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Synthesis and antiangiogenic activity of *exo*-olefinated deoxoartemisinin derivatives

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Abstract—10-*exo*-Bromoalkylidene and benzylidene deoxoartemisinin derivatives with antiangiogenic activity were synthesized from corresponding 10-alkanesulfonyl dihydroartemisinin and 10-phenylmethanesulfonyl dihydroartemisinin using a highly efficient, mild, and simple Ramberg–Bäcklund rearrangement.

Angiogenesis, the formation of new blood vessels from existing host capillaries stimulated by biochemical stimulators, plays a key role in the growth of the solid tumors, their invasion, and metastasis.[1] Therefore, the control of angiogenesis may be a promising therapeutic strategy for the related diseases.[2]

Because of bioavailability, biostability, and effectiveness of endogenous antiangiogenic proteins, it is very important to discover the antiangiogenic small molecules that might be suitable as clinical therapies.[3]

In the course to discover small molecular angiogenesis inhibitors from natural lead compounds, we had reported that coronarin A[4] and artemisinin (**1**) derivatives[5] have an antiangiogenic activity. In particular, thioacetal artemisinin derivatives (**3**) have a strong effect.[5] Recently, Chen and co-workers reported that artemisinin (**1**), dihydroartemisinin (**2**), and artesunate have the antiangiogenic activity as well as the antitumor activity on in vitro models of angiogenesis.[6]

The natural sesquiterpene endoperoxide artemisinin (**1**), which was isolated from *Artemisia annua* L.,[7] has become a potential lead compound in the development of antimalarial[8] and recently anticancer agents.[9]

However, to search for novel types of artemisinin analogues with a high antiangiogenic activity and synthetic effectiveness, we decide to synthesize the C-10 *exo*-olefinated deoxoartemisinin derivatives (**4**) by using the Ramberg–Bäcklund rearrangement of *S*-glycoside for 1-*exo*-methylene glycal (Fig. 1).[10]

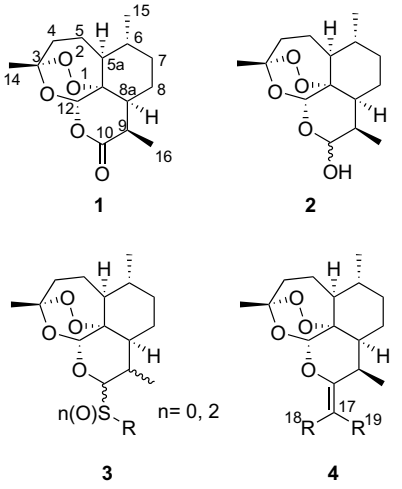

Figure 1.

Keywords: Angiogenesis; Antiangiogenesis; Artemisinin; *exo*-Olefinated deoxoartemisinin.
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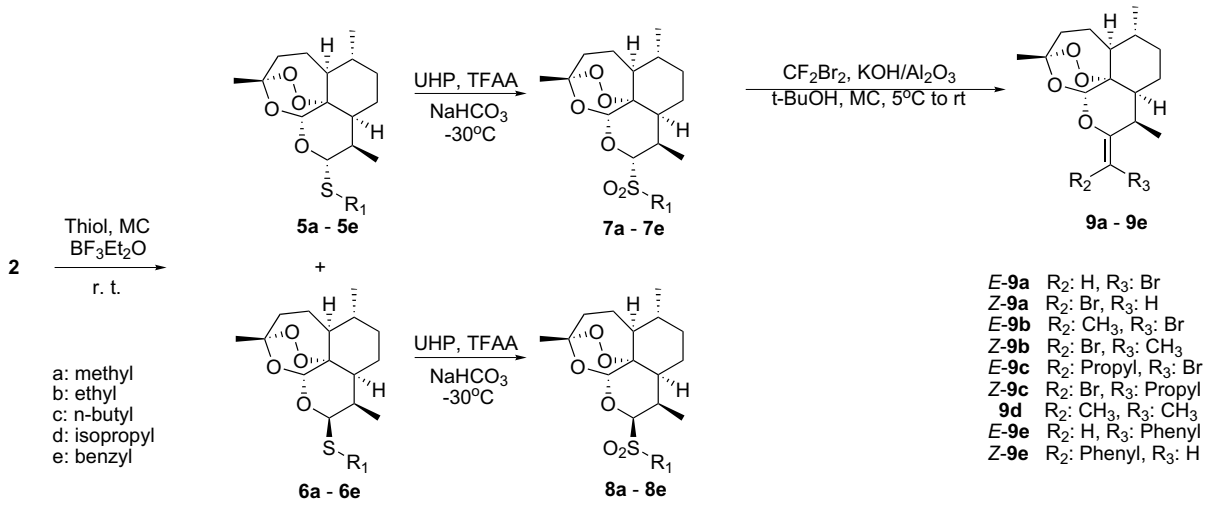

Scheme 1. Synthesis of *exo*-olefinated deoxoartemisinin derivatives by Ramberg–Bäcklund rearrangement: **9a** (*E*:*Z* = 50:50, 74%), **9b** (*E*:*Z* = 84:16, 76%), **9c** (*E*:*Z* = 92:8, 44%), **9d** (26%), **9e** (*E*:*Z* = 70:30, 78%).

Herein, based on the screening of formation assay of HUVEC tube on Matrigel and Chorioallantomic membrane (CAM) assay as well as growth inhibition activity against HUVEC, we report that C-10 *exo*-olefinated deoxoartemisinin derivatives (**4**) effectively inhibit the growth of HUVEC and these molecules are noble promising antiangiogenic agents.

As shown in Scheme 1, the reaction of dihydroartemisinin (**2**) with the corresponding thiol reactants[11] in the presence of BF_3Et_2O gave a separable mixture of major thioacetal dihydroartemisinin (**5a**–**e**) with a C-10α stereochemistry, and minor C-10β diastereomers (**6a**–**e**), respectively,[12] which were transformed to produce the corresponding 10α-substituted sulfonyl dihydroartemisinin (**7a**–**e**) and 10β derivatives (**8a**–**e**) by oxidation[13] with H_2O_2/urea (UHP), trifluoroacetic anhydride (TFAA), and $NaHCO_3$ as high yield.[14]

To obtain *exo*-olefinated deoxoartemisinin derivatives (**4**), we used modified Ramberg–Bäcklund rearrangement conditions.[15] The reaction between 10α-methanesulfonyl dihydroartemisinin (**7a**) and CF_2Br_2, and KOH/Al_2O_3 in *t*-BuOH and methylene chloride (2:1) at room temperature gave an inseparable *E* and *Z* mixture of 10-bromomethylene deoxoartemisinin (**9a**) in the same ratio.[16] Under the same reaction conditions, two 10α-alkanesulfonyl dihydroartemisinin **7b** and **7c** stereoselectively produced a separable *E* and *Z* mixture of 10-(1-bromoethylidene) deoxoartemisinin (**9b**) (76% yield, *E*:*Z* = 84:16) and 10-(1-bromobutylidene) deoxoartemisinin (**9c**) with a high stereoselectivity (84% yield, *E*:*Z* = 92:8), which is a very rare case that the bromo-olefinated products could be synthesized from alkanesulfonyl reactants by a Ramberg–Bäcklund rearrangement.

In the case of the secondary alkane substituted sulfonyl dihydroartemisinin (**7d**), 10-isopropylidene deoxoartemisinin (**9d**) was obtained in a very low yield (26%) due to steric hindrance of the isopropyl group.

Unlike the alkane sulfonyl reactants (**7a**–**d**), the 10α-phenylmethanesulfonyl dihydroartemisinin (**7e**) with an aromatic sulfonyl group gave separable *E*- and *Z*-isomers of the 10-benzylidene deoxoartemisinin (**9e**) with no bromide in a 78% yield (*E*:*Z* = 70:30). The stereochemistry of *E*-**9e** and *Z*-**9e** was determined by comparing each chemical shift of H-17 (6.43 ppm for *E* and 5.45 ppm for *Z*) and H-9 (3.45 ppm for *E* and 3.33 ppm for *Z*) of the two isomers.[14,17]

To investigate the antiangiogenic activity of C-10 *exo*-olefinated deoxoartemisinin derivatives (**9a**–**e**), they were examined on a HUVEC proliferation assay using the MTT colorimetric method,[18] tube formation assay on Matrigel[19] and CAM assay.[20] These results were listed in Table 1.

Table 1. Antiangiogenic activity of C-10 *exo*-olefinated deoxoartemisinin derivatives

Compounds	Growth inhibition IC_{50} (μg/mL)[a]	Tube formation inhibition at 10 μg/mL (%)	CAM assay at 10 μg/egg (%)
E/*Z* mixture of **9a**	4.8	25	80
E-**9b**	4.5	65	50
Z-**9b**	25.4	32	43
E-**9c**	1.5	39	57
Z-**9c**	1.9	10	8
9d	2.3	13	29
E-**9e**	1.9	10	10
Z-**9e**	2.3	10	5

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

First, tested C-10 *exo*-olefinated deoxoartemisinin derivatives (**9a**–**e**) showed a strong inhibitory activity upon HUVEC growth except for mildly active *Z*-isomer of **9b**. Especially, all isomer of **9c**, **9d**, and **9e** effectively inhibited the HUVEC growth at the concentration 2 μg/mL level. This result led us to assume that deoxo-type artemisinin derivatives may have an antiangiogenic activity.

Second, to conform our hypothesis, the activity to suppress the growth factor induced tube formation by HUVEC on Matrigel was assessed at the concentration of 10 μg/mL. Among the promising molecules, disappointingly, only *E*-isomer of **9b** showed a good inhibition activity against tube formation. Other compounds (**9a**, *Z*-**9b**, and *E*-**9c**) were mildly effective.

Third, in the CAM assay at the concentration of 10 μg/egg, the *E* and *Z* mixture of **9a** strongly inhibited the formation of new blood vessel on CAM. The *E*- and *Z*-isomer of **9b** and *E*-isomer of **9c** showed a mild inhibitory activity.

In conclusion, with the various screening methods, such as growth inhibition activity against HUVEC, formation assay of HUVEC tube on Matrigel and Chorioallantomic membrane (CAM) assay, we concluded that the C-10 *exo*-olefinated deoxoartemisinin derivatives (**9a**–**e**) can inhibit the angiogenesis and might be angiogenesis inhibitors. The bromo-alkenylidene analogues (**9a**–**c**) were expected to have potential synthetic utilities and might be used to synthesize new multi-substituted deoxoartemisinin derivatives derived from the metalation and successive addition reaction.

Acknowledgements

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17. Spectral data for **9a**: ^{1}H NMR (300 MHz; $CDCl_3$) δ 5.88 (1H, d, $J = 0.9$ Hz, H-17 of *E*-**9a**), 5.48 (1H, s, H-12 of *E*-**9a**), 5.43 (1H, s, H-12 of *Z*-**9a**), 5.24 (1H, d, $J = 2.0$ Hz, H-17 of *Z*-**9a**), 3.31 (1H, m, H-9 of *E*-**9a**), 3.22 (1H, m, H-9 of *Z*-**9a**), 2.46–2.29 (2H, m, H-4α of *E* and *Z*-**9a**), 1.48 (3H, s, H-14), 1.40 (3H, s, H-14), 1.25 (3H, d, $J = 7.5$ Hz, H-16), 1.03 (3H, d, $J = 7.1$ Hz, H-16), 0.98 (3H, d, $J = 4.8$ Hz, H-15), 0.95 (3H, d, $J = 4.8$ Hz, H-15) ppm; for *E*-**9b**: ^{1}H NMR (300 MHz; $CDCl_3$) δ 5.57 (1H, s, H-12), 3.33 (1H, m, $J = 7.7$ Hz, H-9), 2.29 (3H, s, H-18), 1.36 (3H, s, H-14), 1.13 (3H, d, $J = 7.3$ Hz, H-16), 0.97 (3H, d, $J = 6.0$ Hz, H-15) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ 150.0, 106.5, 102.9, 93.1, 81.2, 50.4, 42.8, 37.6, 36.1, 34.2, 32.2, 25.5, 25.0, 24.2, 21.7, 19.8, 14.5 ppm; for *Z*-**9b**: ^{1}H NMR (300 MHz; $CDCl_3$) δ 5.57 (1H, s, H-12), 3.25 (1H, m, $J = 7.5$ Hz, H-9), 2.32 (1H, td, $J = 13.9$, 3.8 Hz, H-4α), 2.28 (3H, s, H-18), 1.45 (3H, s, H-14), 1.09 (3H, d, $J = 7.5$ Hz, H-16), 0.97 (3H, d, $J = 6.0$ Hz, H-15) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ 149.2, 103.4, 102.2, 92.8, 81.2, 50.5, 43.5, 37.5, 36.2, 34.1, 31.0, 25.4, 25.0, 23.8, 22.5, 19.9, 15.8 ppm; for *E*-**9c**: ^{1}H NMR (300 MHz; $CDCl_3$) δ 5.57 (1H, s, H-12), 3.33 (1H, m, $J = 7.7$ Hz, H-9), 2.85 (1H, td, $J = 14.1$, 8.2 Hz, H-18), 2.37–2.26 (2H, m, H-18, H-4α), 1.35 (3H, s, H-14), 1.14 (3H, d, $J = 7.3$ Hz, H-16), 0.97 (3H, d, $J = 6.1$ Hz, H-15), 0.91 (3H, t, $J = 7.3$ Hz, H-20) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ 149.9, 113.0, 103.0, 93.1, 81.1, 50.5, 42.7, 37.6, 36.2, 35.3, 34.2, 32.3, 25.5, 25.0, 24.3, 21.6, 19.9, 14.7, 13.1 ppm; for *Z*-**9c**: ^{1}H NMR (300 MHz; $CDCl_3$) δ 5.58 (1H, s, H-12), 3.25 (1H, m, $J = 7.7$ Hz, H-9), 2.50–2.26 (2H, m, H-18, H-4α), 1.44 (3H, s, H-14), 1.10 (3H, d, $J = 7.5$ Hz, H-16), 0.97 (3H, m, $J = 6.2$ Hz, H-15), 0.96 (3H, t, $J = 7.3$ Hz, H-20) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) 149.4, 109.3, 103.3, 92.9, 81.1, 50.5, 43.4, 37.5, 36.5, 36.2, 34.1, 31.1, 25.4, 25.0, 23.9, 21.8, 19.8, 16.5, 13.2 ppm; for **9d**: ^{1}H NMR (300 MHz; $CDCl_3$) δ 5.46 (1H, s, H-12), 3.19 (1H, m, $J = 7.5$ Hz, H-9), 2.30 (1H, td, $J = 14.3$, 3.9 Hz, H-4α), 1.72, 1.66 (each 3H, s, H-18, H-19), 1.36 (3H, s, H-14), 1.06 (3H, d, $J = 7.5$ Hz, H-16), 0.96 (3H, d, $J = 6.0$ Hz, H-15) ppm; ^{13}C NMR

(75 MHz; $CDCl_3$) δ 145.4, 112.5, 102.9, 92.8, 81.1, 50.8, 43.9, 37.6, 36.4, 34.3, 30.2, 25.7, 25.1, 24.0, 20.0, 18.0, 17.8, 16.1 ppm; *E*-**9e**: ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.38–7.17 (5H, m, phenyl), 6.43 (1H, s, H-17), 5.50 (1H, s, H-12), 3.45 (1H, m, H-9), 2.38 (1H, td, $J = 13.4$, 3.1 Hz, H-4α), 1.46 (3H, s, H-14), 0.98 (3H, d, $J = 6.2$ Hz, H-16), 0.91 (3H, d, $J = 7.3$ Hz, H-15) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) 153.5, 136.0, 129.3×2, 127.7×2, 126.1, 113.7, 103.9, 93.6, 80.8, 51.0, 45.6, 37.4, 36.2, 33.9, 31.6, 25.7, 24.8, 23.0, 20.0, 15.7 ppm; for *Z*-**9c**: ^{1}H NMR (300 MHz; $CDCl_3$) δ 7.69 (2H, d, $J = 7.3$ Hz, phenyl), 7.28 (2H, dd, $J = 7.5$, 7.7 Hz, phenyl), 7.16 (1H, d, $J = 7.3$ Hz, phenyl), 5.48 (1H, s, H-12), 5.45 (1H, d, $J = 1.3$ Hz, H-17), 3.33 (1H, m, H-9), 2.44 (1H, td, $J = 13.2$, 3.8 Hz, H-4α), 1.51 (3H, s, H-14), 1.10 (3H, d, $J = 7.0$ Hz, H-16), 0.94 (3H, m, $J = 5.9$ Hz, H-15) ppm; ^{13}C NMR (75 MHz; $CDCl_3$) δ 154.1, 135.8, 128.5×2, 128.2×2, 126.0, 109.0, 104.6, 92.9, 80.8, 51.3, 46.6, 37.4, 36.2, 33.8, 31.7, 25.8, 24.8, 21.9, 20.2, 14.1 ppm.

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