Structure–Activity Relationship of Anti-malarial Allylpyrocatechol Isolated from *Piper betle*

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Malaria disease remains a serious worldwide health problem. In South-East Asia, one of the malaria infection “hot-spots,” medicinal plants such as *Piper betle* have traditionally been used for the treatment of malaria, and allylpyrocatechol (1), a constituent of *P. betle*, has been shown to exhibit anti-malarial activities. In this study, we verified that 1 showed in vivo anti-malarial activity through not only intraperitoneal (i.p.) but also peroral (p.o.) administration. Additionally, some analogs of 1 were synthesized and the structure–activity relationship was analyzed to disclose the crucial sub-structures for the potent activity.

**Key words** anti-malarial; allylpyrocatechol; *Piper betle*; 4-d suppressive test

**Introduction**

Malaria disease is an infection induced by protozoa *Plasmodium* sp. and was estimated to account for 228 million cases and 405000 deaths worldwide in 2018. Even though this was slightly lower than the number of cases reported in 2010 (239 million cases), the policy to effectively suppress global malaria has not been going well, judging by the 217 and 219 million malaria cases in 2016 and 2017, respectively. Children younger than 5 years old are most susceptible to malaria infection and account for about 60% of victims. Malaria is chiefly distributed around tropical and subtropical zones, with more than 90% of malaria cases being found in the African region. The part of Africa with the highest incidence of cases, often called sub-Saharan Africa, has shown an increase in malaria cases and deaths in 2017 compared to 2016, whereas India was the only country exhibiting a significant decrease. WHO has warned that nearly half of the world population has been exposed to the risk of malaria, including not only sub-Saharan Africa, but also all of Africa, South-East Asia, the Eastern Mediterranean, the Western Pacific, and the Americas. Moreover, the risk of malaria spread to other regions by travelers cannot be overlooked. In order to overcome malaria, many kinds of drugs have been developed, but in the present state of affairs, all patients and all people living in the regions at risk cannot benefit from them. The current anti-malarial drugs are better at preventing malaria infection than curing the disease. The artemisinin-based combination therapy is recommended as a treatment against malaria disease, but exacerbation and resistance still remain as major issues. Furthermore, although a possible vaccine has been developed through the great efforts of many researchers, it has not entered the test phase yet.1–4 Under these circumstances, there continues to be a strong need for new anti-malarials, and thus research into the control of malaria must be continued.

We have been engaged in the search for anti-malarial agents from natural sources. In a previous study, we reported the isolation of allylpyrocatechol (1) as the principle anti-malarial compound from *Piper betle*, a medicinal plant used for treatment of malaria, and found that palmitic acid enhanced the anti-malarial activity of 1 synergistically.5 In this study, we investigate the in vivo anti-malarial effect of 1 to clarify the potential of 1 and the structure–activity relationship by using synthetic analogs to disclose the key parts of the structure for the potent anti-malarial activity.

**Results and Discussion**

Traditional medicinal plants such as *Piper betle* have been used to treat malaria disease in South-East Asia, one of the epidemic areas of malaria. In addition, methanolic leaf extract of *P. betle* has been reported to exhibit effective anti-malarial activity.6 In a previous study, we used bioassay-guided separation to isolate the anti-malarial compounds from the MeOH extract of leaves of *P. betle*. In brief, MeOH extract was divided into four fractions using a synthetic resin, Diaion HP-20, followed by sequential column chromatographies using the following as elution solvents: H2O, 50% aqueous MeOH, MeOH, and acetone. The MeOH elution showing the strongest activity was subjected to SiO2 column chromatography with an n-hexane–EtOAc gradient solvent system to furnish allylpyrocatechol (1) as an active principle in n-hexane–EtOAc = 7:3 elution (Fig. S1). In order to examine the in vivo anti-malarial activity of 1, a 4-d suppressive test using a mouse malaria parasite, *Plasmodium berghei*, was carried out. Through the intraperitoneal administration, 1 showed about 70% inhibition of the parasitemia (i.e., the percentage of infected cells) of *P. berghei* at a dose of 100 mg/kg and more than 50% inhibition even at 12.5 mg/kg (Table 1). Moreover, 1 still exhibited about 40% inhibition by peroral treatment at a dose of 200 mg/kg (Table 2). It was notable that 1 did not cause any toxic symptoms, such as diarrhea or weight loss, via either route of administration at any dose. Thus, the results revealed that 1 has promise as a potential new anti-malarial agent with sufficient efficacy through peroral (p.o.) administration.

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Next, we planned to analyze the structure–activity relationships using synthetic analogs in order to clarify the crucial functional groups and sub-structures for the anti-malarial activity of 1. We designed two analogs possessing a methyl group on the hydroxy moiety (2, 3) to assess the importance of hydroxy moieties on the aromatic ring. To disclose the role of the catechol structure, analogs bearing different numbers or positions of hydroxy moieties (4, 5, 6) on the aromatic ring were designed. In addition, in order to investigate the contribution of the allyl group, we prepared the allyl-lacking (7), alkyl group-saturated (8), differently-positioned allyl (9), 1,1'-dimethyl (10), and prolonged chain (11–14) analogs (Fig. 1).

Among the designed analogs, eugenol (2) and catechol (7) are available on the Japanese market. At first, the introduction of an allyl moiety to 7 was performed by the treatment of allyl chloride with Na₂CO₃ in the presence of a catalytic amount of CuCl to afford 1.⁹ The obtained 1 was subjected to methylation by trimethylsilyldiazomethane under a basic condition to give chavibetol (3), although the regio-selectivity and the yield were insubstantial. In the same manner as for the preparation for 1, resorcinol (15) was converted to 4 by allyl chloride, Na₂CO₃, and CuCl as the catalyst. On the other hand, 4-allylanisol (16) and 4-allyl-2,6-dimethoxyphenol (17) were also commercially available, and were subjected to de-methylation by BBr₃ to furnish 5 and 6 in moderate yield, respectively (Chart 1).

The syntheses of the analogs bearing a catechol structure are depicted in Chart 2. The saturated analog, 4-propylcatechol (8), was readily prepared from 1 through the hydrogenation catalyzed by palladium on carbon, quantitatively. The preparation of 3-allyl catechol (9) was accomplished by Claisen rearrangement of allyl ether 18 synthesized from

Table 1. In Vivo Anti-malarial Activity of 1 in Mice Infected with P. berghei through i.p. Administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Parasitemia (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>31.9 ± 3.0</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>8.5 ± 2.3</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>10.3 ± 0.1</td>
<td>68</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>12.2 ± 2.7</td>
<td>62</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>14.2 ± 0.5</td>
<td>56</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>5</td>
<td>15.6 ± 0.0</td>
<td>51</td>
</tr>
</tbody>
</table>

*Data represent the ratio of infection on day 4 as means ± standard deviation (S.D.) (n = 5).

Table 2. In Vivo Anti-malarial Activity of 1 in Mice Infected with P. berghei through p.o. Administration

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Parasitemia (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td></td>
<td>43.7 ± 1.9</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>200</td>
<td>25.0 ± 0.4</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30.1 ± 0.7</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>25.9 ± 0.7</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>32.0 ± 1.5</td>
<td>27</td>
</tr>
<tr>
<td>Artemisinin</td>
<td>15</td>
<td>17.2 ± 0.5</td>
<td>61</td>
</tr>
</tbody>
</table>

*Data represent the ratio of infection on day 4 as means ± S.D. (n = 5).

Fig. 1. Designed Analogs for Structure–Activity Relationship of Anti-malarial Allylpyrocatechol 1

Chart 1. Preparation for Analogs of 1 Bearing Allylphenol Sub-structure
7 through allylation under the basic condition. In case of the 1’,1’-dimethyl analog (10), 3,4-dihydroxyphenylacetic acid (19) as a starting material was converted into the corresponding methyl ester, and then both of its hydroxy groups were protected as 2-tetrahydropyranyl (THP) ether. 10) The introduction of two methyl moieties into the benzyl position of methyl ester 20 was achieved one-by-one through repeated treatment with methyl iodide in the presence of lithium diisopropylamide (LDA) to furnish 21. Subsequently, the carbomethoxy group in 21 was reduced by lithium aluminium hydride followed by oxidation by Dess–Martin periodinane (DMP) to give the corresponding aldehyde 22. After a one-carbon increase through a Wittig reaction using methyltriphenylphosphonium bromide, two THPs were finally deprotected by p-toluenesulfonic acid (TsOH) to furnish 10 in 17% yield from 20. In addition, the prolonged chain analogs were synthesized from 20, which was prepared on the way to 10. In a manner similar to the transformation from 21 to 22, methyl ester 20 was converted into aldehyde 24. The common intermediate 24 was treated with the corresponding Wittig salt under the basic condition to build up the alkene side chains. However, the typical condition using sodium hexamethyldisilazide (NaHMDS) as a base produced only Z-olefin when we attempted to prepare the trans-congener 11. For this reason, Schlosser’s methodology,11) known as the modified Wittig reaction for the selective construction of E-olefin, was applied. As a result, the desired trans-alkene side chain was obtained even though the yield and the selectivity remained to be improved. Lastly, the THP groups on the phenolic hydroxy moieties were removed to afford 11, 12, 13, and 14, respectively. The E or Z configuration of 11 and 12 was determined by the chemical shift of the methyl moiety in 13C-NMR spectra; the methyl signal of Z congener was observed at a higher magnetic field because of the steric compression.

The anti-malarial in vitro potency of the synthetic analogs was evaluated against the Plasmodium falciparum CDC-I strain (cycloguanil-resistant) by measuring the lactate dehydrogenase (LDH) activity of the parasites, and the data are displayed in Table 3 and Fig. S2 as IC50 (µM) values. Reduction of anti-malarial activity was observed by the introduction of a methyl group into either of the catechol hydroxy moieties (Table 3, 2, 3). Additionally, the increase nor the decrease of number of aromatic hydroxy moieties also derived lower potency as well as the alteration of their arrangement. This attenuation was prone to be extreme when the hydroxy one at the meta-position from allyl substitution was disrupted (Table 3, 4–6). Meanwhile, among the congeners possessing catechol partial structures, the analogs without the allyl chain and with the saturated alkyl group showed less activity than 1 by one digit of IC50, whereas the analog 9, which carried the allyl chain in the different position, exhibited almost the same potency as 1. This analog 9 had a phenolic hydroxy moiety at the meta-position of the allyl group, and therefore it was consistent with the above observation that the meta-position between the hydroxy and the allyl functions played an important role in the potent anti-malarial activity. The introduction of the two methyl groups at the benzyl position on the allyl moiety had little influence on the activity (Table 3, 7–10). Moreover, the analogs with the extended alkenyl chain generally presented a similar potency to 1. Although the analog possessing the additional C1-unit with a Z-configuration showed slightly weaker activity, those having C1, C4, or C8-units as E-isomers displayed more potent effects than 1. It was observed that the longer side chain tended to give a little more potency, which might have depended on the permeability into the parasites infecting in red blood cells according to the lipophilicity of the compounds (Table 3, Fig. S2).

Hence, the catechol sub-structure and the unsaturated...
side chain were revealed to be important for the potent anti-malarial activity. The phenolic hydroxy group at the meta-position of the alkenyl chain was thought to be especially essential. However, the induction of methyl groups, the elongation, and the E–Z geometry about the allyl chain were found to have almost no effect on the activity.

In conclusion, we disclosed that allylpyrocatechol 1, the main anti-malarial compound in the medicinal plant *Piper betle*, was a promising anti-malarial agent in an in vivo experiment. Moreover, through the study of structure–activity relationship by using the synthetic analogs of 1, the crucial structural features for potent anti-malarial activity were clarified. The typical approved anti-malarial agents are majorly classified into quinoline derivatives such as quinine, chloroquine, and mefloquine, and benzimidazole relatives such as proguanil, and peroxide agents such as artemisinin, artemether and artesunate. Apparently, allylpyrocatechol analogs do not belong to any of these categories, thus our study is believed to provide the novel type of anti-malarials. Further chemical modifications to search for derivatives of 1 with much greater potency and investigations of the mode of action of 1 will be needed to develop more comprehensive anti-malarials, which are now ongoing.

### Experimental

**Evaluation for in Vivo Anti-malarial Activity**  All animal experiments were approved by the ethics committee of the Research Institute for Microbial Diseases, Osaka University and carried out in accordance with the relevant guidelines. *In vivo* anti-malarial activity of 1 was determined in mice infected with *P. berghei* (NK65 strain) in the 4-d suppressive test.[5] Five-week-old ICR male mice (Charles River Breeding Laboratories, Inc., Japan) were housed under a natural day–night cycle at 25°C with free access to diet and water. After 1 d adaptation, they were selected which weight were ca. 25 g and randomly assigned to treated groups (n = 5). The test compound 1 was prepared at doses of 12.5, 25, 50, and 100 mg/kg in dimethylsulfoxide (DMSO) for intraperitoneally (i.p.) and 25, 50, 100, and 200 mg/kg in 0.5% aqueous carboxymethylcellulose for *p.o.* As a positive control, artemisinin (5 mg/kg for i.p. and 15 mg/kg for *p.o.*) was used.

Five animals were treated with each dose. The parasites were collected by cardiac puncture from a donor mouse harboring about 20% parasitemia. After treatment with 3.2% trisodium citrate solution (14% v/v) as anticoagulant, the collected blood was diluted with D-PBS (−) to a final concentration of 1 × 10⁶ infected erythrocytes per 0.2 mL. Each mouse was inoculated intravenously in the tail vein with 0.2 mL of infected suspension with 1 × 10⁶ parasitized erythrocytes per one thin blood film. The suppression of parasitemia for each dose of 1 was calculated by the formula: [1 – (average % of parasitemia in treated mice/average % of parasitemia in control <vehicle treatment>)] × 100 (%). The data shown are the mean values from five mice in one test.

**Preparation of Allylpyrocatechol (1) from Catechol (7)**  To a solution of catechol (7, 200 mg, 1.8 mmol) in H₂O (0.5 mL) were added CuCl (3.5 mg, 0.036 mmol), allyl chloride (0.146 mL, 1.8 mmol), and 14% aqueous Na₂CO₃ (0.32 mL), successively. After stirring for 30 min at room temperature, brine was added to reaction mixture then the whole was washed with brine and dried over MgSO₄. The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure to afford the residue (231 mg). The crude product (33 mg) was purified by HPLC (column: Cosmosil 5C₁₈ AR (10 mm i.d. × 250 mm, Nacalai Tesque, Inc.), mobile phase: 60% aqueous MeOH, flow rate: 4.0 mL/min, detection: UV (λ = 250 nm)) to give 1 (21 mg, 54%). The obtained products was identified by the previously reported spectroscopic data.[12]

**Preparation of Chavibetol (3) from 1**  To a solution of allylpyrocatechol (1, 11.2 mg, 0.075 mmol) in CH₃CN–MeOH (9:1, 0.3 mL) were added Pr₂NEt (0.025 mL, 0.113 mmol) and TMSC–HN₃ (2.0 M in n-hexane, 0.057 mL, 0.113 mmol) at 0°C. After stirring overnight at room temperature, EtOAc was added to reaction mixture then the whole was washed

### Table 3. *In Vitro* Anti-malarial Activity of Analogs of 1

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ (µM)</th>
<th>Position</th>
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<tbody>
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<td></td>
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<td>6</td>
</tr>
<tr>
<td>1</td>
<td>3.5</td>
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<tr>
<td>2</td>
<td>370</td>
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<td>3</td>
<td>43</td>
<td>H</td>
</tr>
<tr>
<td>4</td>
<td>590</td>
<td>H</td>
</tr>
<tr>
<td>5</td>
<td>310</td>
<td>H</td>
</tr>
<tr>
<td>6</td>
<td>21</td>
<td>OH</td>
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<td>7</td>
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<td>H</td>
</tr>
<tr>
<td>14</td>
<td>2.5</td>
<td>H</td>
</tr>
</tbody>
</table>
with 5% HCl, saturated aqueous NaHCO₃, and brine, sequentially. The organic layer was dried over MgSO₄, filtered and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (n-hexane–EtOAc = 5:1) and subsequently HPLC [column: Cosmosil SSL (10 mm i.d. × 250 mm, Nacalai Tesque, Inc.), mobile phase: n-hexane–EtOAc = 5:1, flow rate: 4.0 mL/min, detection: UV (λ = 250 nm)] to give 3 (5.7 mg, 46%). The obtained products was identified by the previously reported spectroscopic data.¹⁵

Preparation of 4-Alllylresorcinol (4) from Resorcinol (15) Resorcinol (15, 130 mg, 1.2 mmol) was converted to 4 (110 mg, 62%) in the same manner as preparation of I. The obtained products was identified by the previously reported spectroscopic data.¹⁴

Preparation of 4-Alllylphenol (5) from 4-Alllylanisol (16) To a solution of 4-alllylanisol (16, 100 mg, 0.67 mmol) in CH₂Cl₂ (1.0 mL) was added BBr₃ (1.0 M in CH₂Cl₂, 1.0 mL, 1.0 mmol) at room temperature. After stirring for 30 min at room temperature, the reaction was quenched by adding H₂O. The mixture was extracted with EtOAc (10.8 mg, 46%). The obtained products was identified by the previously reported spectroscopic data.¹⁵

Preparation of 3-Allylcatechol (9) from 7 A solution of the crude product in CH₂Cl₂ (4.0 mL) were added pyridinium p-toluenesulphonate (PPTS, 35.5 mg, 0.14 mmol) and 3,4-dihydro-2H-pyran (DHP, 2.2 mL, 25.4 mmol) at room temperature. After stirring for 12 h at room temperature, the reaction was quenched by adding brine. The mixture was extracted with EtOAc (×3) and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (n-hexane–EtOAc = 9:1) to give 5 (76 mg, 85%). The obtained products was identified by the previously reported spectroscopic data.¹⁵

Preparation of 5-Allyl-1,2,3-trihydroxybenzene (6) from Methyl Ether 17 In the same manner as preparation of 5, 5-allyl-1,3-dimethoxy-2-hydroxybenzene (17, 45 mg, 0.23 mmol) was converted to 6 (26 mg, 68%).

Preparation of 4-Propylcatechol (8) from 1 To a solution of allylpyrocatechol (1, 6.7 mg, 0.044 mmol) in MeOH (0.3 mL) was added 10% palladium on carbon (Pd–C) (2.0 mg) at room temperature. After stirring for 1 h at room temperature under H₂ atmosphere, the reaction mixture was filtered through Celite. The filtrate was concentrated under reduced pressure to afford the crude product, which was purified by silica gel column chromatography (EtOAc only) to give 8 (6.8 mg, quant.). The obtained products was identified by the previously reported spectroscopic data.¹²

Preparation of 3-Allyl catechol (9) from 7 To a solution of catechol (7, 141 mg, 0.13 mmol) in acetone (2.0 mL) were added K₂CO₃ (10.6 mg, 0.078 mmol) and allyl bromide (0.012 mL, 0.14 mmol) at room temperature. After stirring for 18 h at 50°C, the reaction was quenched by adding saturated aqueous NH₄Cl. The mixture was extracted with EtOAc (×3) and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (n-hexane–EtOAc = 20:1) to give 18 (19.8 mg, 91%). The obtained products was identified by the previously reported spectroscopic data.¹⁶

A solution of 18 (18.7 mg, 0.12 mmol) in N,N-dimethylformamide (DMF, 0.15 mL) was stirred for 10 h at 150°C. After cooling to room temperature, H₂O was added to the mixture. The whole was extracted with EtOAc (×3) and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (n-hexane–EtOAc = 10:1) and subsequently HPLC [column: Cosmosil 5SIL (10 mm i.d. × 250 mm, Nacalai Tesque, Inc.), mobile phase: n-hexane–EtOAc = 5:1, flow rate: 4.0 mL/min, detection: UV (λ = 250 nm)] to give 9 (10.8 mg, 58%). The obtained products was identified by the previously reported spectroscopic data.¹⁷

Preparation of Methyl Ester 20 from 19 To a solution of 19 (238 mg, 1.41 mmol) in MeOH (4.0 mL) with conc. H₂SO₄ (0.1 mL) was stirred for 1 h at 60°C. After cooling to room temperature, EtOAc was added to reaction mixture, then the whole was washed with saturated aqueous NaHCO₃ and brine, sequentially. The organic layer was dried over MgSO₄, filtered and concentrated under reduced pressure to yield a crude product. To a solution of the crude product in CH₂Cl₂ (4.0 mL) were added pyridinium p-toluenesulphonate (PPTS, 35.5 mg, 0.14 mmol) and 3,4-dihydro-2H-pyran (DHP, 2.2 mL, 25.4 mmol) at room temperature. After stirring for 12 h at room temperature, the reaction was quenched by adding brine. The mixture was extracted with EtOAc (×3) and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (n-hexane–EtOAc = 10:1 to 5:1) to give 20 (498 mg, quant.). The obtained products was identified by the previously reported spectroscopic data.¹⁰

Preparation of a,a-Dimethyl Methyl Ester 21 from 20 To a solution of 20 (498 mg, 1.41 mmol) in tetrahydrofuran (THF) (1.6 mL) was added LDA (0.38 M in THF, 14.0 mL, 5.32 mmol) by dropwise, then the mixture was stirred at −40°C for 30 min. To the reaction mixture was added iodomethane (MeI) (1.8 mL, 12.7 mmol), then stirred at −40°C for further 30 min. The reaction was quenched by adding saturated aqueous NH₄Cl. After warming up to room temperature, the whole was extracted with EtOAc (×3) and the combined organic layers were washed with 5% HCl, saturated aqueous NaHCO₃, and brine, sequentially, dried over MgSO₄, filtered and concentrated under reduced pressure to afford a crude mono-methylated product. The repeated same procedure was applied to the crude product to give an unrefined dimethyl product 21 (252.6 mg).

Preparation of Aldehyde 22 from 21 To a solution of unrefined 21 (21.6 mg, approx. 0.057 mmol) in THF (0.3 mL) was added LiAlH₄ (13.0 mg, 0.34 mmol) at 0°C. After stirring at 0°C for 10 min, the reaction was quenched by adding brine. After warming up to room temperature, the whole was extracted with EtOAc (×3) and the combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure to afford a crude alcohol (17.3 mg). To a solution of the crude alcohol (17.3 mg, approx. 0.049 mmol) in CH₂Cl₂ (0.3 mL) was added Dess–Martin periodinane (31.8 mg, 0.75 mmol) at room temperature. After stirring at room temperature for 1 h, the reaction was quenched by adding saturated aqueous NaHCO₃ and saturated aqueous Na₂S₂O₅. The whole was extracted with EtOAc (×3) and the combined organic layers were dried over MgSO₄, filtered and concentrated under reduced pressure to give a crude aldehyde 22 (17.3 mg).
Preparation of \(a,a\)-Dimethyl Analog 10 from 22 To a solution of methyltriphenylphosphonium bromide (35.7 mg, 0.10 mmol) in THF (0.5 mL) was added NaHMDSS (1.0 M in THF, 0.075 mL, 0.075 mmol) at 0°C by dropwise. After stirring at room temperature for 15 min, to the reaction mixture was added a solution of crude aldehyde 22 (17.3 mg, approx. 0.049 mmol) in THF (0.4 mL) at 0°C, then stirred at 0°C for further 10 min. The reaction was quenched by adding saturated aqueous NH\(_4\)Cl. After warming up to room temperature, the whole was extracted with EtOAc (×3) and the combined organic layers were washed with saturated aqueous NH\(_4\)Cl and brine, sequentially, dried over MgSO\(_4\), filtered and concentrated under reduced pressure to afford a crude exo-olefin 23 (32.8 mg). To a solution of the crude exo-olefin 23 (14.1 mg, approx. 0.021 mmol) in MeOH (0.2 mL) was added \(p\)TsOH H\(_2\)O (1.6 mg, 0.008 mmol) at room temperature. At stirring room temperature for 30 min, the reaction was directly concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (n-hexane–EtOAc = 3:1) and subsequently HPLC [column: Cosmosil 5SL (10 mm i.d. × 250 mm, Nacalai Tesque, Inc.), mobile phase: n-hexane–EtOAc = 5:1, flow rate: 4.0 mL/min, detection: UV (\(\lambda = 250\) nm)] to give 10 (1.8 mg, 17% from 20 for 6 steps).

Analog 10: a yellow oil. IR (KBr) \(\nu\): 3354, 1604 cm\(^{-1}\). \(^1\)H-NMR (500 MHz, CDCl\(_3\) \(\delta\): 6.82 (1H, d, \(J = 2.4\) Hz), 6.73 (1H, d, \(J = 8.5\) Hz), 6.66 (1H, dd, \(J = 2.4, 8.5\) Hz), 5.98 (1H, dd, \(J = 11.0, 17.1\) Hz), 5.00 (1H, dd, \(J = 1.8, 17.1\) Hz), 4.96 (1H, dd, \(J = 1.8, 11.0\) Hz), 1.31 (6H, s). HR-FAB-MS \(m/z\): [M + H] 179.0999 (Calcd for C\(_{11}\)H\(_{15}\)O\(_3\)).

Preparation of Aldehyde 20 from a methyl ester 24 (443 mg, 1.26 mmol), the crude corresponding aldehyde 24 (354 mg) was prepared in the same manner as the conversion from 21 to 22.

Preparation of Analogs 11 and 12 from 24 To a solution of ethyltriphenylphosphonium bromide (56.9 mg, 0.15 mmol) in THF–Et\(_2\)O (5:3, 1.6 mL) was added \(BuLi\) (2.6 M in n-hexane, 0.06 mL, 0.16 mmol) by dropwise. After stirring at room temperature for 15 min, to the reaction mixture was added a solution of crude aldehyde 24 (49.1 mg, approx. 0.153 mmol) in Et\(_2\)O (1.0 mL) at −78°C, then stirred at −78°C for further 10 min. Next, extra \(BuLi\) (2.6 M in n-hexane, 0.06 mL, 0.16 mmol) was added to the reaction, which was stirred at −30°C for 5 min. Finally, 0.6% HCl–Et\(_2\)O (0.1 mL, 0.16 mmol) was added to the reaction, which was stirred at −30°C for 5 min. After stirring at room temperature for 40 min, the reaction was quenched by adding H\(_2\)O. The whole was extracted with EtOAc (×3) and the combined organic layers were dried over MgSO\(_4\), filtered and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (n-hexane–EtOAc = 20:1) to afford the \(cis\)--trans mixture of 25 (30.8 mg). Afterwards, a \(cis\)--trans mixture 25 (20.8 mg) was converted to analog 11 and 12 in the same manner as preparation of analog 10, which were purified by silica gel column chromatography (n-hexane–EtOAc = 5:1) and subsequently HPLC [column: Cosmosil 5SL-300AR (10 mm i.d. × 250 mm, Nacalai Tesque, Inc.), mobile phase: 40% aqueous CH\(_3\)CN containing 0.05% trifluoroacetic acid, flow rate: 4.0 mL/min, detection: UV (\(\lambda = 250\) nm)] to give 11 (1.2 mg, 4% from 20 for 4 steps) and 12 (8.4 mg, 28% from 20 for 4 steps).

Analog 11: a yellow oil. IR (KBr) \(\nu\): 3352, 1604 cm\(^{-1}\).

Preparation of Analog 13 from 24 From a crude aldehyde 24 (81.9 mg, approx. 0.256 mmol), the crude intermediate 26 (287 mg) was prepared in the same manner as the conversion from 22 to 23 by use of n-pentyliphenolphosphenium bromide in place of methyltriphenylphosphonium bromide. Afterwards, a crude 26 (287 mg) was converted to analog 13 in the same manner as preparation of analog 10, which was purified by silica gel column chromatography (n-hexane–EtOAc = 5:1) and subsequently HPLC [column: Cosmosil 5SL (10 mm i.d. × 250 mm, Nacalai Tesque, Inc.), mobile phase: n-hexane–EtOAc = 5:1, flow rate: 4.0 mL/min, detection: UV (\(\lambda = 250\) nm)] to give 13 (12.0 mg, 20% from 20 for 4 steps).

Analog 13: a yellow oil. IR (KBr) \(\nu\): 3356, 1604 cm\(^{-1}\). \(^1\)H-NMR (500 MHz, CDCl\(_3\) \(\delta\): 6.78 (1H, d, \(J = 8.1\) Hz), 6.71 (1H, d, \(J = 2.0\) Hz), 6.63 (1H, dd, \(J = 2.0, 8.1\) Hz), 5.55 (2H, m), 3.29 (2H, d, \(J = 4.4\) Hz), 1.71 (3H, t, \(J = 4.9\) Hz). \(^13\)C-NMR (125 MHz, CDCl\(_3\) \(\delta\): 143.4, 141.5, 134.3, 130.2, 126.1, 120.8, 115.6, 115.3, 38.3, 17.8. HR-FAB-MS \(m/z\) [M + H]: 165.0830 (Calcd for C\(_8\)H\(_{12}\)O\(_2\): 165.0837).

Preparation of Analog 14 from 24 From a crude aldehyde 24 (81.9 mg, approx. 0.256 mmol), the crude intermediate 27 (413 mg) was prepared in the same manner as the conversion from 22 to 23 by use of n-onyloniphenolphosphenium bromide in place of methyltriphenylphosphonium bromide. Afterwards, a crude 27 (413 mg) was converted to analog 14 in the same manner as preparation of analog 10, which was purified by silica gel column chromatography (n-hexane–EtOAc = 5:1) and subsequently HPLC [column: Cosmosil 5SL (10 mm i.d. × 250 mm, Nacalai Tesque, Inc.), mobile phase: n-hexane–EtOAc = 5:1, flow rate: 4.0 mL/min, detection: UV (\(\lambda = 250\) nm)] to give 14 (17.1 mg, 25% from 20 for 4 steps).

Analog 14: a yellow oil. IR (KBr) \(\nu\): 3354, 1604 cm\(^{-1}\). \(^1\)H-NMR (500 MHz, CDCl\(_3\) \(\delta\): 6.78 (1H, d, \(J = 7.9\) Hz), 6.71 (1H, s), 6.63 (1H, d, \(J = 7.9\) Hz), 5.50 (2H, m), 3.28 (2H, d, \(J = 5.5\) Hz), 2.12 (2H, dt, \(J = 6.4, 6.7\) Hz), 1.28 (12H, m), 0.89 (3H, t, \(J = 6.7\) Hz). HR-FAB-MS \(m/z\) [M + H]: 263.1939 (Calcd for C\(_{11}\)H\(_{19}\)O\(_2\): 263.1933).

Evaluation for in Vitro Anti-malarial Activity A strain of \(P. falciparum\) (CDC1) was used in sensitivity test. Cultivation of malarial parasite was performed referring to the previous report.\(^{10}\) Asynchronously cultivated parasites by sorbitol treatment\(^{19}\) was used for the assay. The infectious ratio was estimated based on the LDH activity of \(P. falciparum\).\(^{20,21}\) Briefly, 90 \(\mu\)L of erythrocytes suspension (0.5% parasitemia and 2% hematocrit) infected by the parasites synchronized at ring stage was inoculated to each well in 96-ewll microculture plate. The DMSO solution of the test sample with the corresponding concentration was 20-fold diluted by the complete medium, then 10 \(\mu\)L was added to malaria parasites. After in-
cubation at 37°C for 72 h, the incubated medium in each well was sufficiently suspended, then 20 µL of it was transferred to another 96 well plate and mixed up with 100 µL of Malstat reagent (Flow Laboratories). By shaking at room temperature for 15 min, erythrocytes were completely hemolysed. Subsequently, it was treated with 10 µL of 1 mg/mL nitroblue tetrazolium (Sigma) and 10 µL of 0.1 mg/mL phenylethyl sulfate (Sigma) for 2 h at room temperature in a dark. Finally, the absorbance at 650 nm of each well was measured. The anti-malarial activity (%) was calculated by the formula: \[ \frac{\text{ABS (control)} - \text{ABS (sample)}}{\text{ABS (control)} - \text{ABS (intact)}} \times 100 \]. The condition of “intact” is only erythrocytes without the parasites. The average of the inhibition ratio was plotted at each concentration and IC_{50} value was assessed. Artemisinin was used as positive control which showed the anti-malarial activity of 80.0 ± 4.9% inhibition (mean ± S.D.) at 3 ng/mL and 4.4 ± 1.1% at 1 ng/mL, basically.

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References