Antimalarial and Cytotoxic Activities of Bicyclo[6.4.0]dodecenones

Hiroshi FUJISHIMA,[a] Hiroshi TAKESHITA,[a] Masahiro TOYOTA,[a] Hye-Sook KIM,[b] Yusuke WATAYA,[b] Motohiro TANAKA,[c] Takuma SASAKI,[c] and Masataka IHARA*,[a]

Department of Organic Chemistry, Graduate School of Pharmaceutical Sciences, Tohoku University,[a] Aobayama, Sendai 980–8578, Japan, Faculty of Pharmaceutical Sciences, Okayama University,[b] Okayama 700–8530, Japan, and Department of Experimental Therapeutics, Cancer Research Institute, Kanazawa University,[c] Kanazawa 920–0934, Japan.
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Biological evaluations of bicyclo[6.4.0]dodecenone derivatives on antimalarial activity *in vitro* against *Plasmodium falciparum* and cytotoxicity against human KB cells were made. (±)-(1*R,4*S**,7*R**,8*S**)-4-*tert*-Butyldimethylsiloxy-5,5-dimethyl-1-methyl-9-methylene-7-phenylsulfonylbicyclo[6.4.0]dodec-2,11-dien-10-one (15) exhibited potent antimalarial activity, whereas (±)-(1*R**,7*R**,8*S**)-1-methyl-9-methylene-7-phenylsulfonylbicyclo[6.4.0]dodec-2,11-dien-10-one (14) showed significant cytotoxic activity in human KB cells. Both 14 and 15 possess, as a structural character, the *exo*-methylene moiety in their 6-membered ring of the 8-6 fused ring system.**

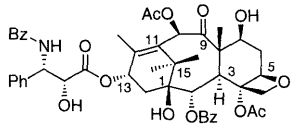
Key words bicyclo[6.4.0]dodecenone; antimalarial activity; cytotoxicity

Since the identification of the structure of taxol (**1**) (Fig. 1) by Wani and co-workers[1] and the elucidation of its unusual mode of action,[2] structural features of the taxane diterpens consisting of the 6–8–6 fused ring system and promising activities against several important human cancers[3] have stimulated the attention of numerous organic and biological chemists. In the past decade, six groups succeeded in the total synthesis of **1**[4] including the enantioselective synthesis,[5] and a number of structure–activity relationships (SAR) of **1** and its modified compounds have been reported by several groups.[6]

Our attention has been focused on the establishment of a novel synthetic route to **1**, and we found that the reaction of sulfone (**4** or **7**) with potassium hexamethyldisilazide ($KN(SiMe_3)_2$) provided the bicyclo[6.4.0]dodecenone (**8** or **9**) (Chart 1).[7] Although many SAR studies between biological activities and functional groups of taxol have been reported,[6] there is little research describing the influence of the 6–8–6 fused ring skeleton on these biological activities. Expecting that it would be worth biologically evaluating bicyclo[6.4.0]dodecenone compounds, we investigated their activities on malaria (*Plasmodium falciparum*)[8] and human KB cells.[9] The former is one of the most serious human malaria parasites in the tropical areas, and the rapid acquisition of resistance by *P. falciparum* to the drug chloroquine has recently become a significant problem. In the *in vitro* assay of malaria, mouse mammary tumor FM3A cells in culture were used as a control for mammalian cell cytotoxicity. We now report interesting biological activities of bicyclo[6.4.0]dodecenone derivatives.

Results and Discussion

Syntheses of Substrates Sulfones (**4**, **7**) were prepared through the corresponding intermediates (**3**, **6**) which were derived from a common material, cyclohexadienyl aldehyde (**2**).[7a] The intramolecular Michael reaction of **4** or **7** with $KN(SiMe_3)_2$ in tetrahydrofuran (THF) provided the *cis*-fused bicyclo[6.4.0]dodecenone compound (**8** or **9**) in quantitative yield, respectively. **9** was reacted with L-Selectride® (1 M in THF solution) to produce the allylic alcohol (**10**) with high diastereoselectivity[7b] (Chart 2). Treatment of enone (**8** or **9**) with electrophiles, such as *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) or methoxymethyl chloride (MOMCl), under the basic conditions furnished the TBS enolether (**11**) in 63% yield or the MOM enolether (**12**) in 59% yield. In the latter reaction, the C-alkylated product (**13**) was also obtained in 18% yield. In addition, it was found that quenching the cyclization reaction of **4** or **7** with excess amounts of MOMCl afforded the *exo*-olefinated product (**14** or **15**) in high yield.[7b] Oxidation of the *exo*-olefin moiety with hydrogen peroxide (30% aqueous solution) in the mixture of 6 M NaOH aqueous solution and MeOH at room temperature provided the epoxide (**16**) in 66% yield as a single



taxol (**1**)

Fig. 1. Structure of Taxol (**1**)

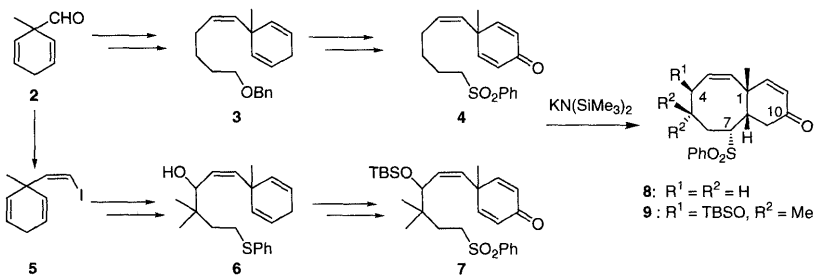
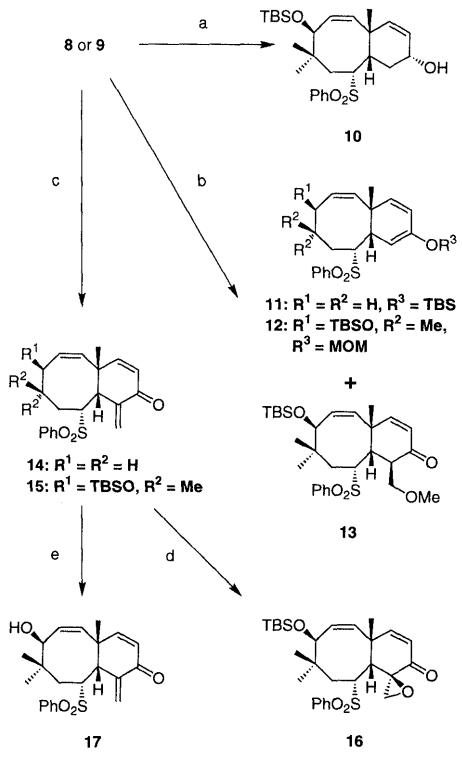


Chart 1

* To whom correspondence should be addressed. e-mail: mihara@mail.pharm.tohoku.ac.jp



a) L-Selectride, THF, 0 °C, b) $KN(SiMe_3)_2$, R^3-X, THF, 0 °C, c) $KN(SiMe_3)_2$, MOMCl (excess), DME, 45 °C, d) 30% H_2O_2, 6M NaOH, 0 °C, e) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −15 °C.

Chart 2

isomer. Deprotection of TBS group under usual conditions (tetrabutylammonium fluoride in THF) did not afford the desired alcohol (**17**), but furnished complex products. However, employment of Lewis acid (boron trifluoride diethyl etherate ($BF_3{\cdot}OEt_2$), dichloromethane (CH_2Cl_2), −15 °C)[10] allowed deprotection of the silyl group and furnished **17** in 83% yield.

Biological Evaluations

Antimalarial Activity With a series of substrates in hand, antimalarial activity against *P. falciparum* and cytotoxicity against mouse mammary FM3A cells[8] *in vitro* were investigated and the results are outlined in Table 1. In the series of 4,5-non-substituted compounds, enone (**8**) and its TBS enolether (**11**) exhibited similar activity with almost the same EC_{50} value of 4.2×10^{-5} M and 3.1×10^{-5} M, respectively (entries 1 and 4). Compound (**14**) in which *exo*-olefin group was introduced in the 6-membered ring showed a slightly more effective activity than **8** and **11** with the EC_{50} value of 8.4×10^{-6} M (entry 7).

On the other hand, in the series of the 4,5-substituted compounds, enone (**9**) and alcohol (**10**) possessed the same EC_{50} value of 1.6×10^{-5} M (entries 2 and 3). Compound (**12**) having *O*-MOM enolether group and compound (**13**) with *C*-MOM unit demonstrated the EC_{50} value of 3.1×10^{-6} M and 7.4×10^{-6} M, respectively (entries 5 and 6). Interestingly, it was found that cytotoxicity against FM3A of **13** was approximately 10 times greater than that of **12**, though **13** had little predominance in the antimalarial activity. Next, we examined antimalarial activity with **15** having *exo*-olefin group. To our surprise, this activity was about 500 times more potent than enone (**9**) (entry 8), and the EC_{50} value of 1.9×10^{-8} M was found to be almost the same as that of chloroquine (entry 11) (Fig. 2).[11] Compound **16** of which *exo*-olefin group was converted into epoxide group reduced the activity more than 150 fold compared to **15** and also reduced the cytotoxic activity against FM3A (entry 9). Alcohol (**17**) in which TBS group was removed had rather poor activity compared to **15** (entry 10).

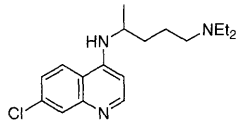
Based upon the consequence obtained in these evaluations, it was inferred that a combination of the *exo*-olefin group and the TBS group in the bicyclo[6.4.0]dodecenone would play an important role in antimalarial activity. Interestingly, the result also showed that the more the antimalarial activity increased, the stronger was the cytotoxicity against FM3A cells.

Cytotoxicity in Human KB Cells Cytotoxicity against human KB cells was also tested for three compounds **14**, **15**, and **17**. As shown in Table 2, **14** was found to be the most effective among these compounds, with an IC_{50} value of 0.33 μg/ml (entry 1). **15** and **17** were less toxic to KB cells with IC_{50} values of 4.1 μg/ml and >20 μg/ml, respectively

Table 1. Antimalarial Activity against *P. falciparum* and Cytotoxicity against FM3A Cells of Bicyclo[6.4.0]dodecenone Compounds

Entry	Compound	EC_{50} values (M)		Selectivity[c]
		P. falciparum[a]	FM3A[b]	
1	**8**	4.2×10^{-5}	NT[d]	—
2	**9**	1.6×10^{-5}	NT	—
3	**10**	1.6×10^{-5}	NT	—
4	**11**	3.1×10^{-5}	NT	—
5	**12**	3.1×10^{-6}	5.0×10^{-6}	1.6
6	**13**	7.4×10^{-6}	5.5×10^{-7}	0.1
7	**14**	8.4×10^{-6}	2.8×10^{-6}	0.3
8	**15**	1.9×10^{-8}	5.0×10^{-8}	2.6
9	**16**	6.4×10^{-6}	1.8×10^{-5}	2.8
10	**17**	1.6×10^{-5}	2.1×10^{-6}	0.1
11[e]	Chloroquine	1.8×10^{-8}	3.2×10^{-5}	1778

a) Chloroquine-sensitive (FCR-3 strain). *b*) Mouse mammary tumor FM3A cells in culture as a control for mammalian cell cytotoxicity. *c*) Selectivity=(mean of EC_{50} value for FM3A cells)/(mean of EC_{50} value for *P. falciparum*). *d*) NT, not tested. *e*) See ref. 11.



chloroquine

Fig. 2. Structure of Chloroquine

Table 2. Cytotoxicity of Bicyclo[6.4.0]dodecenones against Human KB Cells

Entry	Compound	IC_{50} values (μg/ml)[a] KB cells[b]
1	**14**	0.33
2	**15**	4.1
3	**17**	>20

a) IC_{50} value was measured from the dose–response curve with at least 3 drug concentrations. *b*) 2000 cells in 198 μl of RPMI-1640 medium supplemented with 10% fetal bovine serum.

(entries 2 and 3). It was very interesting that elimination of the functional groups in the 8-membered ring resulted in an increase of activity (entry 2), whereas removal of the TBS group from **14** led to reduction of cytotoxicity (entry 3). Judging from the results observed here, it was obvious that there would be correlation between the appearance of cytotoxicity against human KB cells and functional groups in the bicyclo[6.4.0]dodecenone framework.

Conclusion

We have studied the biological activities of the bicyclo[6.4.0]dodecenones against malaria (*P. falciparum*), FM3A cells and human KB cells. It is worth mentioning that the antimalarial potency against the chloroquine-sensitive strain of *P. falciparum* is related to some of the bicyclo[6.4.0]dodecenone compounds comprising both the functionalized 8-membered ring and the *exo*-olefinated 6-membered ring, while the compound consisting of the non-substituted 8-membered ring and the *exo*-olefinated 6-membered ring is responsible for the cytotoxicity against human KB cells.

Experimental

General All moisture- or air-sensitive reactions were carried out under an atmosphere of nitrogen or argon. All reagents and solvents were used as obtained from commercial suppliers except for the following: THF was distilled from benzophenone ketyl under argon. CH_2Cl_2 and 1,2-dimethoxyethane (DME) were distilled from CaH_2 prior to use. Melting points are uncorrected. ^{1}H-NMR spectra were recorded by a VARIAN Gemini-2000 at 300 MHz in $CDCl_3$ using tetramethylsilane as the internal standard. Column chromatography was carried out on silica gel (230—400 mesh). IR spectra were recorded with a JASCO IR Report-100 spectrophotometer. ^{1}H-NMR data are described in the following order: chemical shift, multiplicity [s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broadened)], coupling constant(s) (Hz), and integration. ^{13}C-NMR spectra were also recorded using a VARIAN Gemini-2000 at 75 MHz in $CDCl_3$. Mass spectra were taken on a JEOL-DX-300 spectrometer. The synthetic methods of compounds **8**—**10**, **12**—**15** were described in the previous papers.[7b]

(±)-(1*R,7*R**,8*S**)-10-*tert*-Butyldimethylsiloxy-1-methyl-7-phenyl-sulfonylbicyclo[6.4.0]dodec-2,9,11-triene (11)** To a mixture of $KN(SiMe_3)_2$ (242 μl, 121 μmol) in THF (200 μl) was added dropwise a THF solution (400 μl) of sulfone (**4**)[7] (20 mg, 61 μmol) at −78 °C. The temperature was allowed to warm to 0 °C over 2 h and then TBSOTf (28 μl, 120 μmol) was added. After 30 min at 0 °C, the reaction mixture was quenched by the addition of saturated ammonium chloride solution, and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (EtOAc) and the combined extracts were washed with brine, dried over magnesium sulfate ($MgSO_4$) and concentrated. The residue was purified by column chromatography (hexanes/EtOAc=8 : 1) to afford **11** (17 mg, 63%) as a solid; mp 145—146 °C. IR (neat) cm^{-1}: 1650, 1600, 1300, 1150. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.21 (3H, s), 0.22 (3H, s), 0.76—0.82 (1H, m), 0.95 (9H, s), 1.21 (3H, s), 1.46—1.58 (1H, m), 1.81—2.06 (3H, m), 2.35—2.51 (1H, m), 3.15 (dd, J=6.0, 2.7 Hz, 1H), 3.52—3.60 (1H, m), 5.17—5.23 (1H, m), 5.34 (dd, J=11.5, 1.4 Hz, 1H), 5.63—5.78 (3H, m), 7.50—7.66 (3H, m), 7.82—7.90 (3H, m). ^{13}C-NMR ($CDCl_3$, 75 MHz) δ: −4.7, −4.4, 17.6, 18.0, 24.3, 25.7, 26.9, 27.0, 29.9, 39.4, 42.3, 67.3, 100.9, 123.3, 128.6, 129.3, 133.1, 133.4, 138.0, 139.4, 140.0, 149.2. MS *m/z*: 444 (M^+), 314, 303. HRMS: Calcd for $C_{25}H_{36}O_3SSi$: 444.2155. Found: 444.2144.

(±)-(1*R,4*S**,7*R**,8*S**,9*R**)-4-*tert*-Butyldimethylsiloxy-5,5-dimethyl-1-methyl-7-phenylsulfonylbicyclo[6.4.0]dodec-2,11-dien-10-one-9-spiro-2′-oxirane (16)** To a solution of **15**[7] (17 mg, 34 μmol) in MeOH (0.5 ml) was added hydrogen peroxide (30% aqueous solution, 6 μl, 51 μmol) followed by aqueous 6 M-NaOH (2 μl, 9 μmol) at room temperature. After 0.5 h at room temperature, the reaction mixture was poured into water and extracted with ether. The combined extracts were washed with brine, dried ($MgSO_4$), and concentrated. The residue was purified by column chromatography (hexanes/EtOAc=20 : 1) to give **16** (12 mg, 66%) as a solid, whose recrystallization from methanol provided needles; mp 154—156 °C. IR (neat) cm^{-1}: 1690. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: −0.24 (3H, s), −0.03 (3H, s), −0.02 (3H, s), 0.69 (3H, s), 0.82 (9H, s), 1.25 (3H, s), 1.74 (dd, J=15.9, 1.1 Hz, 1H), 1.91 (dd, J=16.1, 6.6 Hz, 1H), 3.06—3.15 (1H, m), 3.59 (d, J=4.4 Hz, 1H), 3.62—3.72 (1H, m), 3.73 (d, J=4.4 Hz, 1H), 4.34 (dd, J=7.0, 1.1 Hz, 1H), 5.41 (dd, J=11.7, 1.1 Hz, 1H), 5.53 (dd, J=11.7, 7.0 Hz, 1H), 6.31 (d, J=10.6 Hz, 1H), 6.83 (dd, J=10.6, 1.1 Hz, 1H), 7.50—7.70 (3H, m), 7.80—7.90 (2H, m). MS *m/z*: 516 (M^+), 459, 375. *Anal.* Calcd for $C_{28}H_{40}O_5SSi$: C, 65.08; H, 7.96%. Found: C, 65.11; H, 7.96.

(±)-(1*R,4*S**,7*R**,8*S**,10*R**)-5,5-Dimethyl-4-hydroxy-1-methyl-9-methylene-7-phenylsulfonylbicyclo[6.4.0]dodec-2,11-dien-10-ol (17)** To a solution of **15**[7] (27 mg, 54 μmol) in CH_2Cl_2 (1 ml) was added $BF_3 \cdot OEt_2$ (15 μl, 81 μmol) at −15 °C. After being stirred for 1 h, the reaction mixture was quenched by the addition of water (50 μl), and the resulting mixture was dried over $MgSO_4$. After usual work up, the residue was purified by column chromatography (hexanes/EtOAc=3 : 1) to give **17** (17 mg, 83%) as a solid; mp 189—190 °C. IR (KBr) cm^{-1}: 3480, 1694. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 0.76—0.96 (2H, m), 1.03 (3H, s), 1.25 (3H, s), 1.57 (s, 3H), 1.82 (br, 1H), 2.44 (dt, J=13.2, 6.0 Hz, 1H), 2.63 (br, 1H), 3.55 (br, 1H), 3.60—3.84 (br, 1H), 5.53—5.73 (m, 1H), 5.95 (br d, J=12.6 Hz, 2H), 6.46 (br, 1H), 7.57—7.69 (m, 3H), 7.82—7.89 (m, 2H). MS *m/z*: 386 (M^+), 277, 245. HRMS Calcd for $C_{22}H_{26}O_4S$: 386.1545. Found: 386.1572.

***In Vitro* Antimalarial Activity of Bicyclo[6.4.0]dodecenones** Antimalarial activities of bicyclo[6.4.0]dodecanes against *P. falciparum in vitro* were assayed as previously described.[8a] Asynchronously cultivated *P. falciparum* were utilized. Various concentrations of compounds in dimethyl sulfoxide (DMSO) were prepared. Five microliters of each solution was added to individual wells of 24 well dishes. Erythrocytes with 0.3% parasitemia were added to each well containing 995 μl of culture medium to give a final hematocrit level of 3%. The plates were incubated at 37 °C for 72 h in a CO_2–O_2–N_2 incubator (5%, CO_2, 5% O_2, and 90% N_2 atmosphere). To evaluate the antimalarial activity of a test compound, we prepared thin blood films from each culture and strained them with Giemsa (E. Merck, Germany). A total of 1×10^4 erythrocytes per one thin blood film were examined under a microscope. All of the test compounds were assayed in duplicate at each concentration. Drug-free control cultures were run simultaneously. All data points represent the mean of three experiments. Parasitemia in control reached between 4% and 5% at 72 h. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in parasite density at 72 h by 50% of control.

Toxicity against Mammalian Cell Lines FM3A cells grew with a doubling time of about 12 h.[8b] Prior to exposure to drugs, cell density was adjusted to 5×10^4 cells/ml. A cell suspension of 995 μl was dispensed to the test plate, and a compound at various concentrations suspended in DMSO (5 μl) was added to individual wells of 24 well dishes. The plates were incubated at 37 °C in a 5% CO_2 atmosphere for 48 h. All of the test compounds were assayed in duplicate at each concentration. Cell numbers were measured using a microcell counter CC-130 (Toa Medical Electric Co., Japan). All data points represent the mean of three experiments. The EC_{50} value refers to the concentration of the compound necessary to inhibit the increase in cell density at 48 h by 50% of control. Selectivity refers to the mean of EC_{50} value for FM3A cells per the mean of EC_{50} value for *P. falciparum*.

Toxicity against Human KB Cell Lines Compounds were dissolved in DMSO. The cytotoxic effects of compounds on human KB cells were measured by the tetrazolium-based colorimetric assay (MTT assay). Cells (2000 cells in 198 μl of RPMI-1640 medium supplemented with 10% fetal bovine serum) were seeded in a 96-well flat bottom micro test plate (InterMed, Roskilde, Denmark), and 2 μl of a drug solution at graded concentrations was simultaneously added in triplicate to each well. The plate was incubated for 3 d at 37 °C in a humidified atmosphere of 5% CO_2. The MTT reagent, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma, St. Louis, MO, U.S.A.), was prepared at a concentration of 2 mg/ml in Dulbecco's phosphate-buffered saline (PBS) without calcium or magnesium. On day 3, 25 μl of the MTT reagent was added to each well. After another 4 h of incubation, the medium was removed by aspiration. To solubilize the resulting MTT-formazan crystals, 0.2 ml of DMSO was added to each well and thoroughly mixed using a mechanical plate mixer. Absorbance at 540 nm (OD540) was measured with an Immuno Reader NJ-2000 (InterMed Japan, Tokyo). The percentage of cell growth inhibition was calculated by the following formula: % of cell growth inhibition=(1−T/C)×100, where C is the mean OD540 of the control group and T is that of the treated group. The 50% inhibitory drug concentration (IC_{50} value) was measured graphically from the dose–response curve with at least 3 drug concentration points.

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