# New Semisynthetic Quassinoids with in Vivo Antimalarial Activity

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On the basis of a comparative analysis for stability in mouse serum between 15-*O*-acetylbruceolide and bruceolide 15-methyl carbonate, several 3,15-dialkyl carbonates of bruceolide were synthesized and their in vitro antimalarial activity was assessed. Methyl, ethyl, and isopropyl carbonates with pronounced in vitro activity were further evaluated for in vivo antimalarial potency. Both the methyl and ethyl carbonates significantly increased the life span of mice as compared with 3,15-di-*O*-accetylbruceolide and chloroquine.

## Introduction

Malaria remains the greatest human killer among parasitic infections, despite worldwide efforts to combat the disease and attempts at eradication of the causative organisms. The emergence and ongoing spread of the drug-resistant strains of *Plasmodium falciparum*, including multidrug resistant strains, the most lethal malaria parasite, poses a serious health-care problem in the malaria-endemic countries, and among international travelers.[1]

Some species of the Simaroubaceae family have been traditionally used as a cure for malaria.[2] Under the assumption that this potency is attributed to the major constituents of these plant medicines, the antimalarial activity of several naturally occurring quassinoids was investigated. As a result, some quassinoids were disclosed to inhibit proliferation of *P. falciparum* potently in vitro.[3] However, intensive assessment of antimalarial potency by bioassay test in vivo has been performed with respect to only limited quassinoids because of the scarcity of natural supply.[4] Among the antimalarial active quassinoids, bruceolide (**1**) is one of the representative compounds as a common core skeleton.

In this regard, we undertook to establish a practical procedure for preparing bruceolide (**1**) from a readily available Chinese crude drug, "Ya-tan-tzu" and to explore antimalarial semisynthetic quassinoids using **1** as the starting material.[5] Previously, it was found that 3,15-*O*-diaccetylbruceolide (**5**) showed potent in vitro antimalarial activity as well as a high selective index against mouse mammary tumor FM3A cells, representing a model of host cells.[5] Furthermore, **5** was also shown to possess potent in vivo antimalarial activity against *P. berghei* infected mice.[6] As a part of a program aimed at more promising quassinoid derivatives with high antimalarial potency in vivo, several carbonates of bruceolide (**1**) were designed and evaluated for their biological properties. This paper describes several new semisynthetic quassinoids with in vivo antimalarial potency.

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**Synthesis and Biological Property of Bruceolide Derivatives.** Despite potent antimalarial activity in vivo ($ED_{50}$ = 0.46 mg/kg), 3,15-*O*-diacetylbruceolide (**5**) was unable to completely remove malaria parasites in the blood stream in the 4-day suppressive test. Bruceolide (**1**) displayed a slightly weaker antimalarial effect in vivo ($ED_{50}$ = 1.1 mg/kg) than **5**.[6] This finding led to the consideration that the acetyl functions in **5** were readily metabolized in mice. Thus, the stability of the acetyl residues, linked to the hydroxyl groups on C-3 and C-15, in mouse serum was examined. Both 3-*O*-acetyl (**4**) and 15-*O*-acetylbruceolide (**11**, = brucein B)[7] were respectively synthesized from the precursor triethylsilyl (TES) ethers (**2** and **3**), as shown in Scheme 1. Synthesis of 3-*O*-acetylbruceolide (**4**) was conducted using the following protocol. Protection of the 3- and 15-hydroxyl groups in **1** by treatment with TESCl in pyridine at 50 °C gave 3,15-*O*-diTES ether, in which the 3-*O*-TES group was selectively deprotected using *n*-$Bu_4$NF at − 20 °C to afford the 15-*O*-TES ether **3** in 92% yield for the two steps. Acetylation of **3** with $Ac_2O$/pyridine followed by removal of the TES residue with HF−pyridine in THF at 0 °C furnished 3-*O*-acetylbruceolide (**4**) in 89% yield from **3**. 15-*O*-Acetylbruceolide (**11**, = bruceine B) was prepared as follows. The 3-hydroxyl group in **1** was treated with TESCl under dilute conditions to give 3-*O*-triethylsilylbruceolide (**2**) selectively in 90% yield. Successive acetylation with $Ac_2O$ in pyridine and desilylation with HF-pyridine in THF at 0 °C provided **11** in 88% yield for the two steps.

The stability of bruceolide (**1**), and the monoacetyl (**4**, **11**) and diacetyl (**5**) derivatives of **1** in mouse serum was assessed and their time-course of disappearance is depicted in Figure 1. Both **4** and **5** with the 3-*O*-acetyl residue decreased in the mouse serum at nearly the same rate and disappeared completely after 3 h treatment. On the other hand, about 50% of **11** was preserved after exposure in mouse serum for 3 h. This finding indicates that retention of the acetyl residue at the 15-hydroxyl group in **5** is crucial to exert the in vivo antimalarial potency of **5**. In addition, the carbonyl residue attached to the 15-hydroxyl group was shown to play an important role in the antimalarial activity of bruceolide derivatives during the course of an extensive

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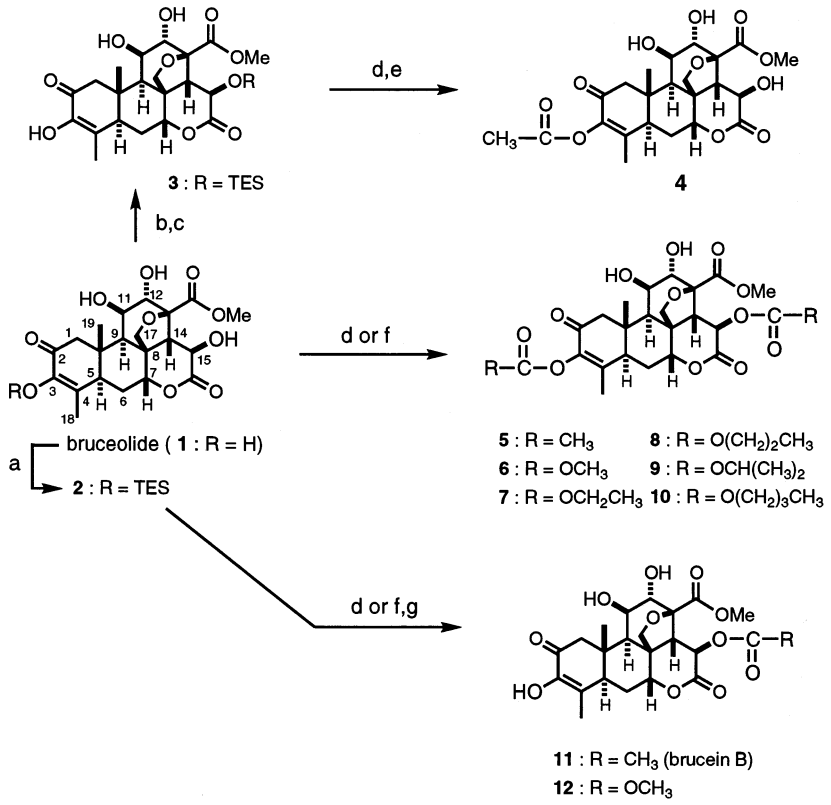
**Scheme 1**[a]




[a] Conditions; (a) TESCl, pyridine, 50 °C, (0.015 mM of **1** in pyridine); (b) TESCl, pyridine, 50 °C, (0.24 mM of **1** in pyridine); (c) *n*-$Bu_4NF$, $CH_2Cl_2$, −20 °C; (d) $Ac_2O$, pyridine); (e) HF-pyridine, THF; (f) alkyl chloroformate, pyridine, 0 °C; (g) HF−pyridine, THF, 0 °C.

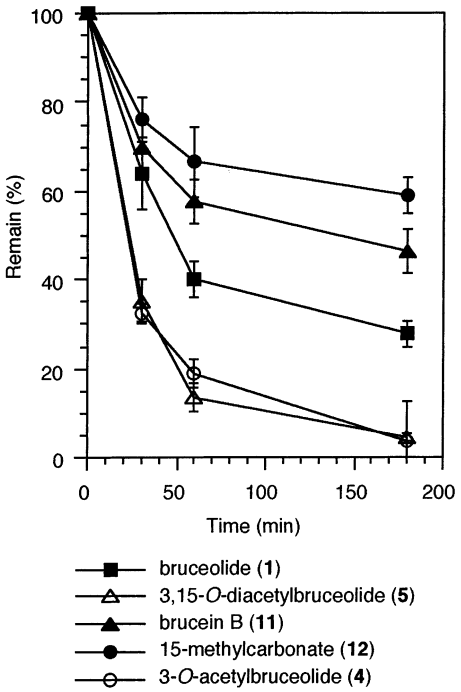



**Figure 1.** Stability of bruceolide (**1**) and its derivatives (**4**, **5**, **11**, and **12**) in mouse serum.

analysis of their structure−activity relationships.[8] This structural requirement led to the proposal of 3,15- and/or 15-alkyl carbonates as further candidates. Therefore, the 15-methyl carbonate (**12**) was synthesized in order to compare the antimalarial activity, as well as the stability in serum, between **11** and **12**. Treatment of 3-*O*-TES-bruceolide (**2**) with methyl chloroformate in pyridine at 0 °C provided the corresponding carbonate, which was submitted to the same deprotection procedure as in the preparation of **11** to furnish bruceolide 15-methyl carbonate (**12**) in 82% yield for the two steps. Notably, the methyl carbonate **12** showed higher stability than 15-*O*-acetylbruceolide (**11**) after exposure in mouse serum for 3 h, and similar antimalarial potency to **11** in vitro, as shown in Figure 1 and Table 1. This interesting biological outcome encouraged the design and synthesis of several 3,15-dialkyl carbonates and an evaluation of their antimalarial activity.

For the purpose of rationally designing carbonate derivatives of **1** in expectation of in vivo potency, effective use was made of calculated logP (clogP) values.[9]

**Table 1.** In Vitro Antimalarial Activities of Bruceolide Derivatives

| compound | $EC_{50}$ values (nM)[a] | | selectivity index[d] | clogP |
|---|---|---|---|---|
| | *P. falciparum*[b] | FM3A[c] | | |
| **4** | 880 | $>3.2 \times 10^4$ | >36 | |
| **5**[e] | 39 | $1.6 \times 10^4$ | 410 | −0.67 |
| **6** | 90 | $>6.3 \times 10^4$ | >700 | −1.08 |
| **7** | 64 | $3.6 \times 10^4$ | 563 | −0.01 |
| **8** | 100 | $6.6 \times 10^3$ | 66 | 1.05 |
| **9** | 80 | $1.1 \times 10^4$ | 138 | 0.61 |
| **10** | 14 | $7.0 \times 10^2$ | 50 | 2.13 |
| **11**[e] | 24 | $8.0 \times 10^3$ | 333 | |
| **12** | 53 | $8.7 \times 10^3$ | 164 | |
| chloroquine | 18 | $3.2 \times 10^4$ | 1780 | |

[a] In vitro antimalarial activities and cytotoxicities are described in the Experimental Section. [b] Chloroquine-sensitive (FCR-3 strain). [c] Mouse mammary tumor FM3A cells are used as a control for mammalian cell cytotoxicity. [d] Selectivity index = (mean of $EC_{50}$ value for FM3A cells)/(mean of $EC_{50}$ value for *P. falciparum*). [e] Biological scores were reported in our preliminary communication.[5]

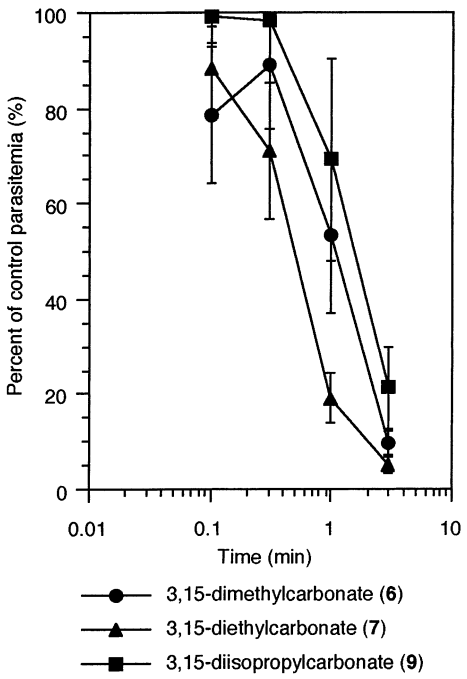



**Figure 2.** The dose-response curve for antimalarial potency of the three carbonates (**6**, **7**, and **9**) in *P. berghei*-infected mice. Parasitemia was determined by the following formula: (number of infected erythrocytes/number of total erythrocytes).

ClogP is a parameter correlated to the permeability of drugs, and is thus believed to be an important index in predicting biological activity in animal models. Generally, clogP values larger than 3 and less than −3 tend to reduce in vivo pharmacological efficacy significantly regardless of a good in vitro biological score.[10] Thus, easily accessible 3,15-dialkyl carbonates with moderate clogP values were explored. In the first instance, the logP values of bruceolide (**1**), 3,15-*O*-diacetyl (**5**), 3-*O*-hexanoyl,[5] and 3-*O*-ethyl[8] derivatives were experimentally measured. On the basis of the observed logP values and the estimated clogP values of the four compounds, which are determined by commercially available software,[11] five 3,15-dialkyl carbonate derivatives (**6**−**10**) of bruceolide (**1**) were designed as promising candidates. The carbonates (**6**−**10**) were prepared in 71 to 100% yield from **1** through the treatment with the corresponding alkyl chloroformate in pyridine.

**Antimalarial Activity of the Carbonate Derivatives of Bruceolide.** The semisynthetic quassinoid derivatives were tested for in vitro antimalarial activity against *P. falciparum*. Furthermore, their cytotoxicities against FM3A cells, the model cells of the host animal, were also assessed in order to determine the selectivity index.[12] Table 1 summarizes the in vitro biological activity of the carbonate derivatives. All of the carbonate derivatives potently inhibited proliferation of the malaria parasite and exhibited similar $IC_{50}$ values, regardless of the difference in the alkyl residues. In particular, two of the carbonates (**6** and **7**) demonstrated outstanding selectivity indices, as well as antimalarial potency. By comparing the biological outcome between the 15-methyl carbonate (**12**) and 3,15-dimethyl carbonate (**6**), the introduction of a carbonate function to the 3-hydroxyl group is likely to diminish cytotoxicity against host cells. In contrast with brucein B (**11**) and 3,15-*O*-diacetylbruceolide (**5**), 3-*O*-acetylbruceolide (**4**) showed reduced antimalarial activity, suggesting that the presence of a carbonyl residue adjacent to the 15-hydroxyl group is beneficial to antimalarial potency. With respect to the length of unbranched alkyl residues in the carbonates, the carbonates with longer carbon chains showed stronger cytotoxic activity against host cells. This biological behavior due to the carbon chains linked to the 3,15-hydroxyl functions was also observed in the case of the acyl derivatives of **1**.[5]

**Table 2.** In Vivo Antimalarial Activity of the Carbonate Derivatives of Bruceolide

| compound | $ED_{50}$ (mg/kg) | $ED_{90}$ (mg/kg) | increase of life span (ILS, %)[a] |
|---|---|---|---|
| **5** | 0.46 | 0.98 | 49 [dose: 1 mg/kg] |
| **6** | 1.3 | 3.0 | 196 [dose: 3 mg/kg] |
| **7** | 0.49 | 1.9 | 182 [dose: 3 mg/kg] |
| **9** | 1.4 | >3.0 | 86 [dose: 3 mg/kg] |
| chloroquine | 2.0 | 3.3 | 15 [dose: 3 mg/kg] |

[a] Antimalarial potency was evaluated by the increase of life span (ILS): $(T/C - 1) \times 100\%$, where "*T*" is the mean survival days (MSD) of the treated group and "*C*" is the MSD of the control group.

Based on this encouraging in vitro biological activity, the antimalarial potential of the three carbonate derivatives was examined in vivo with the 4-day suppressive test using *P. berghei* infected mice. The dose−response curves given in Figure 2 indicate that the antimalarial effects of all of the derivatives tested on mice are enhanced dose-dependently. Judging from the $ED_{50}$ and $ED_{90}$ values, the three carbonates exhibited similar or slightly less potent antimalarial activity in vivo in comparison with 3,15-*O*-diacetylbruceolide (**5**), as depicted in Table 2. However, all of the carbonates showed superior $ED_{50}$ values to chloroquine ($2.0 \pm 0.2$ mg/kg), a clinically used antimalarial drug. No obvious signs of toxicity, such as diarrhea, body weight loss, and mortality were observed during the treatment at doses of 3.0 mg/kg per day among the three carbonates. Notably, the mean survival days of the treated mice with all of the carbonates are significantly prolonged as compared with **5** and chloroquine. In particular, it should be noted that the methyl and the ethyl carbonates (**6**, **7**) showed about four times longer increase of life span than **5**.

In summary, the new semisynthetic quassinoids, the 3,15-dimethyl and 3,15-diethyl carbonates (**6**, **7**) of bruceolide (**1**), with in vivo antimalarial potency, following the results of stability testing in mouse serum and rational design by use of adequate clogP have been disclosed. Work is now in progress to evaluate curability in detail.

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**Supporting Information Available:** Experimental Section and elemental analysis data. This material is available free of charge via the Internet at http://pubs.acs.org.

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