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Concise synthesis and antiangiogenic activity of artemisinin–glycolipid hybrids on chorioallantoic membranes

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ABSTRACT

Novel hybrids of non acetal and acetal-type derivatives at C-12 of artemisinin and glycolipids were synthesized via efficient coupling reactions. Some of these hybrids showed potent in vivo antiangiogenic activity on the chorioallantoic membrane (CAM) that was higher than or comparable to those of fumagillin and thalidomide at a concentration of 2.5 nmol.

Angiogenesis is a physical process in which new blood vessels are formed from pre-existing vessels. The blockade of vascular endothelial growth factor results in regression of the disease and prolongation of survival when used for anticancer therapy.[1] Discovery of new antiangiogenic agents based on small molecules is an attractive approach for the treatment of cancer.

Artemisinin (**1**), a sesquiterpene endoperoxide isolated from *Artemisia annua L.*,[2] (Fig. 1) and its derivatives have been reported as potential antitumor[3] and virtually nontoxic[3a] agents. Artemisinin has also been reported to have antiangiogenic activity.[4] In a previous report, we described the potent antiangiogenic effects of artemisinin derivatives.[5] Non acetal-type derivates at C-12 of artemisinin and their dimers including a fullerene conjugate were synthesized and some of them showed potent in vivo antiangiogenic activity on the chorioallantoic membrane that was higher than or comparable to those of fumagillin and thalidomide.[5] Furthermore, novel amide derivatives of a C-12 non acetal deoxoartemisinin trimer were synthesized and showed potent in vivo antiangiogenic activity according to the results of mouse matrigel plug assays.[3d] Recently, some studies have reported the antiangiogenic activity of glycolipids.[6] Daumone (**2**), originally isolated from *Caenorhabditis elegans*, was identified by our laboratory (Fig. 1) and its total synthesis was presented.[7] In a continuation of the investigation, we studied the anticancer activity of daumone against human cell lines.[8] Daumone and tri-deoxyrhamnose derivatives containing amide side chains were the most potent anticancer compounds that we surveyed, with effective concentrations in the nanomolar range, which is comparable to that of doxorubicin.[8] Although details of the mechanism of apoptosis remain unclear, lipophilicity may play an important role in glycolipid anticancer activity.[8]

Although various antiangiogenic agents have been developed, adverse side effects and limitations associated with antitumor therapies have recently become apparent. Cancer is a complex disease. In order to improve the activity of anticancer agents, treatment using hybrid drugs, an approach that incorporates two drugs in a single molecule, has been developed.[9] The use of hybrid drugs can impact multiple targets simultaneously.

In the present article, we report the synthesis and the antiangiogenic activity of artemisinin–glycolipid hybrid agents.

Carboxymethyldeoxoartemisinin (**1a**) and various derivatives as outlined in Figure 2 were prepared according to previously-described procedures.[10] Glycolipid (**2a**) and its derivatives (Fig. 2) were synthesized according to the previously-reported

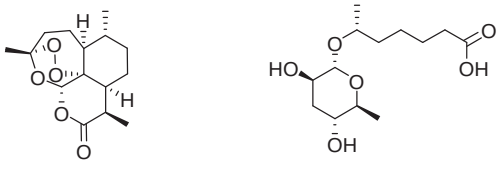

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Figure 1. Structures of artemisinin (**1**) and daumone (**2**).

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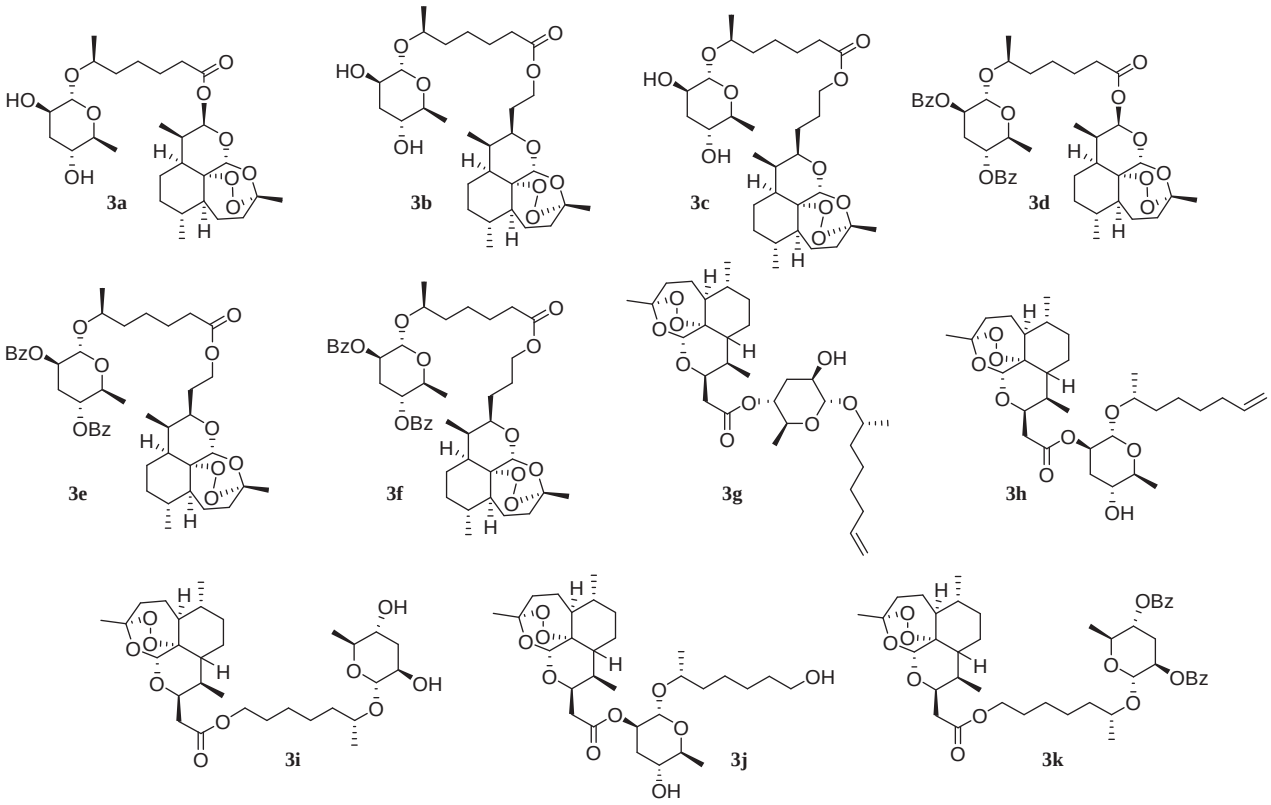
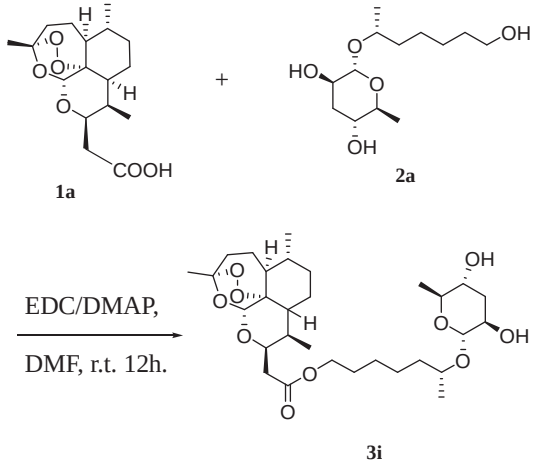


Figure 2. Structures of artemisinin–glycolipid hybrids.

procedures.[7,8] Then, a short series of artemisinin–glycolipid hybrids (Fig. 2) covalently linked were prepared by efficient coupling reactions (EDC/DMAP, rt, DMF, 12 h), as shown in Scheme 1, and their structures were confirmed by spectral analysis (see Supplementary data). The hybrid regioisomers (**3g, 3h, 3j**) were formed with glycolipids containing protected terminal olefin or primary alcohol instead of free carboxylic acid.

The in vivo antiangiogenic activity of the various hybrid compounds was evaluated using the CAM vessel development assay as previously described.[5,11]

Briefly, fertilized eggs (Pulmuone Co., Kyungki-do, Korea) were incubated at 37 °C with 80–90% relative humidity. At day 3, a window was opened after the removal of 2 ml albumin in the eggs.



Scheme 1. Coupling reaction of artemisinin and glycolipid.

At day 5 of incubation, test sample loaded on a quarter size Thermanox coverslip (Nunc, Roskilde, Denmark) was applied to the CAM of each individual embryo at a concentration of 2.5 nmol/egg. After 2 incubation days, a 20% fat emulsion was injected into the CAM for observation of the inhibition avascular zone. If an avascular zone of about 3–6 mm diameter, as indicated with an arrow in Figure 3, was observed, then it was considered to represent effective inhibition on neovascularization. The results of these experiments are listed in Table 1. The standard drugs used for comparison were (−)-fumagillin and (−)-thalidomide.

As shown in Table 1, it is interesting to note that most hybrids exhibited twice the antiangiogenic activity at a concentration of 2.5 nmol/egg than that of fumagillin or thalidomide as the standard drug.

Artemisinin (**1**) showed a weak inhibitory effect at the given concentration, while glycolipid (**2**) remained stronger than standard drugs. Generally hybrids showed higher antiangiogenic activity than artemisinin (**1**) and comparable to that of glycolipid (**2**).

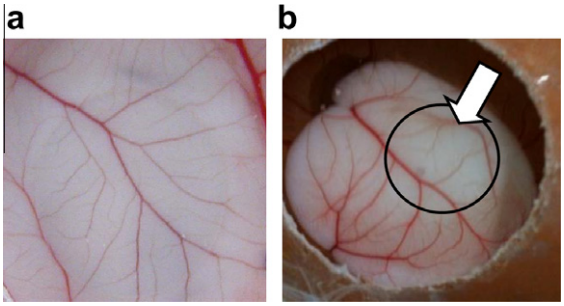


Figure 3. Antiangiogenic effect of artemisinin–glycolipid hybrids on the chick CAM. Membranes were treated with (a) control, (b) artemisinin–glycolipid hybrids in a concentration of 2.5 nmol/egg.

Table 1
Inhibitory effect of artemisinin–glycolipid hybrids on CAM antiangiogenesis in a concentration of 2.5 nmol/egg

Compounds	Positive eggs/eggs tested	Inhibition effect[a]	Inhibition (%)
1	3/10		30
2	7/10	++	70
3a	5/10 (1)[b]	+	50
3b	6/10 (2)	+	60
3c	6/10 (2)	+	60
3d	1/10 (2)		10
3e	6/10 (2)	+	60
3f	8/10	+++	80
3g	7/10 (2)	++	70
3h	5/10 (5)	Low toxic	50
3i	10/10	+++	100
3j	5/10	+	50
3k	5/10 (2)	+	50
(−)-Fumagillin	4/10 (1)		40
(−)-Thalidomide	4/10 (4)	Low toxic	40
Control[c]	0/10		0

[a] Inhibition effect; antiangiogenic effect of plus (+) is similar to thalidomide or fumagillin, double plus (++) is stronger and triple plus (+++) is the strongest.
[b] Number in parentheses describes eggs in which the embryo died.
[c] Control; solvent only (chloroform) to embryo.

However, C-12 acetal-type artemisinin–glycolipid hybrids (**3a** and **3d**) exhibited weaker activity than non-acetal type hybrids. A benzoyl protected hybrid (**3d**) with acetal function at C-12 of artemisinin displayed the weakest inhibitory activity, while a hybrid (**3i**) with free hydroxyl groups of glycolipid with non-acetal function of artemisinin showed complete (100%) inhibition of angiogenesis.

Interestingly, terminal olefin of the aliphatic side chain of a compound (**3h**) that has a good antitumor activity displayed dramatically increased toxicity, and 50% of tested chicken embryos died at the given concentration. However, hybrid **3g** different from its regioisomer **3h** only by the hydroxyl site connection, showed less toxicity but better activity. The regioisomers (**3h**, **3j**) showed only comparable antiangiogenic activity, thus suggesting the coupling position of the C-12 side function of artemisinin should link with the terminal carboxylic acids of glycolipids. It is noteworthy that the hybrid compound (**3i**) that does not exhibit cytotoxicity has the most potent antiangiogenic activity in this assay. The requirement for the presence of the peroxide bond for antiangiogenesis needs to be determined by preparation and in vivo screening of desoxy derivatives of artemisinin.

In summary, hybrids of nonacetal and acetal types of artemisinin and glycolipid were synthesized in one-step reactions and most showed one to two times more potent in vivo antiangiogenic activity than standard drugs. It is also noteworthy that new hybrid compounds showed drastic increase of the antiangiogenic activity in comparison with non coupled molecules, artemisinin **1** or daumone **2** alone.

Among the 11 synthetic compounds tested, hybrids **3f**, **3g** and **3i** showed the most potent antiangiogenic activity, twice as much potency as fumagillin and thalidomide, known as antiangiogenic agents. In particular, hybrid **3i** showed complete inhibition at 2.5 nmol/egg with no toxicity. Compounds **3a** and **3h** showed similar activity to that of fumagillin. Evidence that acetal-type analogs at C-12 of artemisinin are more neurotoxic in animal studies than non acetal-type analogs is also emerging,[12] and may thus lead to the future abandonment of the currently clinically used acetal-type potential anticancer drug candidates. Therefore, nonacetal 12β (C–C)-type derivatives of artemisinin–glycolipid hybrids deserve further evaluation as possible anticancer drug candidates because of their high acid stability,[3b] low toxicity and high in vivo antiangiogenesis. The synthesis and antiangiogenic activity of hybrids between glycolipids and other anticancer drugs are under investigation and will be reported in due course.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.013.

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