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Synthesis and anticancer activity of novel amide derivatives of non-acetal deoxoartemisinin

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ABSTRACT

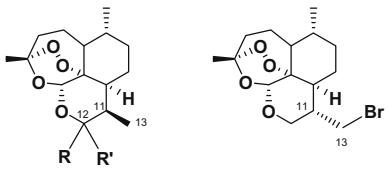
Novel amide derivatives of C-12 non-acetal deoxoartemisinin were synthesized. Some of the derivatives had potent in vitro anticancer activity against major human cancer cell lines. The deoxoartemisinin amide trimer had potent in vivo antiangiogenic activity, according to the mouse matrigel plug assay.

Artemisinin (**1a**), a sesquiterpene isolated from *Artemisia annua L.*,[1] and its derivatives have been used clinically to treat drug-resistant malaria.[2] The pharmacology and pharmacokinetics of artemisinin and its derivatives have been well studied.[3] Artemisinin contains an endoperoxide that reacts with an iron ion to form a carbon-based free radical. When formed intracellularly, this free radical could cause macromolecular damage, leading to cell death. Since tumor cells uptake a large amount of iron compared to normal cells,[3g] they are more vulnerable to the cytotoxic effect of artemisinin. As we describe previously,[4] a variety of researchers have reported on the potential antitumor properties of artemisinin and its derivatives.[5] Of the derivatives, some dimeric chemical structures exhibited the high anticancer activity.[4a] Non-acetal 12β(C–C)-type derivatives of artemisinin exhibited higher anticancer activity,[4a] along with 20 times more acid stability in oral administration, than acetal (C–O)-type derivatives of artemisinin.[4b] Recently, artemisinin has also been reported to have antiangiogenic activity.[6] Chen et al. reported that artesunate exhibits antiangiogenesis, as well as apoptic activity in human endothelial cells.[6b]

In this study, we report on the synthesis of novel amide derivatives of non-acetal deoxoartemisinin. These derivatives exhibit in vitro anticancer activity against major human cancer cell lines, and further in vivo antiangiogenic activity.

We synthesized previously reported[4a,7] artemisinin derivatives for comparison with new amide derivatives, including dihydroartemisinin (**1b**), deoxoartemisinin (**1c**), artemether (**1d**), and 11-α-13-bromodeoxoartemisinin (**2a**), all shown in Figure 1. The various other derivatives (**6–7, 9**), shown in Figure 2, were prepared according to the procedure described by Jung et al.[4a,7] 12-Carboxyethyl deoxoartemisinin (**2b**) was also prepared from artemisinin, as previously presented by the same authors.[8]

New amide derivatives **3**, **4**, **5** and **8** were prepared by reacting **2b** with various amines, in the presence of HOBt and EDC as coupling agents, as outlined in Scheme 1. The new amide derivatives[9] 3–**5** and **8** were obtained in 64–87% yield. The 1,4-propylpiperidine-linked dimer **8** was synthesized with the same coupling conditions as the other derivatives, with the exception that 2 equiv of compound **2b** were used. In amide coupling conditions, the endoperoxide functional group remains intact.



1a: R, R'= O
1b: R=H, R'=OH
1c: R= R'= H
1d: R=H, R'=OCH_3

2a

Figure 1. Structure of artemisinin (**1a**), dihydroartemisinin (**1b**), deoxoartemisinin (**1c**), artemether (**1d**) and 11-α-13-bromodeoxo-artemisinin (**2a**).

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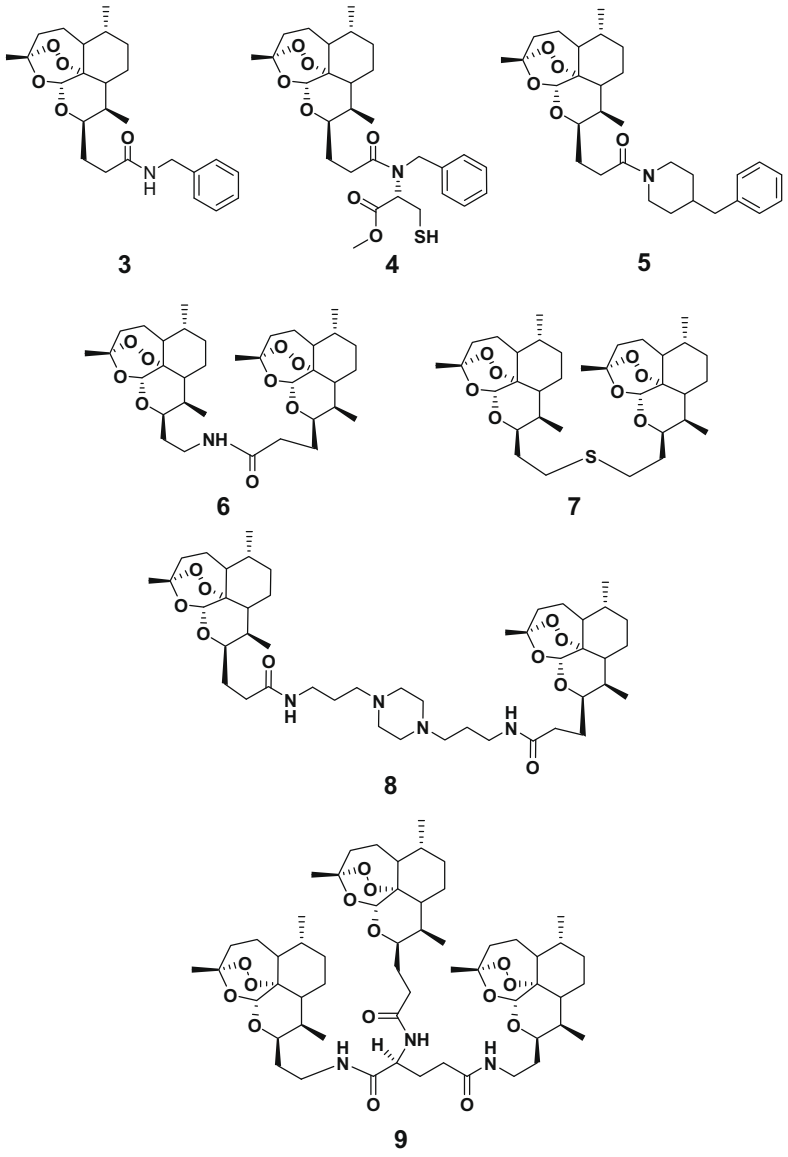


Figure 2. Structure of amide and other derivatives of non-acetal type deoxoartemisinin.

To determine the in vitro anticancer activity of the amide derivatives and their dimers, a SRB (sulforhodamin B) assay was performed, as previously described.[10]

The results are listed in Table 1. The standard drug used for comparison was doxorubicin. Although dihydroartemisinin **1b** exhibited high anticancer activity against all tested human cell lines, its relative instability in acid, due to acetal formation at C-12, rules it out as a potential anticancer drug candidate. Most amide derivatives had high anticancer activity, although artemisinin, 11-α-bromo-deoxoartemisinin **2a** and amide trimer **9** showed only moderate activity. Particularly, the low anticancer activity of trimer **9** could be due to its low solubility.

In comparison, the sulfide dimer **7** showed anticancer activity comparable to that of the amide derivatives. As shown in Table 1, dihydroartemisinin (**1b**), and the amide-linked dimer (**8**) had high vitro anticancer activity. Furthermore, the HCl salt form of the piperadine moiety on compound **8** increases its water solubility, making it suitable for injection.

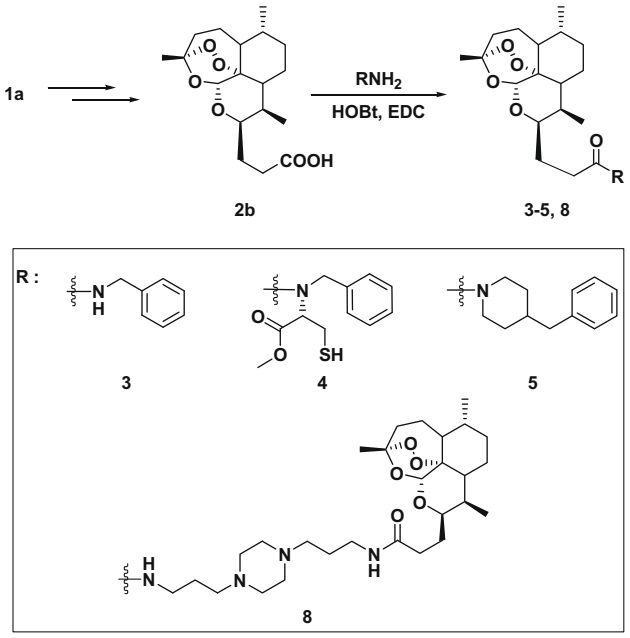
Due to the potent ex vivo antiangiogenic activity (75–95%) of 11-α-13-bromodeoxoartemisinin **2a**, and the amide trimer **9**, the in vivo antiangiogenic activity of each derivative[11] was tested with the mouse matrigel plug assay. Results are shown in Figures 3 and 4. Compound **2a** and the amide trimer **9** showed excellent antiangiogenic activity at 10 μM, with 95% and 80% inhibition, respectively, as shown in Figures 3 and 4. These results are comparable to that of the (−)-thalidomide standard.

There is no direct correlation of the antiangiogenic and anticancer activities for this group of compounds. The results indicate these high potent activities independently.

The proposed mechanism for the anticancer activity of artemisinin derivatives is alkylation to cellular proteins of carbon-centered radical formed with homolytic cleavage of the endoperoxide bridge catalyzed by Fe^{+2} ions, while that of the antiangiogenic activity is unknown.

Therefore, we conclude that the trimer amide and 13-bromodeoxoartemisinin showed potent in vivo antiangiogenic activity, although the in vitro anticancer activity was not as high as other artemisinin derivatives.

In summary, non-acetal type amide derivatives of deoxoartemisinin and their novel derivatives were synthesized. Some of the derivatives exhibited potent in vitro anticancer activity. Compound **2a** and the amide trimer **9** had very high in vivo antiangio-



Scheme 1. Synthesis of novel amide derivatives (**3**–**5**, and **8**) of deoxoartemisinin.

Table 1
In vitro anticancer activity of nonacetal type amide derivatives of deoxoartemisinin on human cancer cell lines

Compds	IC_{50} values (μM)				
	A549	SK-V3	SK-MEL-2	XF498	HCT15
1a	15.2	14.3	12.9	16.2	17.2
1b	0.63	0.85	0.75	0.54	0.46
1d	8.54	8.03	7.24	9.10	9.66
2a	11.2	15.4	14.2	16.4	11.2
3	5.13	4.75	8.36	3.46	6.22
4	3.12	2.35	3.15	2.66	1.54
5	1.35	1.65	1.25	1.95	1.45
6	7.63	4.36	9.94	5.76	7.83
7	5.63	4.86	7.56	5.44	3.53
8	0.85	0.63	0.45	0.46	0.84
9	13.5	12.4	18.2	11.6	19.8
Doxorubicin	0.075	0.046	0.028	.0.35	0.037

Compound **1a**, artemisinin; **1b**, dihydroartemisinin; **1d**, artemether; A594: non small cell lungcarcinoma, SK-OV-3:adenocarcinoma, ovary malignant ascites, SK-MEL-2: malignant melanoma, metastasis to skin of thigh, XF498: central nerve system tumor, HCT15: colon adenocarcinoma.

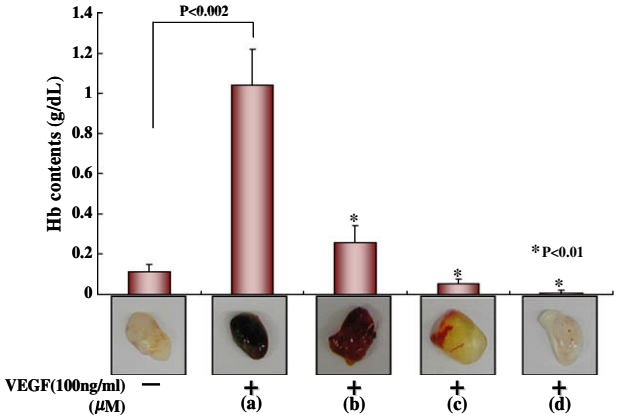


Figure 3. Inhibition of VEGF-induced angiogenesis by 11-α-13-bromodeoxo artemisinin (**2a**) with mouse matrigel plug assay: (a) control; (b) **2a**: 5 μM; (c) **2a**: 10 μM; (d) (−)-thalidomide: 10 μM.

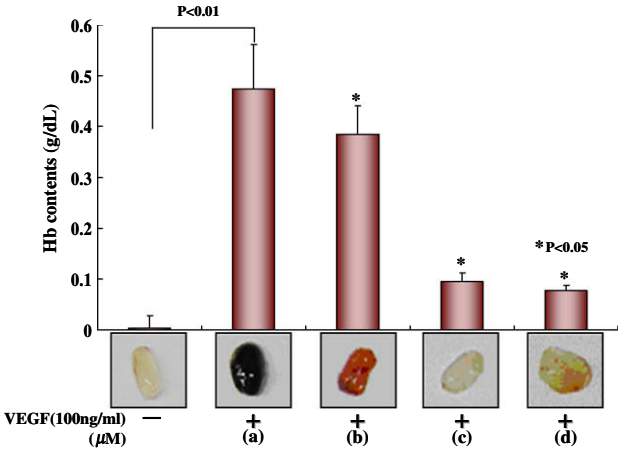


Figure 4. Inhibition of VEGF-induced angiogenesis by deoxoartemisinin amide trimer (**9**) with mouse matrigel plug assay: (a) control; (b) **9**: 5 μM; (c) **9**: 10 μM; (d) (−)-thalidomide: 10 μM.

genic activity. We conclude that non-acetal 12β(C–C)-type amide derivatives of deoxoartemisinin deserve further evaluation as possible anticancer drug candidates, because of their high acid stability and low toxicity, coupled with high in vitro and in vivo anticancer activity.

Acknowledgements

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9. All new compounds exhibited physical and spectroscopic properties consistent with their structure.*Spectral data for compound* **3**: R_f 0.22 (*n*-hexane/EtOAc = 5:1 v/v); $[\alpha]_D^{24} = +91.6$ (*c* 0.095, $CHCl_3$); ^1H NMR (250 MHz, $CDCl_3$) δ 7.30 (m, 5H, aromatic), 6.06 (br s, 1H), 5.28 (s, 1H, C-5), 4.44 (d, 2H), 4.05 (m, 1H, C-12), 2.72 (m, 1H, C-11), 2.48 (m, 1H), 2.34 (m, 2H), 1.37 (s, 3H, C-15), 0.96 (d, 3H, C-14), 0.88 (d, 3H, C-13); ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.9, 138.4, 128.7, 128.7, 128, 128, 127.5, 103.5, 88.8, 81.2, 76.1, 52.5, 44.5, 43.8, 37.4, 36.6, 34.7, 34.5, 30.2, 26.2, 25, 24.9, 24.7, 20.3, 13.3; IR (KBr, cm^{-1}) ν max 3460, 2913,

2046, 1740, 1650, 1540, 1450, 1376, 1258, 1094, 1053, 1017, 874, 698, 526, 457; MALDI-TOF MS: found 430.2171 [(M+H)$^+$]. For compound **4**: R_f 0.37 (*n*-hexane/EtOAc = 1:1 v/v); $[\alpha]_D^{24} = +100$ (*c* 0.03, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.30 (m, 5H, aromatic), 6.29 (d, 1H), 5.29 (s. 1H, C-5), 4.80 (m, 1H), 4.05 (m, 1H, C-12), 3.74 (s, 3H), 3.71 (s, 2H), 2.97 (d, 2H), 1.40 (s, 3H, C-15), 0.95 (d, 3H, C-14), 0.89 (d, 3H, C-13); ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.8, 171.5, 137.8, 129, 129, 128.7, 128.7, 127.4, 103.5, 88.7, 81.2, 76.2, 55.1, 52.7, 52.5, 51.6, 44.6, 37.4, 36.7, 36, 34.5, 33.5, 30.2, 29.8, 26.2, 25, 24.8, 20.3, 13.3; IR (KBr, cm^{-1}) ν max 3476, 2925, 2843, 2471, 2353, 2304, 1732, 1650, 1450, 1380, 1217, 1017, 874, 763, 665, 498; MALDI-TOF MS: found 548.2216 [(M+H)$^+$]. For compound **5**: R_f 0.39 (*n*-hexane/EtOAc = 1:1 v/v); $[\alpha]_D^{24} = +45.5$ (*c* 0.055, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.29–7.03 (m, 5H, aromatic), 5.30 (s, 1H, C-5), 4.62 (d, 1H), 4.04 (m, 1H, C-12), 3.91 (d, 1H), 2.91 (m, 1H, C-11), 2.76 (m, 1H), 2.53 (m, 2H), 2.31 (m, 2H), 1.42 (s, 3H, C-15), 0.95 (d, 3H, C-14), 0.91 (d, 3H, C-13); ^{13}C NMR (63 MHz, $CDCl_3$) δ 171.2, 140.1, 129.2, 128.4, 126.1, 103.6, 88.6, 81.3, 76.6, 52.6, 45.7, 44.7, 43.1, 42.1, 38.4, 37.4, 36.6, 34.5, 32.6, 31.9, 31.4, 30.2, 26.3, 24.9, 24.7, 24.5, 20.4, 13.5; IR (KBr, cm^{-1}) ν max 3293, 2917, 2843, 2651, 1985, 1732, 1638, 1450, 1376, 1258, 1094, 1049, 1012, 910, 878, 767, 632, 481; MALDI-TOF MS: found 498.2810 [(M+H)$^+$]. For compound **8**: R_f 0.42 (only MeOH v/v); $[\alpha]_D^{24} = +204$ (*c* 0.025, $CHCl_3$); ^{1}H NMR (250 MHz, $CDCl_3$) δ 6.89 (br s, 1H), 5.29 (s, 1H, C-5), 4.04 (m, 1H, C-12), 3.27 (m, 2H), 2.75 (m, 1H, C-11), 1.47 (s, 3H, C-15), 0.96 (d, 3H, C-14), 0.86 (d, 3H, C-13); ^{13}C NMR (63 MHz, $CDCl_3$) δ 172.8, 103.4, 100.1, 88.7, 81.3, 76.3, 57.3, 53.4, 52.5, 44.6, 39.3, 37.4, 36.6, 34.9, 34.5, 30.2, 26.3, 25.8, 24.9, 24.7, 20.3, 13.4; IR (KBr, cm^{-1}) ν max 3444, 2913, 2843, 1732, 1646, 1384, 1249, 1021, 874, 767, 620, 477; MALDI-TOF MS: found 845.5177 [(M+H)$^+$].

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