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Growth Inhibition Activity of Thioacetal Artemisinin Derivatives against Human Umbilical Vein Endothelial Cells

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Abstract—Thioacetal artemisinin derivatives, in particular, 10α-phenylthiodihydroartemisinins (**5**), 10β-benzenesulfonyl-9-*epi*-dihydroartemisinin (**9**) and 10α-mercaptodihydroartemisinin (**11**), exhibit good growth inhibition activity against HUVEC proliferation at the concentration level of 1 μM.

Introduction

Angiogenesis, the formation of new blood vessels from existing host capillaries stimulated by biochemical stimulators, in normal vascular system is involved in wound healing, embryonic development, and the female reproductive cycle under elaborate regulations. On the other hand, in abnormal systems, angiogenesis is believed to be responsible for rheumatoid arthritis, ocular retinopathy and tumors.[1] In particular, tumor angiogenesis caused by angiogenic inducers[2] plays a key role in the growth of the solid tumors, their invasion, and metastasis. Therefore, the control of angiogenesis may be a promising therapeutic strategy for the related diseases.[3]

Strategies for regulating angiogenesis have been carried out mainly in molecular biology.[4] However, in spite of the settlement of bioavailavility, biostability and effectiveness, it has been insufficiently carried out to develop small molecule antiangiogenic agents. Therefore, it is very important to discover the antiangiogenic small molecules that might be suitable as clinical therapies.

The initial and key steps in tumor angiogenesis are mainly the roles of the endothelial cells (ECs), such as the migration, differentiation and tube formation of the ECs.[5] Therefore, in order to search for a novel small angiogenesis inhibitor, we must investigate the inhibitory effects of various promising molecules against the proliferation of human umbilical vein endothelial cells (HUVEC) in response to the various growth factors.

The natural sesquiterpene endoperoxide artemisinin (**1**), which was isolated from *Artemisia annua* L.,[6] has become a potential lead compound in the development of antimalarial[7] and recently anticancer agents.[8] The semi synthetic acetal-type artemisinin derivatives (**3**), ether and ester derivatives of trioxane lactol dihydroartemisinin (**2**), were developed for their higher antimalarial efficacy and are now widely used to treat malarial patients[9] (Fig. 1).

Recently, Chen et al. reported that artemisinin (**1**) and dihydroartemisinin (**2**) have the antiangiogenic activity as well as the antitumor activity on in vitro models of angiogenesis.[10] However, in our laboratory, we have already examined that artemisinin, dihydroartemisinin and acetal-type artemisinin derivatives (**3**) have the antiangiogenic activity.

Herein, we report that synthetic thioacetal artemisinin derivatives (**4**) showed a strong inhibitory effect against the proliferation of HUVECs.

Chemistry

As seen in Scheme 1, separable diastereomeric mixture of 10α- (**5**) and 10β-phenylthiodihydroartemisinins (**6**)

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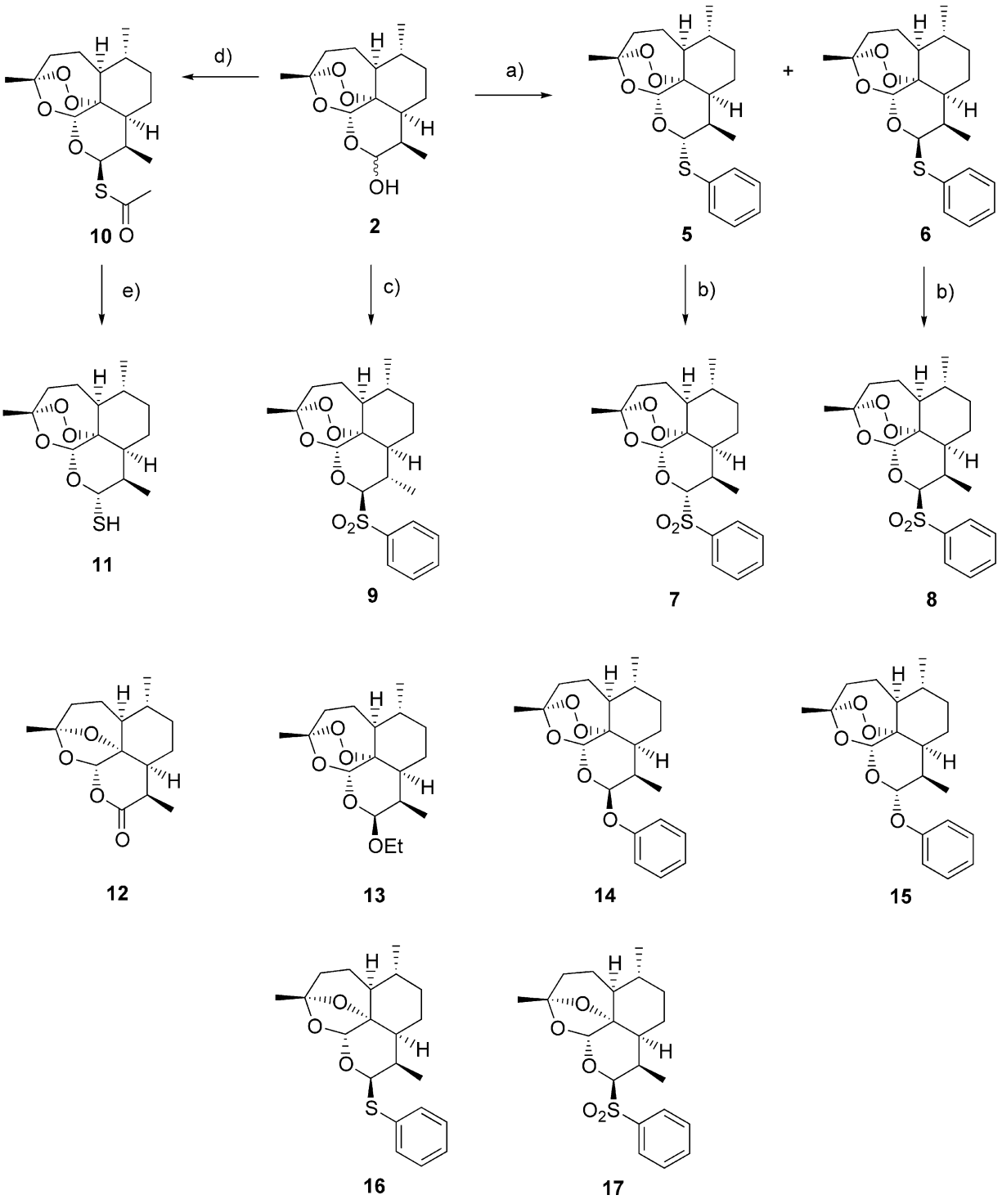
were prepared by reacting dihydroartemisinin (**2**) with thiophenol (2 equiv) under the catalysis of BF_3Et_2O (1 equiv) at room temperature during 10 min.[11,12] Unlike the *O*-acetalization of dihydroartemisinin (**2**) using various alcohol under the same condition, the *S*-acetalization of **2** diastereoselectively produced an α-anomer (**5**) as the major product in the ratio of 10:1. If reaction time is exceeded, desoxy-isomer of **5** (**16**) was formed from the thioacetal reaction as the side product because of the reductive property of thiol group. The thioacetal products (**5** and **6**) and desoxyside product (**16**) was transformed to produce 10α- (**7**) and 10β-benzenesulfonyldihydroartemisinins (**8**) and 10α-benzenesulfonyldesoxydihydroartemisinins (**17**) using oxidation with H_2O_2/urea complex (UHP), trifluoroacetic anhydride (TFAA) and $NaHCO_3$.[13] To compare growth inhibition



Scheme 1. (a) Thiophenol (2 equiv), BF_3Et_2O (1 equiv), CH_2Cl_2, reflux, 10 min (95%, **5**/**6** = 10:1); (b) UHP (3 equiv), TFAA (3 equiv), $NaHCO_3$ (5 equiv), CH_3CN, −30 °C (95% for **7** and 93% for **8**); (c) benzenesulfinic acid (2 equiv), BF_3Et_2O (1 equiv), CH_2Cl_2, rt (75%); (d) thiolacetic acid (1 equiv), BF_3Et_2O (1 equiv), CH_2Cl_2, rt, 10 min (84%); (e) NaOEt (5 equiv), EtOH, rt, 1 h (62%).

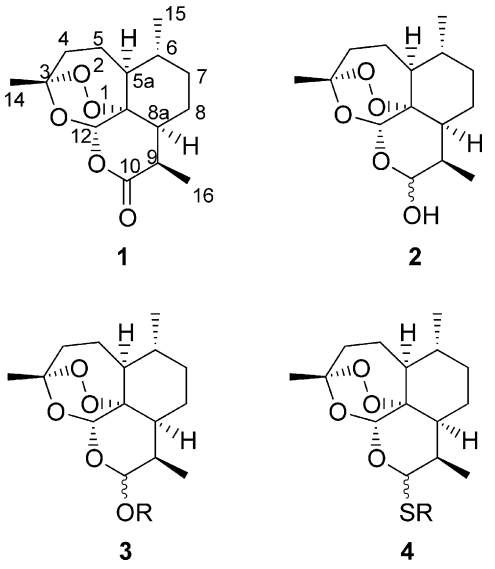


Figure 1.

Table 1. HUVEC proliferation inhibition assay results for artemisinin derivatives

Compd	Growth inhibition IC_{50} (μM)[a]
1	> 50
2	8.91
5	0.93
6	5.24
7	12.77
8	18.21
9	1.74
10	4.56
11	1.29
12	> 50
13	> 50
14	33.81
15	31.03
16	> 50
17	> 50

[a]IC_{50} was calculated from nonlinear regression by GraphPad Prism software ($r^2 > 0.9$).

activity against HUVEC between *S*-acetal and *O*-acetal analogues, 10α- (**14**) and 10β-phenoxydihydro-artemisinin (**15**) were synthesized the known method.[14] The stereochemistry of the products (**5**, **6**, **7**, **8**, **16** and **17**) was confirmed with coupling constants between H-9 and H-10 in ^{1}H NMR. Dihydroartemisinin (**2**) with benzenesulfinic acid under the condition shown in Scheme 1 was directly converted 10β-benzenesulfonyl-9-*epi*-dihydroartemisinin (**9**) in 75% yield, which stereochemistry was determined by ^{1}H and ^{13}C NMR in comparing with its diastereomeric sulfone compounds (**7** and **8**).[15] 10α-Mercaptodihydroartemisinin (**11**), *S*-acetal analogue of **2**, was prepared from the base catalytic deacetylation of 10β-thioacetoxydihydroartemisinin (**10**). In the course of deacetylation, the stereochemistry of C-10 position between **10** and **11** was converted from β to α caused by basic catalyst.[16]

Biology

The growth inhibition effect of thioacetal-type artemisinin derivatives and known acetal type artemisinin compounds were examined on a HUVEC[17] proliferation assay using the MTT colorimetric method.[18] The results are listed in Table 1.

The natural artemisinin (**1**) having endoperoxide ring and desoxyartemisinin (**12**) with no peroxide did not show inhibitory effect at the concentration of 50 μM, while dihydroartemisinin (**2**) was more effective than **1** in inhibiting the HUVEC growth ($IC_{50} = 8.91$ μM). As the Chen's report, we could also make sure that acetal-type derivatives might be a lead compound for use as an antiangiogenic inhibitor.

On the basis of our elementary result, we assumed that all acetal-type artemisinin derivatives (**3**) would have an inhibitory effect on HUVEC growth, but, disappointingly, 10β- (**14**) and 10α-phenoxydihydroartemisinin (**15**) with endoperoxide and aromatic phenyl group were less active than **2**.

So, as the new synthetic approach, we next synthesized a series of *S*-acetal compounds of **2** to determine the inhibitory activity on HUVEC. 10α-Phenylthiodihydroartemisinin (**5**) and 10β-phenylthiodihydroartemisinin (**6**) with endoperoxide and thiophenoxy moiety showed a very strong inhibitory activity with an $IC_{50} = 0.93$ and 5.24 μM, respectively. Among the synthetic sulfonyl compounds, such as 10α- (**7**) and 10β-benzenesulfonyl dihydroartemisinin (**8**) and 10β-benzenesulfonyl-9-*epi*-dihydroartemisinin (**9**), the inhibitory potency of 9-epimer (**9**) was over 7–10 times higher than that of **7** and **8**. It was very interesting that those compounds bearing a same molecular structure but only different C-9 stereochemistry showed a lot of difference in inhibitory effect. Even if desoxy compounds (**16** and **17**) had the thioacetal functionality and β-arteether had the endoperoxide, they have no inhibitory activity for lack of endoperoxide or aromatic functional group.

In particular, a comparison between 10α-mercaptodihydroartemisinin (**11**) and dihydroartemisinin (**2**) suggests that the sulfur atom at the C-10 position was critical for inhibition activity on HUVEC. According as oxygen changed to sulfur, the biological activity improved markedly.

In summary, among the 15 compounds tested, **5**, **9**, and **11** were the most potent, and **2**, **7**, **8** and **10** were moderate potent. So when assuming the HUVEC proliferation inhibitory effect listed in Table 1, it was postulated that the thioacetal dihydroartemisinin derivatives bearing sulfur acetal linkage at C-10 position and aromatic functional group as well as endoperoxide ring showed significantly efficient activity. Currently, with the various screening methods, such as HUVEC tube formation assay, HUVEC migration assay and CAM assay, we have confirmed that the selected molecules from the thioacetal artemisinin derivatives might be

antiangiogenic inhibitors, and are preparing a manuscript to be published on this work.

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12. Spectral data for **5**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.68 (2H, dd, J = 8.25, 1.83 Hz, Ph), 7.26 (3H, m, Ph), 5.35 (1H, s, H-12), 4.73 (1H, d, J = 10.98 Hz, H-10), 2.57 (1H, m, H-9), 2.37 (1H, td, J = 14.46, 4.02 Hz, H-4α), 1.47 (3H, s, H-14), 0.95 (3H, d, J = 6.21 Hz, H-16), 0.90 (3H, d, J = 7.14 Hz, H-15) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 132.9, 132.5, 128.6, 127.3, 104.3, 92.2, 83.5, 80.3, 51.7, 46.0, 37.4, 36.2, 34.1, 31.1, 26.0, 24.8, 21.4, 20.2, 15.1 ppm; for **6**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.54 (2H, dd, J = 8.61, 1.47 Hz, Ph), 7.26 (3H, m, Ph), 5.74 (1H, s, H-12), 5.57 (1H, d, J = 5.31 Hz, H-10), 3.12 (1H, m, H-9), 2.39 (1H, td, J = 14.28, 3.84 Hz, H-4α), 1.44 (3H, s, H-14), 1.05 (3H, d, J = 7.32 Hz, H-16), 0.98 (3H, d, J = 6.42 Hz, H-15) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.9, 130.9, 128.8, 126.7, 104.2, 90.2, 88.3, 81.0, 52.6, 45.1, 37.2, 36.4, 34.4, 32.7, 26.0, 24.6, 24.3, 20.3, 15.0 ppm.
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16. Spectral data for **10**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.12 (1H, d, J = 5.13 Hz, H-10), 5.33 (1H, s, H-12), 3.17 (1H, m, H-9), 2.37 (3H, s, -$SCOCH_3$), 2.41–2.31 (1H, m, H-4α), 1.35 (3H, s, H-14), 0.87 (3H, d, J = 6.03 Hz, H-16), 0.88 (3H, d, J = 7.35 Hz, H-15) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 193.20, 104.37, 89.34, 82.69, 80.46, 52.40, 44.80, 37.25, 36.12, 34.21, 31.25, 31.19, 25.90, 24.48, 23.54, 20.19, 14.19 ppm; for **11**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.29 (1H, s, H-12), 4.73 (1H, d, J = 10.80 Hz, H-10), 2.71 (1H, m, H-9), 2.36 (1H, td, J = 14.28, 3.84 Hz, H-4α), 1.41 (3H, s, H-14), 0.99 (3H, d, J = 7.14 Hz, H-16), 0.94 (3H, d, J = 6.03 Hz, H-15) ppm; ^{13}C NMR (75 MHz, $CDCl_3$) δ 104.17, 92.57, 86.98, 80.51, 51.82, 46.12, 37.34, 36.28, 33.96, 31.88, 26.07, 24.68, 21.89, 20.27, 14.87 ppm.
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18. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55. Assay method: HUVEC in EGM-2 medium containing the growth factor (Cambrex, Walkersville, MD, USA) was plated in a 48-well plate (10,000 cells/well) for 4 h to allow cells to adhere to the plate. The test compounds, diluted in the medium, were added to the appropriate wells and incubated for 72 h at 37 °C in a 5% CO_2 humidified atmosphere. After incubation, the viability of the HUVEC was assayed using a 3-(4,5-dimethylthiazol-2-y1)-2,5-diphenyltetrazolium bromide (MTT) colorimetric proliferation assay.