1H, ddt, J = 13.4, 6.7 Hz, CONHC*H*<sub>2</sub>), 3.53 (1H, ddt, J = 13.4, 7.3, 6.1 Hz, CONHC*H*<sub>2</sub>), 4.27 (1H, m, 6-H), 7.17 (2H, d, J = 4.9 Hz, Py), 8.46 (2H, d, J = 4.9 Hz, Py). FAB-MS *m/z*: 351 (M + H)<sup>+</sup>. FAB-HRMS *m/z*: calcd for C<sub>19</sub>H<sub>31</sub>N<sub>2</sub>O<sub>4</sub>: 351.2284, found: 351.2284.

# 4.23. *N*-[2-(1*H*-Imidazol-4-yl)ethyl]-2-(3-methoxy-3-pentyl-1,2-dioxan-6-yl)acetamide (25)

As described in procedure F, **39** (7.6mg, 0.018mmol) was converted to **25** (6.1mg, quant.).

Compound **25**: colorless solid; *syn:anti* = 3.8:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1647, 1543. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)

 $\delta$ : 0.82 (3H, t, J = 6.7 Hz), 1.14–1.40 and 1.48–1.86 (total 12H, m), 2.24 (1H, dd, J = 15.3, 4.9 Hz, 1'-Ha), 2.29 (1H, dd, J = 15.3, 7.9 Hz, 1'-Hb), 2.73 (1H, ddd,J = 15.3, 6.7, 6.1 Hz, CH<sub>2</sub>-Imi), 2.79 (1H, ddd,  $J = 15.3, 7.3, 6.7 \text{ Hz}, \text{ CH}_2\text{-Imi}$ , 3.19 (3H, s, 3-OCH<sub>3</sub>, syn), 3.22 (3H, s, 3-OCH<sub>3</sub>, anti), 3.38 (1H, ddd, J = 12.8, 7.3, 6.7 Hz, CONHC $H_2$ ), 3.48 (1H, ddd,  $J = 12.8, 6.7, 6.1 \text{ Hz}, \text{ CONHC}H_2), 4.38 (1H, m, 6-H),$ 6.77 (1H, br s, Imi), 7.54 (1H, br s, Imi). FAB-MS m/z:  $(M + H)^{+}$ . 340 FAB-HRMS m|z: calcd for C<sub>17</sub>H<sub>30</sub>N<sub>3</sub>O<sub>4</sub>: 340.2236, found: 340.2236.

### 4.24. 2-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)-*N*-[4-[{2-(3-methoxy-3-pentyl-1,2-dioxan-6-yl)acetylamino}propyl]tetrahydropyrazin-1-(2*H*)-yl]propylacetamide (26)

As described in procedure F, **39** (7.6 mg, 0.018 mmol) was converted to **26** (12 mg, quant.).

Compound **26**: colorless solid; *syn:anti* = 3.9:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1647, 1541. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.82 (3H × 2, t, J = 6.7Hz), 1.13–1.40 and 1.48–1.86 (total 28H, m), 2.21 (1H × 2, dd, J = 15.3, 4.3 Hz, 1'-Ha), 2.26 (1H × 2, dd, J = 15.3, 7.9 Hz, 1'-Hb), 2.34–2.76 (12H, br s, NCH<sub>2</sub>), 3.19 (3H × 2, s, 3-OCH<sub>3</sub>, *syn*), 3.21 (3H × 2, s, 3-OCH<sub>3</sub>, *anti*), 3.23–3.35 (2H × 2, m, CONHCH<sub>2</sub>), 4.37 (1H × 2, m, 6-H). FAB-MS *m*/*z*: 657 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>34</sub>H<sub>65</sub>N<sub>4</sub>O<sub>8</sub>: 657.4803, found: 657.4796.

#### 4.25. Methyl (E)-6-cyclohexyl-6-oxohex-2-enoate (41)

As described in procedures A, B, and C, 40 (150 mg, 1.7 mmol) was converted to 41 (171 mg, four steps 45%).

Compound **41**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1714, 1659. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.11–1.23 and 1.60–1.83 (total 10H, m), 2.30 (1H, tt, J = 11.0, 3.7 Hz, 7-H), 2.43 (2H, dt, J = 7.3, 6.7 Hz, 4-H), 2.57 (2H, t, J = 7.3Hz, 5-H), 3.69 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 5.80 (1H, d, J = 15.9 Hz, 2-H), 6.91 (1H, dt, J = 15.9, 6.7 Hz, 3-H). FAB-MS *m*/*z*: 255 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>13</sub>H<sub>21</sub>O<sub>3</sub>: 225.1491, found: 225.1486.

### 4.26. Methyl 2-(3-cyclohexyl-3-methoxy-1,2-dioxan-6-yl)-acetate (27)

As described in procedures D and E, **41** (100 mg, 0.45 mmol) was converted to **27** (1.3 mg, two steps 1.1%). The 3-cyclohexyl analogue **27** was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-OCH<sub>3</sub> was definitely separated between the two isomers.

Compound **27**: colorless oil; *syn:anti* = 15:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1728. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.93–1.38 and 1.42–1.86 (total 15H, m), 2.35 (1H, dd, J = 15.9, 5.5 Hz, 1'-Ha), 2.48 (1H, dd, J = 15.9, 7.6 Hz, 1'-Hb), 3.24 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.26 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.68 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 4.42 (1H, m, 6-H). FAB-MS *m*/*z*: 295 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>14</sub>H<sub>24</sub>NaO<sub>5</sub>: 295.1521, found: 295.1538.

### 4.27. Methyl (E)-6-oxo-6-phenylhex-2-enoate (42)

As described in procedures A, B, and C, **40** (150mg, 1.7mmol) was converted to **42** (234mg, four steps 63%).

Compound **42**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1730, 1576. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 2.64 (2H, dt, J = 7.3, 7.3 Hz, 4-H), 3.13 (2H, t, J = 7.3 Hz, 5-H), 3.70 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 5.88 (1H, d, J = 15.9 Hz, 2-H), 7.02 (1H, dt, J = 15.9, 7.3 Hz, 3-H), 7.45 (2H, t, J = 7.3 Hz, Ph), 7.55 (1H, t, J = 7.3 Hz, Ph), 7.93 (2H, d, J = 7.3 Hz, Ph). FAB-MS *m*/*z*: 219 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>13</sub>H<sub>15</sub>O<sub>3</sub>: 219.1021, found: 219.1035.

### 4.28. Methyl 2-(3-methoxy-3-phenyl-1,2-dioxan-6-yl)acetate (28)

As described in procedures D and E, 42 (11 mg, 0.051 mmol) was converted to 28 (5.7 mg, two steps 42%). The 3-phenyl analogue 28 was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-OCH<sub>3</sub> was definitely separated between the two isomers.

Compound **28**: colorless oil; *syn:anti* = 3.8:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1742. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.69, 1.83, 1.95, and 2.09 (total 4H, m), 2.44 (1H, dd, J = 15.9, 4.9 Hz, 1'-Ha), 2.68 (1H, dd, J = 15.9, 7.9 Hz, 1'-Hb), 3.15 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.17 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.72 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 4.65 (1H, m, 6-H), 7.25–7.41 (5H, m, Ph). FAB-MS *m*/*z*: 289 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>14</sub>H<sub>18</sub>NaO<sub>5</sub>: 289.1052, found: 289.1055.

### 4.29. Methyl (E)-6-oxo-7-phenylhept-2-enoate (43)

As described in procedures A, B, and C, 40 (150 mg, 1.7 mmol) was converted to 43 (232 mg, four steps 59%).

Compound **43**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1717, 1601. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 2.47 (2H, dt, J = 7.3, 6.7 Hz, 4-H), 2.64 (2H, t, J = 7.3 Hz, 5-H), 3.73 (2H, s, CH<sub>2</sub>Ph), 3.74 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 5.81 (1H, d, J = 15.9 Hz, 2-H), 6.91 (1H, dt, J = 15.9, 6.7 Hz, 3-H), 7.23 (2H, t, J = 7.3 Hz, Ph), 7.31 (1H, t, J = 7.3 Hz, Ph), 7.37 (2H, d, J = 7.3 Hz, Ph). FAB-MS *m/z*: 233 (M + H)<sup>+</sup>. FAB-HRMS *m/z*: calcd for C<sub>14</sub>H<sub>17</sub>O<sub>3</sub>: 233.1177, found: 233.1185.

### 4.30. Methyl 2-(3-benzyl-3-methoxy-1,2-dioxan-6-yl)acetate (29)

As described in procedures D and E, 43 (7.1mg, 0.031 mmol) was converted to 29 (2.4mg, two steps 28%). The 3-benzyl analogue 29 was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-CH<sub>3</sub> was definitely separated between the two isomers.

Compound **29**: colorless oil; *syn:anti* = 4:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1742. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.55–1.83 (4H, m), 2.39 (1H, dd, J = 15.9, 5.5 Hz, 1'-Ha), 2.53 (1H, dd, J = 15.9, 7.3 Hz, 1'-Hb), 2.74 and 3.02 (1H × 2, d, J = 14.0 Hz, CH<sub>2</sub>Ph), 3.50 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.51 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.73 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 4.48 (1H, m, 6-H), 7.21–7.36 (5H, m, Ph). FAB-MS *m*/*z*: 303 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>15</sub>H<sub>20</sub>NaO<sub>5</sub>: 303.1209, found: 303.1190.

#### 4.31. Methyl (2*E*)-6-oxoundeca-2,10-dienoate (44)

As described in procedures A, B, and C, 40 (150mg, 1.7 mmol) was converted to 44 (275 mg, four steps 77%).

Compound **44**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1720, 1653. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.62 and 1.99 (total 4H, m), 2.35 (2H, t, J = 7.3 Hz, 7-H), 2.40 (2H, dt, J = 7.3, 6.7 Hz, 4-H), 2.49 (2H, t, J = 7.3 Hz, 5-H), 3.65 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 4.91 (1H, d-like, J =ca. 10 Hz, CH=CH<sub>2</sub>), 4.94 (1H, d-like, J = ca. 17 Hz, CH=CH<sub>2</sub>), 5.69 (1H, ddt, J = 17.1, 10.4, 6.7 Hz, CH=CH<sub>2</sub>), 5.76 (1H, d, J = 15.9 Hz, 2-H), 6.86 (1H, dt, J = 15.9, 6.7 Hz, 3-H). FAB-MS *m*/*z*: 211 (M + H)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>12</sub>H<sub>19</sub>O<sub>3</sub>: 211.1334, found: 211.1343.

# 4.32. Methyl 2-[3-methoxy-3-(pent-4-enyl)-1,2-dioxan-6-yl]acetate (30)

As described in procedures D and E, 44 (9.8 mg, 0.047 mmol) was converted to 30 (7.0 mg, two steps 58%). The 3-pentenyl analogue 30 was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-CH<sub>3</sub> was definitely separated between the two isomers.

Compound **30**: colorless oil; *syn:anti* = 4.8:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1744. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 1.07–1.47 and 1.55–1.90 (total 8H, m), 2.04 (2H, td, J = 7.3, 6.7 Hz, CH<sub>2</sub>CH=CH<sub>2</sub>), 2.35 (1H, dd, J = 15.8, 5.5 Hz, 1'-Ha), 2.48 (1H, dd, J = 15.9, 7.3 Hz, 1'-Hb), 3.23 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.26 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.68 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 4.47 (1H, m, 6-H), 4.95 (1H, dd, J = 9.8, 1.8 Hz, CH=CH<sub>2</sub>), 5.00 (1H, dd, J = 15.3, 1.8 Hz, CH=CH<sub>2</sub>), 5.75 (1H, ddt, J = 15.3, 9.8, 6.7 Hz, CH=CH<sub>2</sub>). FAB-MS *m*/*z*: 281 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>13</sub>H<sub>22</sub>NaO<sub>5</sub>: 281.1365, found: 281.1359.

### 4.33. Methyl (2*E*,9*R*)-9,13-dimethyl-6-oxotetradeca-2,12dienoate (45)

As described in procedures A, B, and C, **40** (150mg, 1.7mmol) was converted to **45** (328mg, four steps 69%).

Compound **45**: colorless oil. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1717, 1659. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.84 (3H, d, J = 6.1 Hz, 9-CH<sub>3</sub>), 1.08–1.41 and 1.85–2.03 (total 7H, m), 1.57 and 1.65 [3H × 2, s, CH=C(CH<sub>3</sub>)<sub>2</sub>], 2.36 (1H, dt, J = 15.3, 6.1 Hz, 7-Ha), 2.39 (1H, dt, J = 15.3, 7.5 Hz, 7-Hb), 2.44 (2H, td, J = 7.3, 6.7 Hz, 4-H), 2.55

(2H, t, J = 7.3 Hz, 5-H), 3.69 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 5.05 [1H, t, J = 7.3 Hz,  $CH = C(CH_3)_2$ ], 5.81 (1H, d, J = 15.9 Hz, 2-H), 6.91 (1H, dt, J = 15.9, 6.7 Hz, 3-H). FAB-MS *m*/*z*: 303 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>17</sub>H<sub>28</sub>NaO<sub>3</sub>: 303.1936, found: 303.1924.

### 4.34. Methyl 2-[3-(3*R*)-(3,7-dimethyloct-6-enyl)-3-methoxy-1,2-dioxan-6-yl]acetate (31)

As described in procedures D and E, 45 (12mg, 0.043 mmol) was converted to 31 (7.2mg, two steps 51%). The 3-citronellyl analogue 31 was obtained as a mixture of *syn* and *anti* isomers. The physicochemical data were analyzed as a mixture. In the <sup>1</sup>H NMR spectra, only the signal due to 3-CH<sub>3</sub> was definitely separated between the two isomers.

Compound **31**: colorless oil; *syn:anti* = 6.8:1. IR  $v_{max}$  (KBr) cm<sup>-1</sup>: 1744. <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$ : 0.85 (3H, d, J = 6.1 Hz, CH<sub>3</sub>), 1.03–1.44 and 1.50–2.03 (total 13H, m), 1.58 and 1.66 [3H × 2, s, CH=C(CH<sub>3</sub>)<sub>2</sub>], 2.36 (1H, dd, J = 15.9, 5.5 Hz, 1'-Ha), 2.49 (1H, dd, J = 15.9, 7.9 Hz, 1'-Hb), 3.23 (3H, s, 3-OCH<sub>3</sub>, *syn*), 3.26 (3H, s, 3-OCH<sub>3</sub>, *anti*), 3.68 (3H, s, CO<sub>2</sub>CH<sub>3</sub>), 4.46 (1H, m, 6-H), 5.06 [1H, t, J = 5.5 Hz, CH=C(CH<sub>3</sub>)<sub>2</sub>]. FAB-MS *m*/*z*: 351 (M + Na)<sup>+</sup>. FAB-HRMS *m*/*z*: calcd for C<sub>18</sub>H<sub>32</sub>NaO<sub>5</sub>: 351.2148, found: 351.2165.

### 4.35. In vitro testing

A strain of *P. falciparum* (FCR3, cycloguanil-resistant from Gambia) was used in sensitivity test. After synchronization by the sorbitol treatment, 50 µL of the parasite culture at the ring stage (0.55%) parasitemia and 2% hematocrit) was added to each well in 96-well microculture plates. The test samples were dissolved in DMSO and diluted to the appropriate concentration using complete medium, then  $50\,\mu\text{L}$  of each sample solution was inoculated. After incubation at 37°C for 48h, the proliferation of P. falciparum was assessed by Giemsa-stained smear by observing 10,000 erythrocytes per one thin blood film in triplicate. Quinine was used as a reference anti-malarial. In this antimalarial assay, quinine inhibited the proliferation of P. falciparum in a concentration-dependent manner with IC<sub>50</sub> of 40 ng/mL and IC<sub>90</sub> of 90 ng/mL. Cytotoxicity was evaluated by the colorimetric MTT assay, in which mitomycin C used as a positive control showed the IC<sub>50</sub> of  $0.1 \,\mu$ g/mL.

### 4.36. Analysis for the stability of esters in mouse serum

Each sample  $(30 \mu L \text{ of } 0.1 \text{ mg/mL} \text{ solution})$  was treated with the fresh mouse serum  $(300 \mu L)$  and incubated at 37 °C for 0, 30, 60, 180, 300 min, respectively. After extraction of the reaction mixture with EtOAc, each extract was concentrated under reduced pressure. The residue was dissolved with  $120 \mu L$  of *n*-hexane–EtOAc (1:1), then an aliquot  $(10 \mu L \text{ for } 7 \text{ and } 14, 5 \mu L \text{ for } 15)$ of the solution was analyzed by SiO<sub>2</sub>-phase HPLC (column: YMC-Pack SIL-06 4.6 mm i.d. × 150 mm, mobile phase: *n*-hexane–EtOH = 200:1, flow rate: 0.5 mL/min, detection: UV 220 nm) to determine the remaining amounts of the test samples by the absolute calibration method in triplicate.

### 4.37. In vivo testing

In vivo anti-malarial activities of the compounds were determined by the 4-day suppressive test using mice infected with P. berghei (NK 65 strain). Five-week-old ddY female mice obtained in sterile containers from Charles River Breeding Laboratories Inc. (Yokohama, Japan) weighing 24–27g were used. They were housed under a natural day-night (12h each) cycle at 25°C. The mice were randomly assigned to treated groups and housed in cages each containing five individuals. Parasites are collected by cardiac puncture from a donor mouse harboring about 15% parasitemia. The blood is diluted with one-seventh volume of 3.2% trisodium citrate solution, then a final concentration of the infected erythrocytes was adjusted to  $5 \times 10^6$  by adding 0.9% NaCl solution. Initially, each mouse was inoculated intravenously in the tail vein with  $1 \times 10^6$  parasitized erythrocytes in 0.2mL of the infected suspension. Test compounds were prepared at doses of 1, 3, 10, and 30 mg/kg in dimethylsulfoxide and administrated by 0.1 mL once a day from day 0 to day 3. The first administration of the test compound started intraperitoneally 2h after parasite inoculation. Parasitemia levels were determined on day 4. To evaluate the anti-malarial activity of the compounds, we prepared tail blood smears and stained them with Giemsa (E. Merck, Germany). Total  $1 \times 10^4$  erythrocytes per one thin blood film were examined under microscopy. On day 4, parasitemia of control mice were between 30% and 35%. The suppression of parasitemia was calculated by the formula: [(average % of parasitemia for control - average % of parasitemia for treated mice)/average % parasitemia for control]  $\times 100$ . Five infected and dimethylsulfoxide-dosed mice were used as a control. The data are determined from the five individuals in duplicate.

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#### **References and notes**

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- 15. In a previous report,<sup>6</sup> we have clarified that the relative and absolute configuration of 3-methoxy-1,2-dioxane moiety have little participation in the anti-malarial activity. So, all in vitro and in vivo testing were executed as a mixture of isomers.