Available online at www.sciencedirect.com

SCIENCE DIRECT®

Bioorganic & Medicinal Chemistry

Bioorganic & Medicinal Chemistry 12 (2004) 5297–5307

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

Motoyuki Kawanishi,[a] Naoyuki Kotoku,[a] Sawako Itagaki,[b] Toshihiro Horii[b] and Motomasa Kobayashi[a,*]

[a]*Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamada-oka, Suita, Osaka 565-0871, Japan*
[b]*Research Institute for Microbial Diseases, Osaka University, 3-1 Yamada-oka, Suita, Osaka 565-0871, Japan*

Received 5 April 2004; revised 29 April 2004; accepted 29 April 2004
Available online 11 September 2004

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 among them, and the imidazole analogue **25** showed desirable in vivo anti-malarial activity against *P. berghei* infected mice.

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.[1] Due to the emergence and ongoing spread of the chloroquine-resistant strains of *Plasmodium falciparum*,[2] 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).[3] In this context, we studied the structure–activity relationship (SAR) of the cyclic peroxide, methyl 2-(3-methoxy-3-pentyl-1,2-dioxan-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 $Sc(OTf)_3$-mediated peroxyhemiacetalization and intramolecular Michael addition.[5] Furthermore, the synthetic peroxide **7** was found to exhibit higher

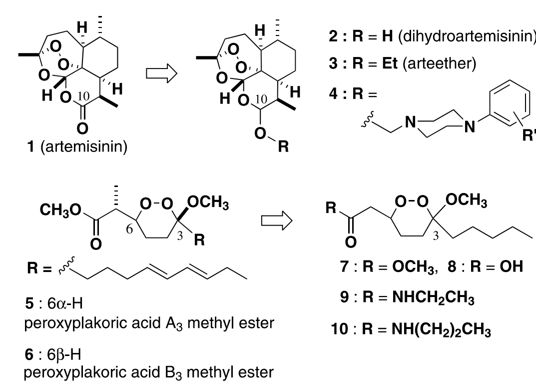


Chart 1.

selectivity index against *P. falciparum* than those of **5** and **6**.[6] 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.[7] 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.
* Corresponding author. Tel.: +81 6 6879 8215; fax: +81 6 6879 8219; e-mail: kobayasi@phs.osaka-u.ac.jp

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.04.051

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_{50} 0.54 μM, **10**: 0.31 μM) in comparison with that of **7** (**7**: IC_{50} 0.12 μM) 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,[7] three ester analogues: α-methyl analogue **13**, *tert*-butyl ester analogue **14**, and phenyl ester analogue **15**, with steric hindrance 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,[6] which was derived from ketoalcohol **32** by Swern oxidation, with methyl 2-(triphenyl-λ^5-phosphanylidene)propionate[8] or commercially available *tert*-butyl (triphenyl-λ^5-phosphanylidene)acetate furnished keto-α,β-unsaturated esters (**33**: 88%, **34**: 92% for two steps), respectively. Conversion from the keto-α,β-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 $Sc(OTf)_3$ and subsequent

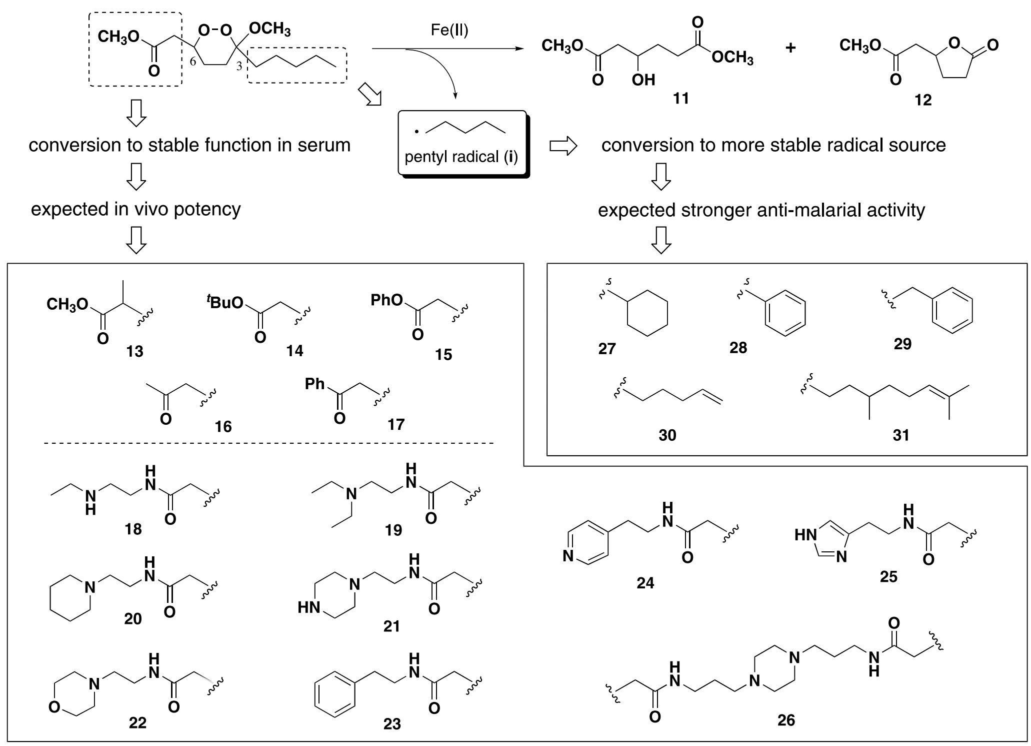
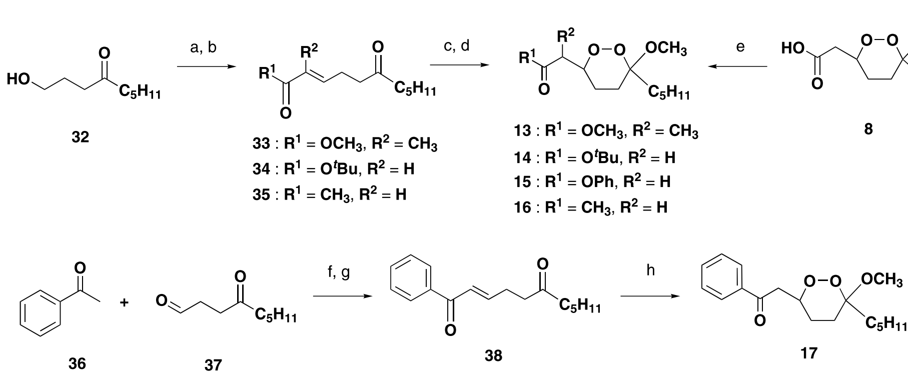


Chart 2. Design for structure–activity relationship study of spongean peroxides.

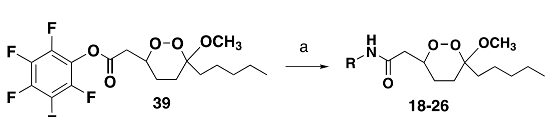


Scheme 1. Reagents and conditions: (a) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2; (b) $Ph_3P{=}C(R^2)COR^1$, CH_2Cl_2, two steps 88% for **33**, two steps 92% for **34**, two steps 89% for **35**; (c) $H_2O_2{\cdot}H_2NCONH_2$, $Sc(OTf)_3$, MeOH, 8.3% for **16**; (d) Et_2NH, CF_3CH_2OH, two steps 38% for **13**, two steps 20% for **14**; (e) PhOH, EDCI·HCl, pyridine, 98% for **15**; (f) NaHMDS, THF, −78 °C; (g) MsCl, Et_3N, CH_2Cl_2, two steps 73%; (h) $H_2O_2{\cdot}H_2NCONH_2$, $Sc(OTf)_3$, MeOH, 17%.

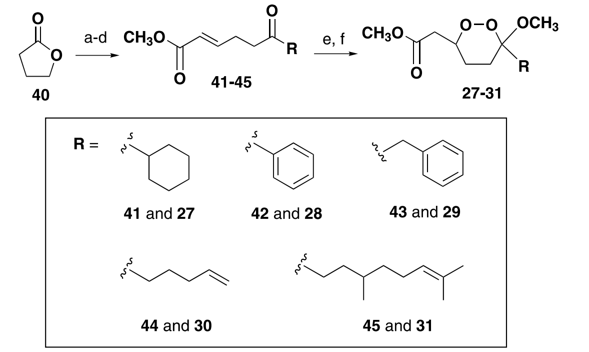
intramolecular Michael addition of the peroxyhemiacetal provided two corresponding ester analogues (**13**: 38%, **14**: 20% for two steps), respectively. Treatment of the ketoaldehyde with commercially available 1-(triphenyl-λ^5-phosphanylidene)propan-2-one furnished a keto-α,β-unsaturated ketone **35** in 89% yield. Then, **35** was further converted to a methyl ketone **16** by peroxyhemiacetalization in the presence of $Sc(OTf)_3$. 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-α,β-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,[9] which is the characteristic acidic organelle (pH 5.0–5.4) in the malaria parasite.[10,11] 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[12] 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.[13] 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 $FeSO_4$ as a model experiment in the food vacuole, **11** and **12** were afforded as major products (Chart 2).[14] 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 anti-malarial 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,[6] 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**, 3-phenyl analogue **28**, 3-benzyl analogue **29**, and two analogues (**30**, **31**) with an olefin portion (Chart 2).



Scheme 2. Reagents and conditions: (a) $R\text{-}NH_2$, 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_2AlCl, CH_2Cl_2; (b) BrMg-R, THF; (c) DMSO, $(COCl)_2$, Et_3N, CH_2Cl_2; (d) $Ph_3P{=}CHCOOCH_3$, CH_2Cl_2, 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) $H_2O_2{\cdot}H_2NCONH_2$, $Sc(OTf)_3$, MeOH; (f) Et_2NH, CF_3CH_2OH, 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 γ-butyrolactone **40**, with each Grignard reagent provided the corresponding ketoalcohols. The Swern oxidation of the ketoalcohols and subsequent Wittig reaction with methyl (triphenyl-λ^5-phosphanylidene)acetate furnished the corresponding keto-α,β-unsaturated esters (**41**: 45%, **42**: 63%, **43**: 59%, **44**: 77%, **45**: 69% for four steps). The peroxyhemiacetalization of the keto-α,β-unsaturated esters (**41–45**) in the presence of $Sc(OTf)_3$ and subsequent intramolecular Michael addition provided the corresponding analogues possessing a different alkyl moiety at the C-3 position of the 3-methoxy-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.[15] 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	R^1	R^2	IC_{50} (μM)		Selectivity index
			P. falciparum	KB 3-1	
13	CH_3O	Me	0.26	26	100
14	*t*-BuO	H	0.12	70	583
15	PhO	H	>3.1	N. T.	
16	Me	H	>4.1	N. T.	
17	Ph	H	>3.3	N. T.	
7	CH_3O	H	0.12	43	360
5			0.15	21	140
6			0.12	28	230

N.T.: not tested.

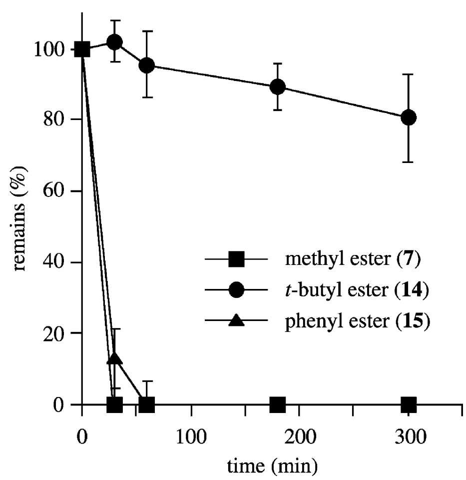


Figure 1. Stability of esters (**7**, **14**, and **15**).

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 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 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.

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

Compd	IC_{50} (μM)		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_{50} (mg/kg) *P. berghei*	*T*/*C*[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

[a] 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.

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 μg/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_{50} (μM)		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.

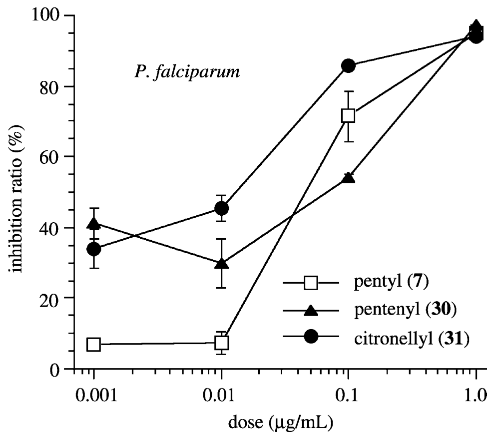


Figure 2. In vitro anti-malarial activity of analogues (**7**, **30**, and **31**).

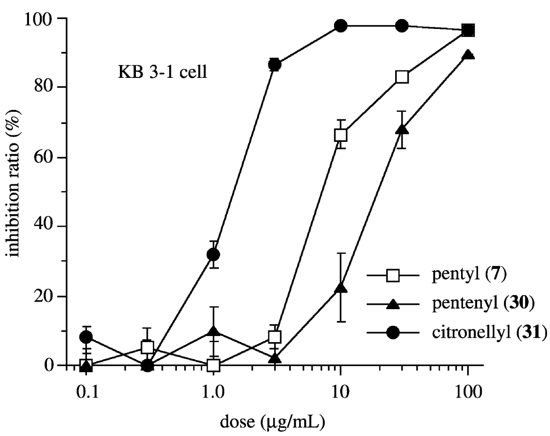


Figure 3. In vitro cytotoxic activity of analogues (**7**, **30**, and **31**).

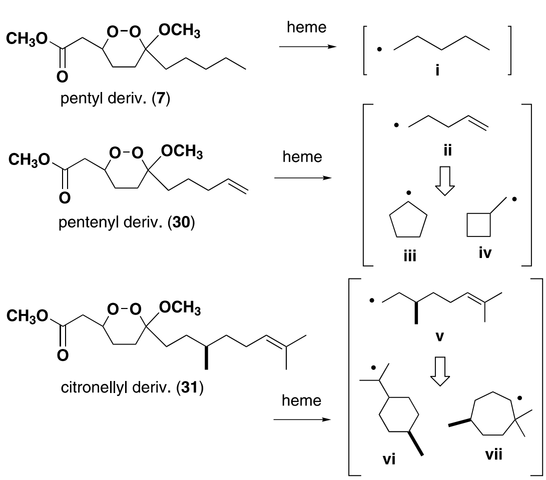


Chart 3. Plausible radical production from 3-methoxy-1,2-dioxane moiety.

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 ^{1}H NMR ($CDCl_3$ 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, $60F_{254}$) 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_2SO_4: 25 mL, AcOH: 5 mL, EtOH: 425 mL) with subsequent heating.

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

Me_2AlCl (1.0 M in *n*-hexane, 1.9 equiv) was treated with the anhydrous CH_2Cl_2 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_2Cl_2 solution of γ-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 $CHCl_3$, and the resulting residue was removed by Celite column. The filtrate was extracted with $CHCl_3$, dried over $MgSO_4$, 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 30 min. The reaction mixture was quenched with 25% aqueous H_2SO_4 at 0 °C, and the whole was stirred for 10 min at room temperature. The reaction mixture was poured into saturated aqueous $NaHCO_3$, and the whole was extracted with EtOAc. The EtOAc extract was washed with saturated aqueous NaCl and dried over $MgSO_4$. Removal of solvent from the EtOAc extract under reduced pressure gave a crude product, which was further purified by SiO_2 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 $(COCl)_2$ (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 2 h. 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 (0.016 M) 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_2 column (*n*-hexane–EtOAc) to furnish a keto-α,β-unsaturated ester.

4.4. General procedure D: preparation of peroxyhemiacetal

An anhydrous MeOH solution of keto-α,β-unsaturated ester (0.025 M) was treated with $Sc(OTf)_3$ (0.003 M) and $H_2O_2{\cdot}H_2NCONH_2$ (7.5 equiv) at room temperature for 48 h. The reaction mixture was diluted with CH_2Cl_2, 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_2 column (*n*-hexane–EtOAc) rapidly to furnish a peroxyhemiacetal.

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

Et_2NH (0.005 M) was added to a CF_3CH_2OH solution of peroxyhemiacetal (0.016 M) at 0 °C, and the whole mixture was stirred at room temperature for 8 h. Removal of solvent from the whole mixture under reduced pressure gave a product, which was purified by SiO_2 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 ν_{max} (KBr) cm^{-1}: 1715, 1646. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 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_3), 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_2CH_3), 6.65 (1H, td, $J = 7.3$, 1.2 Hz, 3-H). FAB-MS *m*/*z*: 249 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{13}H_{22}NaO_3$: 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 α-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 ^{1}H NMR spectra, only the signals due to 3-OCH_3 and CO_2CH_3 were definitely separated between the two isomers of 1′-CH_3. The ratio of *syn* and *anti* isomers was difficult to determine.

Compound **13**: colorless oil. IR ν_{max} (KBr) cm^{-1}: 1744. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.87 (3H, t, $J = 7.3$ Hz, CH_3-a), 0.88 (3H, t, $J = 7.3$ Hz, CH_3-b), 1.22 (3H, d, $J = 7.3$ Hz, 1′-CH_3-a), 1.29 (3H, d, $J = 6.7$ Hz, 1′-CH_3-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_3-a), 3.27 (3H, s, 3-OCH_3-b), 3.67 (3H, s, CO_2CH_3-a), 3.68 (3H, s, CO_2CH_3-b), 4.00 (1H, m, 6-Ha), 4.17 (1H, m, 6-Hb). FAB-MS *m*/*z*: 297 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{14}H_{26}NaO_5$: 297.1678, found: 297.1694.

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

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

Compound **34**: colorless oil. IR ν_{max} (KBr) cm^{-1}: 1717, 1655. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, t, $J = 6.7$ Hz, 11-CH_3), 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 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{15}H_{26}NaO_3$: 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 ^{1}H NMR spectra, only the signal due to 3-OCH_3 was definitely separated between the two isomers.

Compound **14**: colorless oil; *syn*:*anti* = 5:1. IR ν_{max} (KBr) cm^{-1}: 1744. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.87 (3H, t, $J = 6.7$ Hz, CH_3), 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_3, *syn*), 3.26 (3H, s, 3-OCH_3, *anti*), 4.42 (1H, m, 6-H). FAB-MS *m*/*z*: 325 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{16}H_{30}NaO_5$: 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 4 h. Removal of solvent under reduced pressure gave a crude product, which was further purified by SiO_2 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 ^{1}H NMR spectra, only the signal due to 3-OCH_3 was definitely separated between the two isomers.

Compound **15**: colorless oil; *syn*:*anti* = 4.8:1. IR ν_{max} (KBr) cm^{-1}: 1763. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.83 (3H, t, J = 6.7 Hz, CH_3), 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_3, *syn*), 3.25 (3H, s, 3-OCH_3, *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 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{18}H_{26}NaO_5$: 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 ν_{max} (KBr) cm^{-1}: 1715, 1676, 1628. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.86 (3H, t, J = 7.3 Hz, 12-CH_3), 1.19–1.34 (total 4H, m), 1.56 (2H, tt, J = 7.9, 7.3 Hz, 9-H), 2.20 (3H, s, $COCH_3$), 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 $(M + H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{12}H_{21}O_2$: 197.1542, found: 197.1555.

4.12. 1-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)propan-2-one (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 ^1H NMR spectra, only the signal due to 3-OCH_3 was definitely separated between the two isomers.

Compound **16**: colorless oil; *syn*:*anti* = 12.5:1. IR ν_{max} (KBr) cm^{-1}: 1717. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.82 (3H, t, J = 6.7 Hz, CH_3), 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, $COCH_3$), 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_3, *syn*), 3.24 (3H, s, 3-OCH_3, *anti*), 4.43 (1H, m, 6-H). FAB-MS *m*/*z*: 267 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{13}H_{24}NaO_4$: 267.1573, found: 267.1573.

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

NaHMDS (1.0 M in THF, 1.54 mL, 1.54 mmol) was added to the THF solution (3 mL) of 1-phenylethan-1-one (**36**, 221 μL, 1.9 mmol) at −78 °C, and the whole was stirred for 30 min. Ketoaldehyde (**37**, 297 mg, 1.4 mmol) in THF (100 mL) was added to the reaction mixture at −78 °C, and the whole was stirred for 5 h. The reaction mixture was poured into 5% aqueous HCl solution, extracted with EtOAc, washed with brine, and dried over $MgSO_4$. Removal of solvent under reduced pressure gave a crude product. The crude product was dissolved in CH_2Cl_2 (3 mL) and treated with MsCl (164 μL, 2.1 mmol) and Et_3N (793 μL, 5.7 mmol) at room temperature overnight. The reaction mixture was poured into saturated aqueous $NaHCO_3$, extracted with CH_2Cl_2, dried over $MgSO_4$. Removal of solvent under reduced pressure gave a crude product, which was further purified by SiO_2 column (*n*-hexane–EtOAc = 5:1) to furnish **38** (358 mg, two steps 73%).

Compound **38**: colorless oil. IR ν_{max} (KBr) cm^{-1}: 1715, 1671, 1622. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.87 (3H, t, J = 7.3 Hz, 11-CH_3), 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 $(M + H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{17}H_{23}O_2$: 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 ^1H NMR spectra, only the signal due to 3-OCH_3 was definitely separated between the two isomers.

Compound **17**: colorless oil; *syn*:*anti* = 3.9:1. IR ν_{max} (KBr) cm^{-1}: 1687. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.83 (3H, t, J = 6.7 Hz, CH_3), 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_3, *syn*), 3.23 (3H, s, 3-OCH_3, *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 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{18}H_{26}NaO_4$: 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, $CHCl_3$–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 ^1H NMR spectra, only the signal due to 3-OCH_3 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** (7.6 mg, 0.018 mmol) was converted to **18** (5.2 mg, 91%).

Compound **18**: colorless solid; *syn*:*anti* = 4.1:1. IR ν_{max} (KBr) cm^{-1}: 1649, 1543. ^1H NMR (500 MHz, $CDCl_3$) δ: 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_2CH_3), 2.75 (2H, dt, $J = 6.1$, 3.1 Hz), 3.19 (3H, s, 3-OCH_3, *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*: 317 $(M + H)^+$. FAB-HRMS *m/z*: calcd for $C_{16}H_{33}N_2O_4$: 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** (7.6 mg, 0.018 mmol) was converted to **19** (6.2 mg, quant.).

Compound **19**: colorless solid; *syn*:*anti* = 3.9:1. IR ν_{max} (KBr) cm^{-1}: 1672, 1541. ^1H NMR (500 MHz, $CDCl_3$) δ: 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_2), 3.19 (3H, s, 3-OCH_3, *syn*), 3.21 (3H, s, 3-OCH_3, *anti*), 3.33 (2H, br s, $CONHCH_2$), 4.39 (1H, m, 6-H). FAB-MS *m/z*: 345 $(M + H)^+$. FAB-HRMS *m/z*: calcd for $C_{18}H_{37}N_2O_4$: 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 ν_{max} (KBr) cm^{-1}: 1649, 1541. ^1H NMR (500 MHz, $CDCl_3$) δ: 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_2), 3.19 (3H, s, 3-OCH_3, *syn*), 3.20 (3H, s, 3-OCH_3, *anti*), 3.40 (2H, dt, $J = 5.5$, 5.5 Hz, $CONHCH_2$), 4.40 (1H, m, 6-H). FAB-MS *m/z*: 357 $(M + H)^+$. FAB-HRMS *m/z*: calcd for $C_{19}H_{37}N_2O_4$: 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** (7.6 mg, 0.018 mmol) was converted to **21** (5.4 mg, 85%).

Compound **21**: colorless solid. IR ν_{max} (KBr) cm^{-1}: 1651, 1543. ^1H NMR (500 MHz, $CDCl_3$) δ: 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, $J = 15.8$, 3.5 Hz, 1′-Ha), 2.32 (1H, dd, $J = 15.8$, 8.7 Hz, 1′-Hb), 2.35–2.57 (2H, m, NCH_2), 2.68 (4H, br s, NCH_2), 3.11 (4H, br s, NCH_2), 3.20 (3H, s, 3-OCH_3), 3.29 (2H, dt, $J = 6.3$, 5.4 Hz, $CONHCH_2$), 4.32 (1H, m, 6-H). FAB-MS *m/z*: 358 $(M + H)^+$. FAB-HRMS *m/z*: calcd for $C_{18}H_{36}N_3O_4$: 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** (7.6 mg, 0.018 mmol) was converted to **22** (6.4 mg, quant.).

Compound **22**: colorless solid; *syn*:*anti* = 4.3:1. IR ν_{max} (KBr) cm^{-1}: 1649, 1541. ^1H NMR (500 MHz, $CDCl_3$) δ: 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_2), 3.20 (3H, s, 3-OCH_3, *syn*), 3.22 (3H, s, 3-OCH_3, *anti*), 3.32 (2H, dt, $J = 6.1$, 5.5 Hz, $CONHCH_2$), 3.68 (4H, br s, CH_2OCH_2), 4.36 (1H, m, 6-H). FAB-MS *m/z*: 359 $(M + H)^+$. FAB-HRMS *m/z*: calcd for $C_{18}H_{35}N_2O_5$: 359.2545, found: 359.2552.

4.21. 2-(3-Methoxy-3-pentyl-1,2-dioxan-6-yl)-*N*-(2-phenylethyl)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 ν_{max} (KBr) cm^{-1}: 1647, 1543. ^1H NMR (500 MHz, $CDCl_3$) δ: 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_2-Ph), 3.17 (3H, s, 3-OCH_3, *syn*), 3.22 (3H, s, 3-OCH_3, *anti*), 3.44 (2H, m, $CONHCH_2$), 4.30 (1H, m, 6-H), 7.10–7.27 (5H, m, Ph). FAB-MS *m/z*: 350 $(M + H)^+$. FAB-HRMS *m/z*: calcd for $C_{20}H_{31}NNaO_4$: 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** (7.6 mg, 0.018 mmol) was converted to **24** (5.5 mg, 88%).

Compound **24**: colorless solid; *syn*:*anti* = 3.7:1. IR ν_{max} (KBr) cm^{-1}: 1651, 1555. ^1H NMR (500 MHz, $CDCl_3$) δ: 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_2-Py), 2.83 (1H, dd, $J = 13.4$, 6.7 Hz, CH_2-Py), 3.18 (3H, s, 3-OCH_3, *syn*), 3.22 (3H, s, 3-OCH_3, *anti*), 3.42 (1H, ddt, $J = 13.4$, 6.7, 6.7 Hz, $CONHCH_2$), 3.53 (1H, ddt, $J = 13.4$, 7.3, 6.1 Hz, $CONHCH_2$), 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 $(M + H)^+$. FAB-HRMS *m/z*: calcd for $C_{19}H_{31}N_2O_4$: 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** (7.6 mg, 0.018 mmol) was converted to **25** (6.1 mg, quant.).

Compound **25**: colorless solid; *syn*:*anti* = 3.8:1. IR ν_{max} (KBr) cm^{-1}: 1647, 1543. ^1H NMR (500 MHz, $CDCl_3$)

δ: 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_2-Imi), 2.79 (1H, ddd, J = 15.3, 7.3, 6.7 Hz, CH_2-Imi), 3.19 (3H, s, 3-OCH_3, *syn*), 3.22 (3H, s, 3-OCH_3, *anti*), 3.38 (1H, ddd, J = 12.8, 7.3, 6.7 Hz, CONHCH_2), 3.48 (1H, ddd, J = 12.8, 6.7, 6.1 Hz, CONHCH_2), 4.38 (1H, m, 6-H), 6.77 (1H, br s, Imi), 7.54 (1H, br s, Imi). FAB-MS *m*/*z*: 340 $(M + H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{17}H_{30}N_3O_4$: 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 ν_{max} (KBr) cm^{-1}: 1647, 1541. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.82 (3H × 2, t, J = 6.7 Hz), 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_2), 3.19 (3H × 2, s, 3-OCH_3, *syn*), 3.21 (3H × 2, s, 3-OCH_3, *anti*), 3.23–3.35 (2H × 2, m, CONHCH_2), 4.37 (1H × 2, m, 6-H). FAB-MS *m*/*z*: 657 $(M + H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{34}H_{65}N_4O_8$: 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 ν_{max} (KBr) cm^{-1}: 1714, 1659. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 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 = 7.3 Hz, 5-H), 3.69 (3H, s, CO_2CH_3), 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 $(M + H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{13}H_{21}O_3$: 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 ^{1}H NMR spectra, only the signal due to 3-OCH_3 was definitely separated between the two isomers.

Compound **27**: colorless oil; *syn*:*anti* = 15:1. IR ν_{max} (KBr) cm^{-1}: 1728. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 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_3, *syn*), 3.26 (3H, s, 3-OCH_3, *anti*), 3.68 (3H, s, CO_2CH_3), 4.42 (1H, m, 6-H). FAB-MS *m*/*z*: 295 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{14}H_{24}NaO_5$: 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** (150 mg, 1.7 mmol) was converted to **42** (234 mg, four steps 63%).

Compound **42**: colorless oil. IR ν_{max} (KBr) cm^{-1}: 1730, 1576. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 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_2CH_3), 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 $(M + H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{13}H_{15}O_3$: 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 ^{1}H NMR spectra, only the signal due to 3-OCH_3 was definitely separated between the two isomers.

Compound **28**: colorless oil; *syn*:*anti* = 3.8:1. IR ν_{max} (KBr) cm^{-1}: 1742. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 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_3, *anti*), 3.17 (3H, s, 3-OCH_3, *syn*), 3.72 (3H, s, CO_2CH_3), 4.65 (1H, m, 6-H), 7.25–7.41 (5H, m, Ph). FAB-MS *m*/*z*: 289 $(M + Na)^+$. FAB-HRMS *m*/*z*: calcd for $C_{14}H_{18}NaO_5$: 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 ν_{max} (KBr) cm^{-1}: 1717, 1601. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 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_2Ph), 3.74 (3H, s, CO_2CH_3), 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 $(M + H)^+$. FAB-HRMS *m*/*z*: calcd for $C_{14}H_{17}O_3$: 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** (7.1 mg, 0.031 mmol) was converted to **29** (2.4 mg, 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 ^{1}H NMR spectra, only the signal due to 3-CH_3 was definitely separated between the two isomers.

Compound **29**: colorless oil; *syn*:*anti* = 4:1. IR ν_{max} (KBr) cm^{-1}: 1742. ^1H NMR (500 MHz, $CDCl_3$) δ: 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_2Ph), 3.50 (3H, s, 3-OCH_3, *syn*), 3.51 (3H, s, 3-OCH_3, *anti*), 3.73 (3H, s, CO_2CH_3), 4.48 (1H, m, 6-H), 7.21–7.36 (5H, m, Ph). FAB-MS *m/z*: 303 $(M + Na)^+$. FAB-HRMS *m/z*: calcd for $C_{15}H_{20}NaO_5$: 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** (150 mg, 1.7 mmol) was converted to **44** (275 mg, four steps 77%).

Compound **44**: colorless oil. IR ν_{max} (KBr) cm^{-1}: 1720, 1653. ^1H NMR (500 MHz, $CDCl_3$) δ: 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_2CH_3), 4.91 (1H, d-like, J = ca. 10 Hz, CH=CH_2), 4.94 (1H, d-like, J = ca. 17 Hz, CH=CH_2), 5.69 (1H, ddt, $J = 17.1$, 10.4, 6.7 Hz, CH=CH_2), 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 $(M + H)^+$. FAB-HRMS *m/z*: calcd for $C_{12}H_{19}O_3$: 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 ^1H NMR spectra, only the signal due to 3-CH_3 was definitely separated between the two isomers.

Compound **30**: colorless oil; *syn*:*anti* = 4.8:1. IR ν_{max} (KBr) cm^{-1}: 1744. ^1H NMR (500 MHz, $CDCl_3$) δ: 1.07–1.47 and 1.55–1.90 (total 8H, m), 2.04 (2H, td, $J = 7.3$, 6.7 Hz, CH_2CH=CH_2), 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_3, *syn*), 3.26 (3H, s, 3-OCH_3, *anti*), 3.68 (3H, s, CO_2CH_3), 4.47 (1H, m, 6-H), 4.95 (1H, dd, $J = 9.8$, 1.8 Hz, CH=CH_2), 5.00 (1H, dd, $J = 15.3$, 1.8 Hz, CH=CH_2), 5.75 (1H, ddt, $J = 15.3$, 9.8, 6.7 Hz, CH=CH_2). FAB-MS *m/z*: 281 $(M + Na)^+$. FAB-HRMS *m/z*: calcd for $C_{13}H_{22}NaO_5$: 281.1365, found: 281.1359.

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

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

Compound **45**: colorless oil. IR ν_{max} (KBr) cm^{-1}: 1717, 1659. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.84 (3H, d, $J = 6.1$ Hz, 9-CH_3), 1.08–1.41 and 1.85–2.03 (total 7H, m), 1.57 and 1.65 [3H × 2, s, CH=C(CH_3)$_2$], 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_2CH_3), 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 $(M + Na)^+$. FAB-HRMS *m/z*: calcd for $C_{17}H_{28}NaO_3$: 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** (12 mg, 0.043 mmol) was converted to **31** (7.2 mg, 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 ^1H NMR spectra, only the signal due to 3-CH_3 was definitely separated between the two isomers.

Compound **31**: colorless oil; *syn*:*anti* = 6.8:1. IR ν_{max} (KBr) cm^{-1}: 1744. ^1H NMR (500 MHz, $CDCl_3$) δ: 0.85 (3H, d, $J = 6.1$ Hz, CH_3), 1.03–1.44 and 1.50–2.03 (total 13H, m), 1.58 and 1.66 [3H × 2, s, CH=C(CH_3)$_2$], 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_3, *syn*), 3.26 (3H, s, 3-OCH_3, *anti*), 3.68 (3H, s, CO_2CH_3), 4.46 (1H, m, 6-H), 5.06 [1H, t, $J = 5.5$ Hz, CH=C$(CH_3)_2$]. FAB-MS *m/z*: 351 $(M + Na)^+$. FAB-HRMS *m/z*: calcd for $C_{18}H_{32}NaO_5$: 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 µL of each sample solution was inoculated. After incubation at 37 °C for 48 h, 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 anti-malarial assay, quinine inhibited the proliferation of *P. falciparum* in a concentration-dependent manner with IC_{50} of 40 ng/mL and IC_{90} of 90 ng/mL. Cytotoxicity was evaluated by the colorimetric MTT assay, in which mitomycin C used as a positive control showed the IC_{50} of 0.1 µg/mL.

4.36. Analysis for the stability of esters in mouse serum

Each sample (30 µL of 0.1 mg/mL solution) was treated with the fresh mouse serum (300 µL) 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 µL of *n*-hexane–EtOAc (1:1), then an aliquot (10 µL for **7** and **14**, 5 µL for **15**) of the solution was analyzed by SiO_2-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 24–27 g were used. They were housed under a natural day–night (12 h 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 5×10^6 by adding 0.9% NaCl solution. Initially, each mouse was inoculated intravenously in the tail vein with 1×10^6 parasitized erythrocytes in 0.2 mL 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 2 h 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 1×10^4 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.

Acknowledgements

This study was financially supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports, and Culture of Japan.

References and notes

1. Sachs, J.; Malaney, P. *Nature* **2002**, *415*, 680.
2. WHO *Wkly. Epidemiol. Rec.* **1997**, *72*, 269.
3. Jefford, C. W. *Curr. Med. Chem.* **2001**, *8*, 1803.
4. Kobayashi, M.; Kondo, K.; Kitagawa, I. *Chem. Pharm. Bull.* **1993**, *41*, 1324.
5. Murakami, N.; Kawanishi, M.; Itagaki, S.; Horii, T.; Kobayashi, M. *Tetrahedron Lett.* **2001**, *42*, 7281.
6. Murakami, N.; Kawanishi, M.; Itagaki, S.; Horii, T.; Kobayashi, M. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 69.
7. Murakami, N.; Kawanishi, M.; Mostaqul, H. M.; Li, J.; Itagaki, S.; Horii, T.; Kobayashi, M. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4081.
8. Werkhoven, T. M.; Nispen, R.; Lugtenburg, J. *Eur. J. Org. Chem.* **1999**, 2909.
9. Meshnick, S. R.; Jefford, C. W.; Posner, G. H.; Avery, M. A.; Peters, W. *Parasitol. Today* **1996**, *12*, 79.
10. Rosenthal, P. J.; Meshnick, S. R. *Mol. Biochem. Parasitol.* **1996**, *83*, 131.
11. Akompong, T.; Kadekoppala, M.; Harrison, T.; Oksman, A.; Goldberg, D. E.; Fujioka, H.; Samuel, B. U.; Sullivan, D.; Haldar, K. *J. Biol. Chem.* **2002**, *277*, 28923.
12. Hindley, S.; Ward, S. A.; Storr, R. C.; Searle, N. L.; Bray, P. G.; Park, B. K.; Davies, J.; O'Neill, P. M. *J. Med. Chem.* **2002**, *45*, 1052.
13. Roach, M. P.; Ozaki, S.; Watanabe, Y. *Biochemistry* **2000**, *39*, 1446.
14. Murakami, N.; Kawanishi, M.; Itagaki, S.; Horii, T.; Kobayashi, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3515.
15. In a previous report,[6] 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.