Artemisinin, Promising Lead Natural Product for Various Drug Developments

Seokjoon Lee*

Department of Basic Science, Kwandong University College of Medicine, Gangneung 210-701, South Korea

Abstract: Artemisinin and its synthetic derivatives are widely used for antimalarial agents in the world. Moreover, they are discovered to additionally use as anticancer, antiangiogenesis, antiviral, immunosuppressive, and antifungal agents. Recent research results supported that it is a very promising field in drug discovery. In this review, it will discuss the structural and biological features of artemisinin and its derivatives that have published since 2003 except antimalarial, and show that they are useful lead compounds for novel drug discovery.

Key Words: Artemisinin, artemisinin derivatives, natural product, endoperoxide, lead compound, drug discovery.

1. INTRODUCTION

The natural sesquiterpene endoperoxide artemisinin (**1**, Fig. (**1**)), which was isolated from *Artemisia annua L.*, has become a useful lead compound in the development of antimalarial drugs [1]. Semi-synthetic acetal-type artemisinin derivatives such as artemether (**3**), arteether (**4**), sodium artesunate (**5**), and sodium artelinate (**6**) were synthesized from dihydroartemisinin (**2**) that is clinically used for malarial patients for their efficacy [2-5], although dihydroartemisinin (**2**), a metabolite of those drugs, has neurotoxicity in animal model [6, 7]. In particular, artemisinin derived antimalarial agents are effectively active against multidrug-resistant *Plasmodium falciparum* and *Plasmodium vivax* [8]. Since the active principle of *A. annua* was disclosed as artemisinin, the therapeutic potential of artemisinin and their synthetic analogues in treating malaria has been extensively reviewed [9-12]. Recently, as well as there are excellent review paper for pharmacological usefulness and mechanistic study on them [13-17], reviews for combination therapy for amtimalarial are also published [18-20].

Moreover, extensive researches based on structural modification and mechanistic study for improving their potency and bioavailability were used to develop a new kind of approach for different diseases, which are a variety of tumor, viral, fungal infection, and unwanted immune response in transplantation. Since these kinds of problems in human being are also critical to control a quality of human life, novel lead compound to be able to develop potent drugs with high effectiveness, bioavailability and low toxicity is urgent. In my opinion, artemisinin will be the clue to accomplish our goal, especially for medicinal and pharmaceutical chemist.

Fortunately, new trials to use artemisinin as a lead molecule for drug discovery against various diseases are rapidly growing and the researches are on going [21-23]. In this review, I will discuss the structural and biological features of artemisinin and its derivatives that have published since 2003 except antimalarial, and show that they are useful lead compounds for novel drug discovery.

2. ANTICANCER ACTIVITY

It has been reported that artemisinin and its derivatives have a cytotoxic effect against a variety of cancer cell lines and their biological activity including cytotoxicity is variously changed according to kinds of additive drug or molecules for combination therapy.

Recently, there is a good report for treatment against brain tumor using artemisinin derivatives. In particular, since single therapy of artemisinin-related drug candidates is less effective, combination therapy with additive compound to enhance therapeutic efficacy is most common.

A combination treatment of artesunate (**5**) and the epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor OSI-774 (Erlotinib, **7**, Fig. (**2**)) exhibited an interesting cytotoxic activity against glioblastoma multiforme (GBM) including U-87MG transduced with EGFR and non-transduced GBM cell lines [24]. OSI-774 (**7**) is a small molecule quinoline compound that induces apoptosis and cell cycle arrest [25, 26].

Combinational treatment of artesunate (**5**) with OSI-774 (**7**) effectively inhibited the U-87MG,ΔEGFR cell, G-210GM and G-599GM as shown in Fig. (**2**). In U-87MG, ΔEGFR cell, the increment of inhibition by artesunate (**5**) plus OSI-774 (**7**) was 25-folds in comparing to artesunate (**5**) alone, while, in G-210GM and G-599GM, the multiplicity of inhibition by combination treatment was 14.1- and 7.0 folds, respectively [24].

Iron (II) in heme activates the peroxide bond of artemisinin (**1**), and then generates the radical oxygen species responsible for the antimalarial activity [17, 27, 28]. Because similar mechanism can also be applied to increase the cytotoxicity against cancer cell lines, research result for cytotoxicity was already published in the past [29-33].

When compared with cytotoxicity of artemisinin derivatives without iron ion against CCRF-CEM leukemia and U373 astrocytoma cell, the cytotoxicities of artesunate (**5**),

*Address correspondence to this author at the Department of Basic Science, Kwandong University College of Medicine, Gangneung 210-701, South Korea; Tel: +82-33-649-7454; Fax: +82-33-641-1074; E-mail: sjlee@kd.ac.kr

1389-5575/07 $50.00+.00

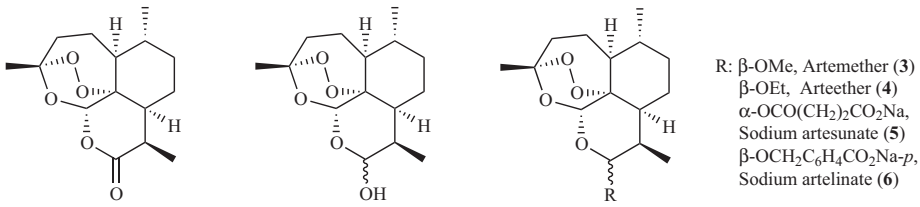


Fig. (1). Artemisinin and its acetal-type artemisinin derivatives.

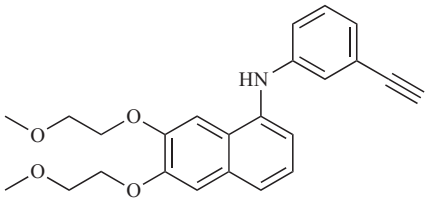
artemisinin microencapsulated in maltosyl-β-cyclodextrin (ART-MCD), and artemisinins with iron(II)-glycine sulfate and transferrin were increased from 1.5-fold to 10.3-fold [34].

As already published report [29-33], iron ion enhanced not only the cytotoxic effect of artemisinin (**1**) and their related derivatives against cancer cell lines, but also the specificity for cancer cell over normal cells [35, 36]. Its enhancement was caused from that iron-carrying plasma glycoprotein transferrin can be easily transported into cell *via* receptor-medicated endocytosis [37] because transferrin receptor was much more expressed on cell surface in cancer cell than normal cell [38].

Interesting conjugate, artemisinin-tagged holotransferrin (**10**, Fig. (**3**)), was obtained from attaching artelinic acid hydrazide (**8**) with lysine residues on the surface of oxidized holotransferrin (**9**), and its cytotoxic activity and specificity were tested on a human leukemia cell line (Molt-4) and normal human lymphocytes. Basically, it was conformed by comparing MALDI-MS data that 4.1 of artemisinin moieties were tagged on one protein on average [39].

After treatment with the tagged-compound (**10**) or dihydroartemisinin (**2**) on Molt-4 and human lymphocytes for 72hrs, the IC_{50} of the each compound was evaluated shown in Fig. (**3**). When comparing to dihydroartemisinin (**2**), the tagged-compound (**10**) is more potent against Molt-4 and less potent upon human lymphocyte [39].

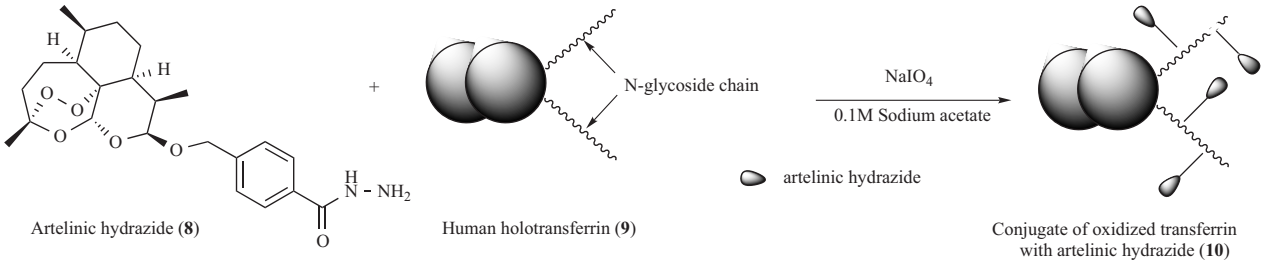
More recently, there was a report that dihydroartemisinin (**2**) enhanced a radiosensitivity of human glioma cell *in vitro* [40]. To determine whether dihydroartemisinin (**2**) improved the radiosensitivity of U373MG cell, *in vitro* clonogenic cell survival assay of cells treated with dihydroartemisinin (**2**) and radiation was performed, and the number of colonies was compared with that treated with dihydroartemisinin (**2**) or radiation alone. As a result, the clonogenic survivals were significantly decreased in parallel with the concentration of dihydroartemisinin (2). For quantitative evaluation, the dose enhancement ratio (DER) was calculated at the surviving fraction of 0.1 by dividing the radiation dose of the radiation alone curve with that of the corresponding dihydroartemisinin (**2**) plus radiation curve. The DER was 1.17, 1.38, and 1.73, respectively, in treatment of radiation with the concen-



OSI-774 (Erlotinib, **7**)

Cell lines	Cyctoxicity (IC_{50}, μM)		Modulation index
	Artesunate alone	Artesunate+ OSI-774	
U-87MG	2.0	2.1	1.0
U-87MG.LUX	2.3	1.9	1.2
U-87MG.DK-2N	3.0	2.1	1.4
U-87MG.WT-2N	4.9	3.7	1.3
U-87MG.DEGFR	11.5	0.5	25.0
G-211GM	1.5	1.5	1.0
G-599GM	4.2	0.6	7.0
G-1301GM	4.2	3.8	1.1
G-750GM	5.2	3.3	1.6
G-1187GM	6.7	5.9	1.1
G-1408GM	7.5	3.2	2.3
G-210GM	15.5	1.1	14.1
G-1265GM	8.5	7.0	1.2
G-1163GM	18.8	15.8	1.2

Fig. (2). Combination therapy of artesunate (**5**) and OSI-774 (**7**) against brain tumor cells.



	IC_{50} μM	
	Molt-4	human lymphocyte
Conjugate (**10**)	0.98	33
Dihydroartemisinin (**2**)	1.64	58.4

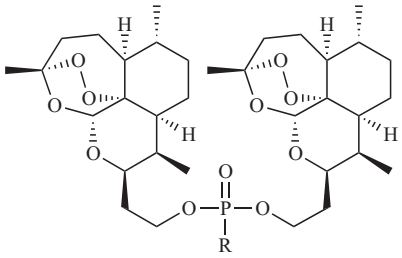
Fig. (3). Cytotoxicity of artemisinin-tagged holotransferrin (**10**).

tration of 0.352, 3.52 and 17.6 of dihydroartemisinin (**2**). When radiating with 6 Gy of γ-radiation to U373MG cell pretreated by 10mM NAC (*N*-Acetyl-L-Cysteine) or 300μM TRION (4,5-dihydroxy-1,3-benzenedisulfonic acid disodium salt), ROS (Reactive oxygen species) blockers, the cell survival rates were increased from 19 to 34% in NAC-treated cultures and 19 to 57% in TRION-treated cultures, which means that block of ROS generation suppressed the cytotoxicity and radiosensitization induced by dihydroartemisinin (**2**). In addition, it was also discovered that GST (Glutathion-s-transferase) activity and expression in the U373MG cell were strongly inhibited by treatment with dihydroartemisinin (**2**). These results proved that dihydroartemisnin improved the radiosensitivity of U373MG cells in a ROS-dependent manner [40].

Since the antimalarial activity of artemisinin derivatives is closely related to iron in hemoglobin, their cytotoxicity is also caused from the interaction with iron to form free radical. Therefore, to investigate the effect of holotransferrin, an iron-loaded tranferrin, on the radiosensitization of dihydroartemisnin upon U373MG cells, clonogenic survival rate in U373MG pretreated with 100μg/ml holotransferrin and then administered dihydroartemisinin and radiation was determined. As a result, pretreated holotranferrin also significantly improved the radiosensitivity of dihydroartemisnin [40], which result is coincidence to some reports that iron in holotransferrin or hemoglobin is critical role for anticancer activity of artemisinin derivatives [41].

From above research results, although artemisinin (**1**), dihydroartemisinin (**2**), and sodium artesumate (**5**) have a growth inhibitory activity against various cancer cell lines and some specificity for cancer cell over normal cell, the cytotoxicity is not strong enough to clinical trial. Important approach to improve the potency of artemisinin derivatives was mainly related to synthesize the dimer or trimer from monomers of artemisinin analog [31, 33].

The phosphate linked dimers (**11** and **12**, Fig. (**4**)) were discovered to have the anticancer activity against leukemia, colon, melanoma, prostate, and breast cancer from a 60 cell lines panel of National Cancer Institute (NCI), which strength was comparable to paclitaxel and doxorubicin in nanomolar to micromolar concentration. However, dimers (**11** and **12**) were little active against lung, central nervous system (CNS), and renal cancer cell lines. Out of preliminary NCI assay, the two phosphate dimers (**11** and **12**) were tested in more detail in HL60 leukemia and Jurkat cell lines comparing to dihydroartemisinin (**2**) and doxorubicin [42]. As shown in Fig. (**4**), two dimers (**11** and **12**) were stronger than doxorubicin in HL60 leukaemea cell but less in Jurkat (human leukemic T-cell lymphoblast). When in considering the toxicity of peroxide dimers was not toxic to lymphocytes at doses of 100μM, they are promising anticancer candidate for clinical trial [42].

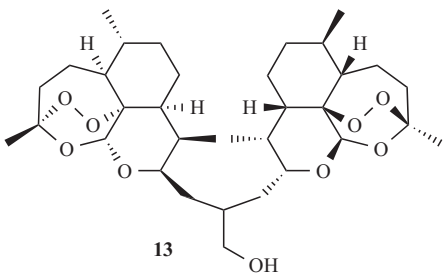


11 R: -Me
12 R: -Ph

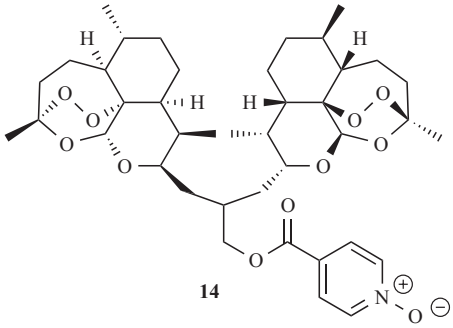
	IC_{50}, μM	
	HL60	Jurkat
11	0.143	7.7
12	0.241	13.2
DHA (**2**)	1.21	101
Doxorubicin	0.51	1.7

Fig. (4). Cytotoxicity of phosphate linked dimers (**11** and **12**).

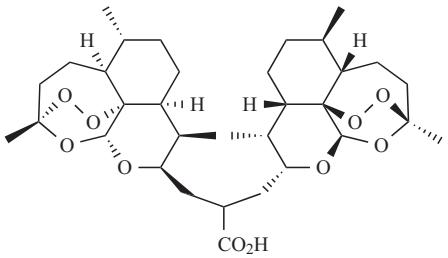
Some carbon chain linked trioxane dimers (**13**, **14**, and **15**, Fig. (**5**)) also showed potent anticancer activity in the transgenic adenocarcinoma of mouse prostate cancer model in nanomolar concentration range [43]. Among the tested prostate cancer cell lines, the C1A and C2D cells are nontumorigenic, and the C2G and C2H cells are tumorigenic and



13



14



15

	IC_{50} of prostate cancer (nM)			
	C1A	C2D	C2G	C2H
13	23.3	18.5	15.4	9.2
14	84.6	62.1	47.4	36.1
15	158.7	231.4	134.0	116.0
Gemzar	9.0	11.9	3.7	4.7
Doxorubicin	75.9	46.5	28.7	30.3

Fig. (5). Cytotoxicity of carbon chain linked trioxane dimers (**13**, **14**, and **15**).

metastatic. As shown in Fig. (**5**), the alcohol dimer **13** was the most effective, while carboxylic acid dimer **15** was the least. Compound **13** showed an IC_{50} of 15.4 and 9.2nM in the cell lines C2G and C2H, respectively. In comparison with Gemzar, chemotherapeutic agent for the treatment of human prostate cancer and non-small-cell lung cancer, alcohol dimer **13** showed a comparable activity. Moreover, it is much stronger than doxorubicin. Because Gemzar and doxorubicin have also a variety of toxicity, these easily synthesizable and low toxic trioxane dimers are worthwhile to further study and clinical trial [43].

Generally, although monomers of artemisinin derivatives have a low cytotoxicity against various cancer cell lines, there was an interesting report that artemisinin derivatives with lipophilic alkyl carbon chains showed a moderate or strong cytotoxicity against human hepatocellular carcinoma (HepG2) cell [44]. This report showed an interesting correlation between lipophilic carbon chain length and cytotoxicity from the initial activity test against HepG2 cell. In this series, the most potent compound was the one bearing *N*-tetradecylacetamide linkage in IC_{50} of 0.46μM. There was a slight activity decrease according to increasing of carbon chain length. Generally speaking from this result, carbon chain length of 12- to 16-carbon exhibited a strong activity. The effect of linkers such as an ester, alcohol, and ketone were also examined on HepG2 cell, but their effect on cytotoxicity is not significant as shown in Fig. (**6**) [44].

However, an increase of lipophilicity decreases the water solubility, which may be an obstacle to develop the drug candidate in the future. To overcome this problem, polar groups were attached at the end of carbon chain. Attaching hydroxyl- (**20b**) and carboxylic acid group (**20c**) at the end of lipophilic chain seriously decreased the cytotoxicty, but its anticancer activity of **21** with amide linker of *N*-polar group was maintained. Fig. (**7**) This result means that a terminal hydrophobic group at the carbon chain has a critical role to their biological activity [44].

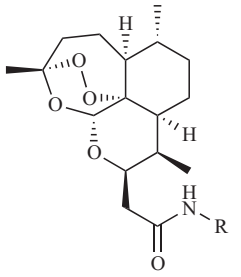
In addition, the cytotoxicity of D-five membered ring artemisinins against HepG2 cell was also comparable to their D-six membered ring analogs. As illustrated in Fig. (**8**), tested D-five membered ring artemisinin derivatives (**22**, **23**, and **24**) with 12- to 18-carbon chain by linked with amide, ester, and ketone exhibited a strong cytotoxicity [44].

Finally, the selectivity between the cancer cell line and normal cell was examined by using normal lung fibroblast (CCD-19Lu). From this selectivity test, it was discovered that the modified artemisinin derivatives (**16f**, **16g**, **17**, and **19**) showed a moderate selectivity between cancere cell and normal cell. In particular, **19** was 10-fold more potent toward HepG2 cancer cell (IC_{50}= 14.5μM) than normal lung fibroblast (IC_{50}= 1.4μM) [44].

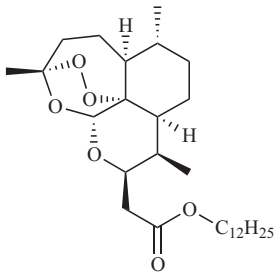
More recently, in preliminary screening by diverse NCI's human cancer cell lines, phthalate dimers **25** was discovered to be highly selective and potent inhibitor against nonsmall cell lung carcinoma HOP-92 cell, melanoma SK-MEL-5 cell, and breast cancer BT-549 cell. Especially, compound **25** had a strong inhibiton activity agaisnt the human cervical cancer cell line in IC_{50} of 500nM. Trioxane diol dimer **26** also showed a high IC_{50} of 46.5nM without any toxicity to primary normal cervical cell (Fig. (**9**)) [45].

3. ANTIANGIOGENESIS ACTIVITY

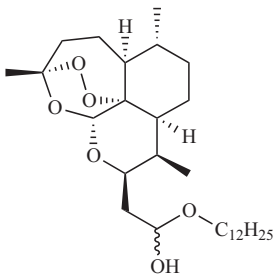
Angiogenesis is the formation of new vascular capillaries from preexisting host vessels by various biological stimulators [46]. In normal system, except during wound healing [47] and embryonic development [48], an elaborate balance between positive and negative regulators tightly controls angiogenesis. On the other hands, in abnormal conditions, angiogenesis occurs in the course of tumor growth [49], dia-



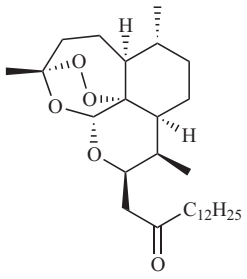
Cytotoxicity against HepG2 (IC_{50}, μM)					
16a	R $-C_2H_5$	>100	**16f**	R $-C_{12}H_{25}$	1.2
16b	$-C_4H_9$	>100	**16g**	$-C_{14}H_{29}$	0.46
16c	$-C_6H_{13}$	17.6	**16h**	$-C_{16}H_{33}$	0.79
16d	$-C_8H_{17}$	9.5	**16i**	$-C_{18}H_{37}$	4.2
16e	$-C_{10}H_{21}$	2.8	Artemisinin (**1**)		97



17



18a (α-OH)
18b (β-OH)

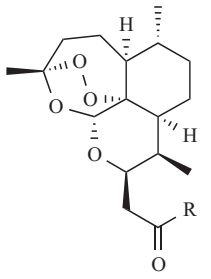


19

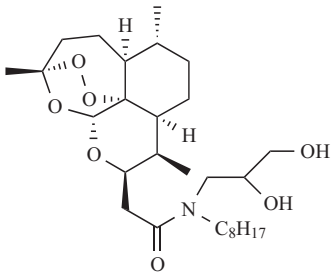
Cytotoxicity	(IC_{50}, μM)
17	0.72
18a	2.8
18b	4.4
19	1.4

Fig. (6). Cytotoxicity of artemisinin derivatives with lipophilic alkyl carbon chains.

betic retinopathy [50], and rheumatoid arthritis [60]. In particular, tumor angiogenesis plays a key role in the growth, invasion, and metastasis of tumors. Therefore, the control of angiogenesis may be a promising therapeutic strategy for the related diseases.



20a R; $-C_8H_{17}$
20b ; $-(CH_2)_7CH_2OH$
20c : $-(CH_2)_7CO_2H$

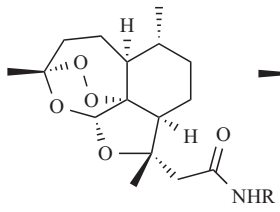


21

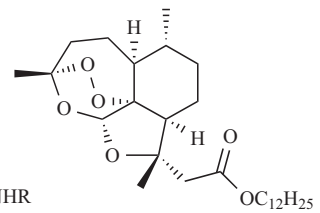
Cytotoxicity	(IC_{50}, μM)
20a	1.8
20b	42.3
20c	>100
21	3.5

Fig. (7). Cytotoxicity of artemisinins with polar group at the end of lipophilic chain.

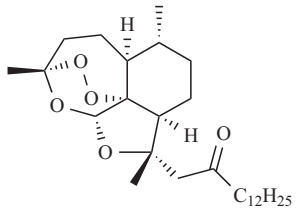
Recently, Chen *et al.* reported that artemisinin (**1**) and dihydroartemisinin (**2**) have an antiangiogenic activity as well as an antitumor activity on *in vitro* models of angiogenesis, such as inhibitory test against the proliferation, migration, and tube formation of human umbilical vein endothelial cells (HUVEC) [52]. Basically, artemisinin (**1**) and dihydroartemisinin (**2**) showed a mild cytotoxicity against various cancer cells. By comparing the cytotoxic effect on normal cells, NIH-3T3 and human endometrium cell, to investigate the cellular toxicity, it was concluded that artemisinin (**1**) and dihydroartemisin (**2**) is more toxic to cancer cells than normal cells, which means they would be safe in clinical trials [52].



22a R; $-C_{12}H_{25}$
22b ; $-C_{14}H_{29}$
22c : $-C_{16}H_{33}$



23

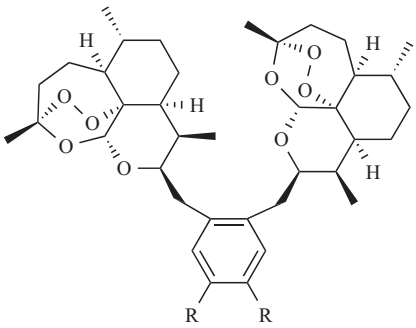


24

Cytotoxicity	(IC_{50}, μM)
22a	1.3
22b	0.77
22c	0.74
23	3.7
24	0.47

Fig. (8). Cytotoxicity of D-five membered ring artemisinins.

Artemisinin (**1**) and dihydroartemisin (**2**) inhibited the HUVEC growth of about 40 and 50%, respectively, at the concentration of 50μM, and completely suppressed HUVEC migration at 50μM (Table **1**). Furthermore, there was a sig-



25 R: -CO_2Me
26 : -CH_2OH

Fig. (9). Highly potent trioxane phthalate dimers **25** and **26**.

nificant decreasing of 70% and 90% in both the number of length of tube formation at 50μM. Although the potency of artemisinin (**1**) and dihydroartemisin (**2**) against growth, migration, and tube formation is mild, it will be possible to start for novel angiogenesis inhibitors from them [52].

Table 1. Antiangiogenesis Activity of Artemisinin (1) and Dihydroartemisin (2)

	Inhibition Percentage (%) Against HUVEC at 50μM		
	Proliferation	**Migration**	**Tube Formation**
Art (**1**)	40	100	70
DHA (**2**)	50	100	80

Artesunate (**5**) also inhibited angiogenesis *in vitro* and *in vivo* screening. In *in vitro* test including inhibition of proliferation, migration, and tube formation assay, Artesunate (**5**) inhibited the HUVEC proliferation with a 20.7 μM of IC_{50}, which potency is more slightly potent than parent compounds **1** and **2**. HUVEC was inhibited 88.7% by artesunate (**5**) in 80μM concentration. In addition, in migration and tube formation assay, it had a similar potency with artemisinin (**1**) and dihydroartemisin (**2**) [53].

More importantly, *in vivo* antiangiogenesis activity of artesunate (**5**) was evaluated by using nude mice implanted by human ovarian cancer (HO-8910) and immunohistochemical stainings for microvessel (CD31), vascular endothelial growth factor (VEGF) and VEGF receptor KDR/flk-1. When artesunate (**5**) was administrated to nude mice of four groups divided by drug concentration, there was no significant change of tumor volume in low dose (10mg/kg) and control group. Tumor growth in two group treated by 50mg/kg and 100mg/kg was reduced by 41% and 62%, respectively, after 15 days administration. The necrotic areas and microvessel density were also changed in three experimental groups according to administrated concentration. In particular, microvessel density as a parameter of antiangiogenic activity was significantly decreased in tested group with 50 and 100mg/kg [53].

In the test of inhibition on VEGF expression, artesunate (**5**) effectively suppressed the expression in ratio of 60% in 50mg/kg and 80% in 100mg/kg. In addition, significantly reduced KDR/flk-1 expression was shown by 70 and 80 % in HO-8910 cells and endothelial cells, respectively. The results demonstrated that artesunate (**5**) has an inhibitory effect in angiogenesis *in vitro* and *in vivo* and may be a promising angiogenesis inhibitor (Table **2**) [53].

In order to deeply evaluate antiangiogenetic activity for artesunate (**5**), additional tests were attempted on chicken chorioallantoic membrane (CAM) neovascularisation model, which is the most widely used vessel development assay *in vivo* [54]. Artesunate (**5**) inhibited the CAM angiogenesis in dose dependent manner, and started to inhibit angiogenesis at 10nmol/100μl/egg. At 20 and 40nmol/100μl/egg, it reduced the CAM blood vessel to about 37 and 63% of the control [54].

Table 2. Antiangiogenesis Activity of Artesunate (5) in *In Vitro* and *In Vivo* Screening

	Test Methods	**Inhibition Activity**
in vitro	HUVEC Proliferation	IC_{50} 20.7 μM
		88.7% in 80 μM
	Tube Formation	75% in 50 μM
in vivo	Tumor Growth	41% in 50mg/kg
		62% in 100mg/kg
	VEGF Expression	40% in 50mg/kg
		80% in 100mg/kg
	KDR/flk-1 on HUVEC	70% in 50mg/kg
		80% in 100mg/kg
	KDR/flk-1 on tumor cells	30% in 50mg/kg
		70% in 100mg/kg

There was an advanced approach that artesunate (**5**) also showed an inhibitory activity the growth of Kaposi's sarcoma (KS) xenograft tumors [55], which is a highly angiogenic multifocal tumor and produces several angiogenic cytokines [56]. In this investigation, artesunate (**5**) effectively suppressed growth and induced apoptosis of KS, but inhibited the growth of HUVEC. Furthermore, it effectively inhibited the tumor xenograft growth *in vivo* [55].

The apoptosis of HUVEC induced by artesunate (**5**) was also investigated by means of nuclear staining, DNA agarose gel electrophoresis, and flow cytometry. The apoptosis rate of HUVEC treated by artesunate (**5**) reached the highest point in concentration of 32μM (Fig. (**12**)) [57]. Dihydroartemisinin (**2**) also downregulated a vascular endothelial growth factor (VEGF) expression and induced an apoptosis in chronic myeloid leukemia K562 cells [58].

Although it is proved that artemisinin (**1**), dihydroartemisinin (**2**), and artesunate (**5**) had a novel antiangiogenesis

effect based on various screening system, such as proliferation, migration, and tube formation assay *in vitro* test, and chicken chorioallantoic membrane (CAM) neovascularisation model and test of inhibition on VEGF expression *in vivo*, there is no any try to discover novel artemisinin derivatives that have angiogenesis inhibitory activity. In my opinion, it is more important to discover small molecular angiogenesis inhibitors from structural modification of artemisinin (**1**) based on previous research results about artemisinins.

I reported that synthetic key intermediates for direct alkylation of artemisinin (**1**), diastereomeric 10-phenylsulfide and 10-benzenesulfonyl dihydroartemisinin [59], showed an inhibition activity against HUVEC proliferation with higher strength than artemisinin (**1**) and dihydroartemisinin (**2**) [60].

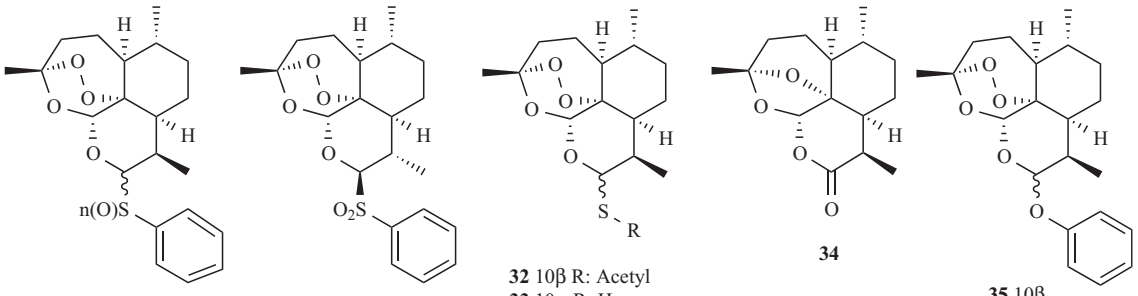
As the Chen's report [52-54], acetal-type derivatives might be a lead compound for use as an antiangiogenic inhibitor. But it is not sufficient for clinical trials in my opinion. For example, the typical acetal-type antimalarial agent, β-arteether (**4**), had no inhibitory activity against HUVEC at the concentration of 50 μM, and 10β- (**35**) and 10α-phenoxydihydroartemisinin (**36**) with endoperoxide and aromatic phenyl group were less active than **2** [60]. Fig. (**10**) However, 10α- (**27**) and 10β-phenylthiodihydroartemisinin (**28**) with endoperoxide and thiophenoxy moiety showed a very strong inhibitory activity with an IC_{50} of 0.93 and 5.24 μM, respectively [60].

In addition, the synthetic sulfonyl compounds, 10α- (**29**) and 10β-benzenesulfonyl dihydroartemisinin (**30**), and 10β-benzenesulfonyl-9-*epi*-dihydroartemisinin (**31**), also showed a high inhibitory activity as shown in Fig. (**10**). In particular, the inhibitory potency of 9-*epimer* (**31**) was over 7–10 times higher than that of **29** and **30**. Even if desoxy compounds (**37** and **38**) had the thioacetal functionality and β-arteether (**4**) had the endoperoxide, they have no inhibitory activity for lack of endoperoxide or aromatic functional group [60].

A comparison between 10α-mercaptodihydroartemisinin (**33**) and dihydroartemisinin (**2**) suggests that the sulfur atom at the C-10 position is critical for inhibition activity on HUVEC. According as oxygen changed to sulfur, the biological activity improved markedly [60].

When compared to thioacetal compounds (**27** and **28**) and sulfonyl derivaitves (**29**, **30** and **31**), sulfone substitution at the C-10 position of tricyclic artemisinin improved the tube formation inhibitory activity. Interestingly, 10β-benzenesulfonyl-9-*epi*-desoxyartemisinin (**38**) and β-arteether (**4**), which have no inhibitory activity against HUVEC growth, exhibited a moderate tube formation inhibitory activity by 32 and 48%, respectively. So, detail study on the structure activity relationship about various functional groups is needed [62].

In vivo inhibitory effect of thio-acetal artemisinin derivatives on angiogenesis was examined by using CAM assay at



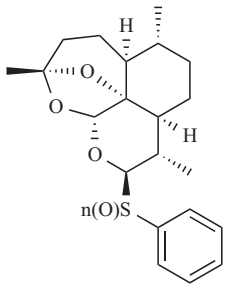
27 10α n=0
28 10β n=0
29 10α n=2
30 10β n=2

31

32 10β R: Acetyl
33 10α R: H

34

35 10β
36 10α



37 n=0
38 n=2

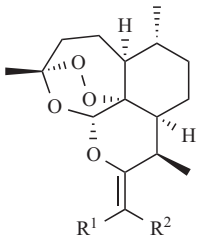
	Growth inhibition IC_{50} μM	Inhibition percentage at 10 μg/mL (%)	Inhibited eggs /Tested eggs
1	>50	0	-
2	8.91	19	-
4	>50	7	-
27	0.93	20	18/20
28	5.24	16	11/16
29	12.77	41	6/16
30	18.21	52	6/15
31	1.74	0	-
32	4.26	32	-
33	1.29	74	16/18
34	>50	0	-
35	33.81	3	-
36	31.03	16	-
37	>50	48	-
38	>50	0	-

Fig. (10). Antiangiogenesis activity of various thio-acetal artemisinin derivatives.

the concentration of 5 μg/egg. As shown in Fig. (**10**), selected thioacetal molecules (**27** and **28**) and 9-*epi*-sulfonyl artemisinin **31** strongly inhibited the formation of new blood vessel on CAM. Particularly, **27** and **31** have a potent activity by 90%. The other analogues (**29** and **30**) were mildly potent [61].

Novel deoxoartemisinins (**39**-**43**, Fig. (**11**).), *exo*-Olefinated deoxoartemisinin derivatives, were synthesized by modified Ramberg-Bäcklund rearrangement conditions [62], and also were examined on a HUVEC proliferation assay using the MTT colorimetric method, tube formation assay on Matrigel and CAM assay [63].

Tested C-10 *exo*-olefinated deoxoartemisinin derivatives (**39**-**43**) showed a strong inhibitory activity upon HUVEC growth except for mildly active *Z*-isomer of **40**. Especially, all isomer of **41**, **42** and **43** effectively inhibited the HUVEC growth at the concentration 2μg/mL level. This result led us to assume that deoxo-type artemisinin derivatives may have an antiangiogenic activity [63].



E-**39** R^1: H, R^2: Br
Z-**39** R^1: Br, R^2: H
E-**40** R^1: CH_3, R^2: Br
Z-**40** R^1: Br, R^2: CH_3
E-**41** R^1: Propyl, R^2: Br
Z-**41** R^1: Br, R^2: Propyl
42 R^1: CH_3, R^2: CH_3
E-**43** R^1: H, R^2: Phenyl
Z-**43** R^1: Phenyl, R^2: H

39 - 43

	Growth inhibition IC_{50} μM	Inhibition percentage at 10 μg/mL (%)	CAM assay at 10 μg/egg (%)
E/Z of 39	4.8	25	80
E-40	4.5	65	50
Z-40	25.4	32	43
E-41	1.5	39	57
Z-41	1.9	10	8
42	2.3	13	29
E-43	1.9	10	10
Z-43	2.3	10	5

Fig. (11). Antiangiogenesis activity of *exo*-olefinated deoxoartemisinin derivatives.

The activity to suppress the growth factor induced tube formation by HUVEC on Matrigel was assessed at the concentration of 10μg/ml. Among the promising molecules, disappointingly, only *E*-isomer of **40** showed a good inhibition activity against tube formation. Other compounds (**39**, *Z*-**40** and *E*-**41**) were mildly effective [63].

In the CAM assay at the concentration of 10μg/egg, the *E* and *Z* mixture of **39** strongly inhibited the formation of new blood vessel on CAM. The *E* and Z isomer of **40** and *E* isomer of **41** showed a mild inhibitory activity [63].

It was concluded that the C-10 *exo*-olefinated deoxoartemisinin derivatives (**39**-**43**) can inhibit the angiogenesis and might be angiogenesis inhibitors. The bromoalkenylidene analogues (**39**, **40** and **41**) 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 [63].

More recently, nonacetal-type derivatives and their dimers were extensively tested on CAM assay to determine antiangiogenesis activity, and, in particular, a deoxoartemisinin-fullerene conjugate was synthesized to expect the drug delivery rate, low toxicity and low dose advantage [64].

As already published results, artemisinin (**1**) and dihydroartemisinin (**2**) exhibited a weak inhibitory activity against blood vessel formation on CAM in a concentration of 5nmol/egg. Two acetal-type artemisinin derivatives (**45** and **46**) and other 10-substituted deoxoartemisinin (**47a**, **47b** and **47f**) also gave a low activity, but, interestingly, **47c**, **47d**, and **47e** showed a comparable antiangiogenesis activity in comparison to fumagillin and thalidomide, positive controls. When considering that 9-substituted deoxoartemisinin derivatives also showed a low inhibitory activity, nonacetal-type deoxoartemisinin derivatives generally have a low activity upon CAM assay. However, 9α-bromoemethyl deoxoartemisinin **49c** and dimer molecules with ether linkage of hydroxymethyl deoxoartemisinin **50** showed extraordinarily a stronger activity than fumagillin and thalidomide, and different linkage type dimers (**51a** and **52b**) are highly toxic to chicken embryos. When comparing these opposite results, it is necessary to synthesize the diverse library of deoxoartemisinin for QSAR study. Interesting conjugate of malonate-linked dimer and fullerene (**54**) showed a moderate activity as similar to that of thalidomide [64].

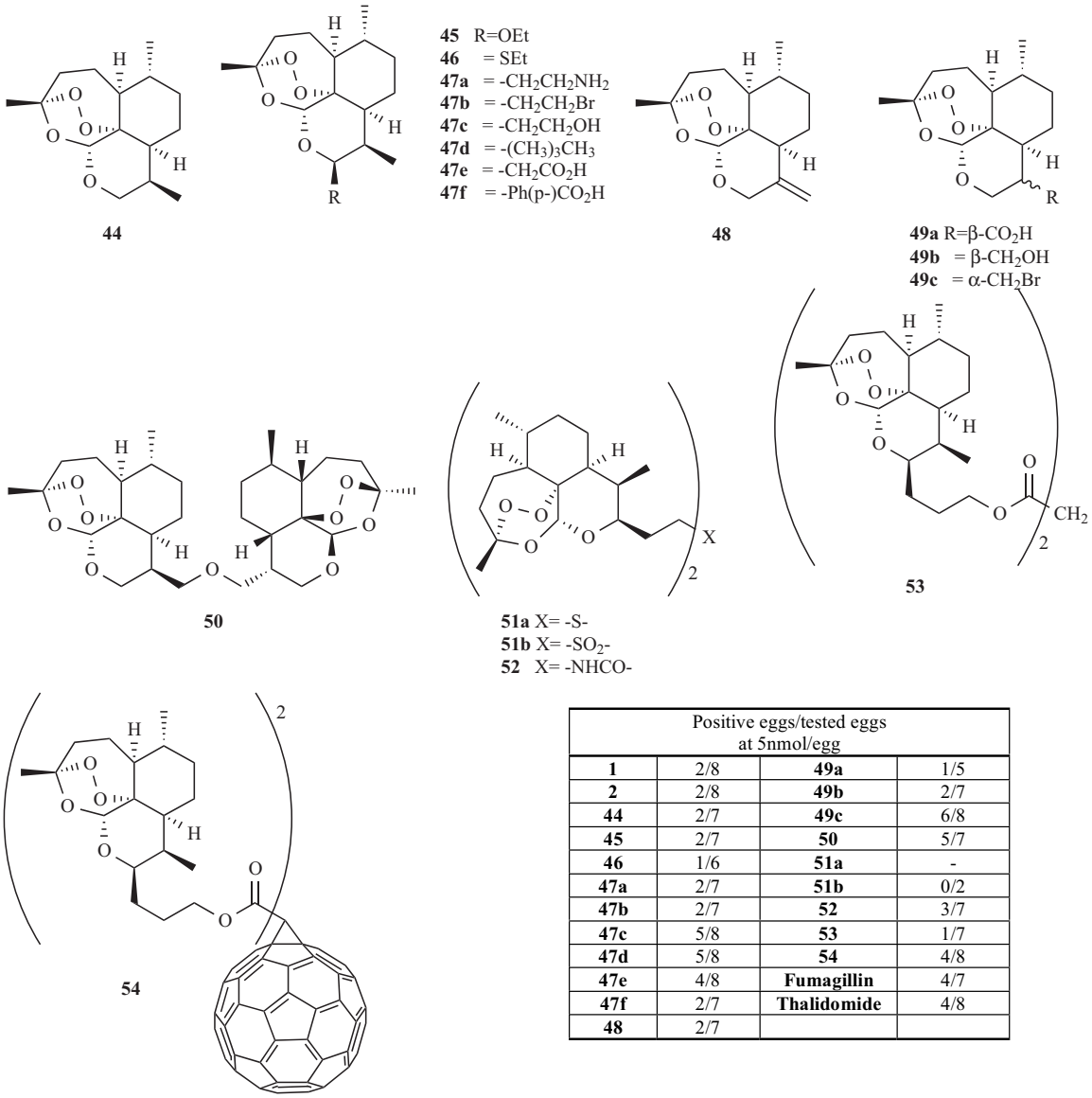
Because some nonacetal-type deoxoartemisinins are more potent than their lead compound, artemisinin (**1**) and dihydroartemisinin (**2**), and these compounds have a high acid stability and low toxicity, it is valuable to intensively study an antiangiogenesis agent from artemisinin library [64].

4. IMMUNOSUPPRESSIVE ACTIVITY

From the early of 1980, there were some reports that artemisnin (**1**), dihydroartemisinin (**2**), artemether (**3**), arteether (**4**), and artesunate (**5**) had an immunosuppressive activity in China. However, it is not well known because a majority of report was published on Chinese [65-68].

An immunosuppressive activity of artemisinin (**1**) on Balb/c mice by delayed type hypersensitivity (DTH) response and fluorescence emission spectrum of calmodulin (CaM) was tested in comparison to cyclosporin A (CsA), which is natural 11 amino acids cyclic peptide used in clinically to prevent organ rejection after transplantation. Artemisinin (**1**) significantly decreased the DTH responses in Balb/c mice against sheep red blood capsule (sRBC) after 72 hours, which was the best time for the assessment of the immune response in tested mice after the injection. In addition, artemisinin (**1**) also induced conformational changes on CaM, and the fluorescent emission of CaM was increased at higher degree of than CsA. Based on *in vivo* and *in vitro* studies, artemisinin (**1**) is more potent immunosuppressant agent than CsA [69].

A series of novel dihydroartemisinin derivatives were synthesized and their immunosuppressive activity was



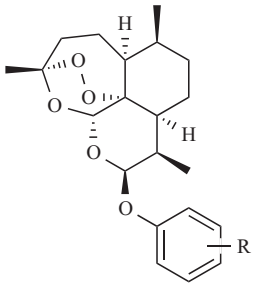
Positive eggs/tested eggs at 5nmol/egg			
1	2/8	**49a**	1/5
2	2/8	**49b**	2/7
44	2/7	**49c**	6/8
45	2/7	**50**	5/7
46	1/6	**51a**	-
47a	2/7	**51b**	0/2
47b	2/7	**52**	3/7
47c	5/8	**53**	1/7
47d	5/8	**54**	4/8
47e	4/8	**Fumagillin**	4/7
47f	2/7	**Thalidomide**	4/8
48	2/7		

Fig. (12). CAM assay result of acetal-type artemisinin derivatives and their dimmers.

evaluated to search for immunosuppressive agents with high efficacy and low toxicity. The new acetal-type artemisinin derivatives with substituted phenol at C-10 position were tested *in vitro* for cytotoxicity against murine, and then inhibitory activity against concnavalin A (ConA) induced T cell proliferation and lipopolysaccharide (LPS) induced B cell proliferation were tested in comparison with CsA, artemisinin (**1**), artemether (**3**), and artesunate (**5**) as the controls [70].

As shown in Fig. (**13**), although known antimalarial agents, artemisinin (**1**), artemether (**3**), and artesunate (**5**), were far less potent than CsA, selected compounds synthesized from acetal-type dihydroartemisnin derivatives (**55**-**60**) generally showed a comparable *in vitro* immunosuppressive activity with CsA. In particular, compound **55** had a high selectivity index (SI=848) on ConA-induced T cell proliferation, which value was comparable to CsA (SI=963). This compound **55**, moreover, had much higher inhibition activity LPS-induced B cell proliferation and extremely high SI value (SI=28473) than CsA (SI=7). The other compounds (**56**-**60**) was about 10- to 100-fold less active than CsA. From the preliminary *in vivo* test in BALB/c mice by DNFB-induced DTH response, compound **55** effectively reduced the ear swelling in DTH mice at a dose of 20mg/kg, whose immunosuppressive activity is much effective than CsA, and also effectively inhibited the hemolysis of SRBC more than dexamethasone (DEX). The authors presumed that high immunosuppressive potency was contributed from the presence of a lipophilic core structure and an appropriate aliphatic side chain of dihydroartemisinin derivatives [70].

For compound **55**, further studies were tried for the evaluation of *in vivo* immune activity [71]. Besides of tests already tried in ConA-induced T cell proliferation and LPS-induced B cell proliferation, mixed lymphocyte reaction



55 R: -*p*-OCH_2COOMe
56 -*p*-OCH(Me)COOEt
57 -*p*-$CH_2CH_2CO_2Me$
58 -*p*-OCH(Me)COOMe
59 -*m*-$OOCCH_2CH_2COOH$
60 -*p*-$CONHCH_2COOEt$

	IC_{50} μM (SI)a		
	Cytotoxicity CC_{50} μM	ConA-induced T-lymphocyte proliferation	LPS-induced B-lymphocyte proliferation
55	207	0.244 [848]	0.00727 [28473]
56	>100	0.26 [>385]	0.086 [>118]
57	41.7	0.138 [302]	0.0178 [2343]
58	6.53	0.013 7[477]	0.78 [8]
59	52.7	0.327 [161]	0.265 [199]
60	130	2.53 [51]	0.0537 [2421]
CsA	1.29	0.00134 [963]	0.184 [7]
1	28.3	4.43 [6]	8.96 [3]
3	86	3.82 [23]	1.78 [48]
5	32.9	4.58 [7]	0.989 [33]

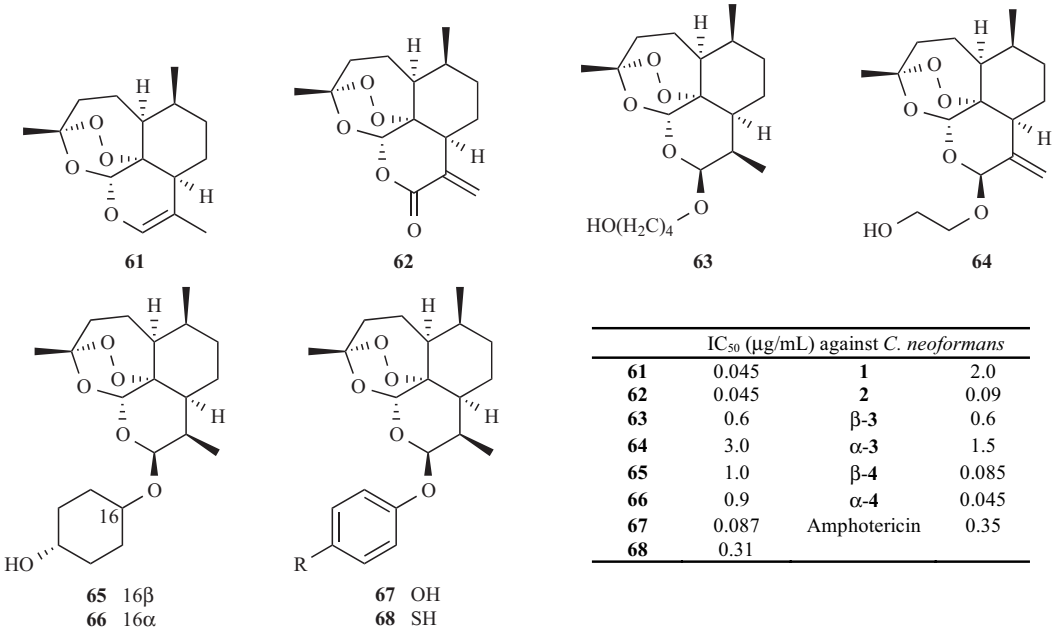
Fig. (13). Immunosuppressive activity of acetal-type dihydroartemisnin derivatives.

(MLR) proliferation assay was carried out. It strongly inhibited T cell growth in MLR in a dose-dependent manner with an IC_{50} of 0.86 μM for 96 hours co-culture. Because MLR is often used clinically for tissue typing for identifying the compatibility of donor organs and recipients, suppression of MLR improves the success of transplation [72, 73]. To conform successful immunosuppression activity, inhibition tests against multiful proinflammatory cytokins, such as interleukins (IL)-2, interferon (IFN)-ɣ and IL-6, were tried, and, in PLS or PMA plus ionomycin stimulation, compound **55** significantly inhibited them. However, when using ConA as a stimulant, the IL-2 level was not changed. It suggested that compound **55** showed completely different suppression mechanism comparing to CsA and FK506 inhibiting IL-2. In additional *in vivo* test by using QHS model, **55** efficiently suppressed antibody-secreting B cell *in vivo*. Although immuno-modulating mechanism is different from CsA and FK506, and not defined, dihydroartemisinin derivatives will be promising immunosuppression agents for their low side effects and toxicity [71].

5. ANTIFUNGAL ACTIVITY

Cryptococcosis caused by *Cryptoccocus neoformans* is dangerous fungal infection in patients with HIV and AIDS [74]. Although the occurrence of *C. neoformans* was decreasing by the treatment of multi-drug HIV therapy, it makes trouble in central nervous system (CNS), and, especially, threatens their life [75].

A series of artemisinin derivatives, mainly acetal type, were studied *in vitro* screening on *Cryptoccocus neoformans* to search for novel antifungal agents as shown in Fig. (**14**). Basically, known antimalarial agents, such as artemisnin (**1**), dihydroartemisinin (**2**), artemether (**3**), and arteether (**4**), have higher antifungal activity than that of amphotericin B, positive control. Dihydroartemisinin (**2**) and α- and β-arteether (**3**) showed 4-10 times much stronger activity in comparing amphotericin B, respectively. However, newly synthesized compounds (**64**, **65**, **66** and **68**) were less active than amphotericin B. Additionally, tested deoxyartemisinins were weakly active or totally inactive. Based on the preliminary structure activity relationship, the typical tetracyclic trioxane ring system of artemisinin is critical for antifungal activity [76].



61 **62** **63** **64**

65 16β
66 16α
67 OH
68 SH

	IC_{50} (μg/mL) against *C. neoformans*		
61	0.045	**1**	2.0
62	0.045	**2**	0.09
63	0.6	β-**3**	0.6
64	3.0	α-**3**	1.5
65	1.0	β-**4**	0.085
66	0.9	α-**4**	0.045
67	0.087	Amphotericin	0.35
68	0.31		

Fig. (14). Antifungal activity of various artemisinin derivatives.

6. ANTIVIRAL ACTIVITY

In spite of an effective and safe vaccine therapy against hepatitis B virus (HBV), viral infection by HBV caused a global health problem in the world, especially the third world. Moreover, because direct antiviral therapy against HBV infection is not yet perfectly developed, it is important to discover the lead compounds for novel anti-HBV agents from the potential library. Recently, there was a report about anti-HBV activity of artemisinin (**1**) and artesunate (**2**) based on the screening by using HBV-transferred HepG2 2.2.15 cell [77], which is derived from hepatoblastoma HepG2 cell [78]. This screening method is a useful *in vitro* model for evaluation of novel anti-HBV drugs, as well as to study several steps of the HBV biology [79].

Artemisinin (**1**), artesunate (**5**), and a variety of purified compounds from traditional Chinese medicine remedy were investigated by measuring the release of surface protein (HBsAg) and HBV-DNA after drug exposure (0.01-100μM) for 21 days [77]. As a result, artesunate (**5**) strongly inhibited the HAsAg secretion with an IC_{50} of 2.3μM and IC_{90} of 16μM, respectively, whereas artemisinin (**1**) had a mild inhibition activity. To evaluate an enhancement in viron production, the amount of the HBV-DNA release to the HepG2 2.2.15 culture medium during different treatments was measured, and it was significantly reduced. In addition, it was discovered that, for artesunate (**5**), toxicity in host cell was shown in drug concentration of 20 μM and therapeutical index (TI) calculated from IC_{50} of HBV-DNA release was 40. When comparing to TI value (500) of lamivudine as positive control, the value of artesunate (**5**) is quite low, but reasonable value for further investigation. Finally, artesunate (**5**) was tried in combination treatment with lamivudine. When both compounds were administered together in each concentration of 20nM, any toxicity was not shown, but a synergic inhibitory effect in HBsAg release was found. It means that it is possible to be potential antiviral agent against infection of lamivudine-resistance HBV strains, frequent problem in clinical treatment [77]. This result was quite similar to potency previously reported for human cytomegaloviruses [80].

CONCLUSION

Recently, although a variety of researches about artemisinin and its synthetic derivatives for their biological phenomena have been performed in many research groups, it is mainly concentrated on antimalarial treatment. As considered in this review, it is still very challenging to discover specific drugs towards various human diseases and utilize them in clinical trials. In this review, I have reviewed a various kind of research for antitumor, antiangiogenesis, immunosuppression, antifungal, and antiviral inhibitors. In particular, we focused on the structural and biological potency of artemisinin and their synthetic analog for each part. Recently published results are the most promising towards the development of selective inhibitor for diverse targets in comparing to antimalarial researches.

ACKNOWLEDGEMENT

I am grateful for the financial support from Kwandong University.

REFERENCES

[1] Klayman, D. L. *Science,* **1985**, *228*, 1049.
[2] Brewer, T.G.; Peggins, J.O.; Grate, S.J.; Petras, J.M.; Levine, B.S.; Weina, P.J.; Swearengen, J.; Heiffer, M.H. *Trans. R.. Soc. Trop. Med. Hyg.*, **1994**, *88*(Suppl. 1), 33.
[3] Lin, A.J.; Lee, M.; Klayman, D.L. *J. Med. Chem.*, **1989**, *32*, 1249.
[4] Lin, A. J.; Klayman, D. L.; Milhous, W. K. *J. Med. Chem*., **1987**, *30*, 2147.
[5] Lin, A. J.; Miller, R. E. *J. Med. Chem.*, **1995**, *38*, 764.
[6] Genovese, R.F.; Newman, D.B.; Brewer, T.G. *Pharmacol. Biochem. Behav.*, **2000**, *67*, 37.
[7] Nontprasert, A.; Pukrittayakamee, S.; Prakongpan, S.; Supanaranond, W.; Looareesuwan, S.; White, N.J. *Trans. R. Soc. Trop. Med. Hyg.,* **2002**, *96*, 99.
[8] Price, R.; van Vugt, M.; Nosten, F.; Luxemburger, C.; Brockman, A., Phaipun, L.; Chongsuphajaisiddhi, T.; White, N. *Am. J. Trop. Med. Hyg.,* **1998**, *59*, 883.
[9] Luo. X.-D.; Shen. C.-C. *Med. Res. Rev.*, **1987**, *7*, 29.
[10] Jung, M. *Curr. Med. Chem.*, **1994**, *1*, 35.
[11] Haynes, R. K.; Vonwiller, S. C. *Acc. Chem. Res.*, **1997**, *30*, 73.
[12] Vroman, J. A.; Alvim-Gaston, M.; Avery, M. A. *Curr. Pharm. Design,* **1999**, *5*, 101.
[13] Borstnik, K.; Paik, I.-K.; Posner, G.H. *Mini Rev. Med. Chem.*, **2002**, *2*, 573.
[14] Borstnik, K.; Paik, I.-K.; Shapiro, T.A.; Posner, G.H. *Int. J. Parasitol.*, **2002**, *32*, 1161.
[15] Sriram, D.; Rao, V.S.; Chandrasekhara, K.V.; Yogeeswari, *P. Nat. Prod. Res.*, **2004**, *18*, 503.
[16] Tang, Y.; Dong, Y.; Vennerstrom, J.L. *Med. Res. Rev.*, **2004**, *24*, 425.
[17] O'Neill, P.M.; Posner, G.H. *J. Med. Chem.*, **2004**, *47*, 2945.
[18] Nosten, F.; Brasseur, P. *Drugs*, **2002**, *62*, 1315.
[19] Olliaro, P.L.; Taylor, W.R. J. *Postgrad. Med.*, **2004**, *50*, 40
[20] Mutabingwa, T.K. *Acta Trop.*, **2005**, *95*, 305.
[21] Jung. M.; Lee, K.; Kim, H.; Park, M. *Curr. Med. Chem.*, **2004**, *11*,1265,
[22] Efferth, T. *Drug Resist. Updat.*, **2005**, *8*, 85.
[23] Efferth, T. *Curr. Drug. Targets,*, **2006**, *7*, 407.
[24] Efferth, T.; Ramirez, T.; Gebhart, E.; Halatsch, M.-E. *Biochem. Pharmacol.*, **2004**, *67*, 1689.
[25] Moyer, J.D.; Barbacci, E.G.; Iwata, K.K.; Arnold, L.; Boman, B.; Cunningham, A.; DiOrio, C.; Doty, J.; Morin, M.J.; Moyer, M.P.; Neveu, M.; Pollack, V.A.; Pustilnik, L.R.; Reynolds, M.M.; Sloan, D.; Theleman, A.; Miller, P. *Cancer. Res.*, **1997**, *57*, 4838.
[26] Norman P. *Curr. Opin. Investig. Drugs*, **2001**, *2*, 298.
[27] Meshnick, S.R.; Jefford, C.W.; Posner, G.H.; Avery, M.A.; Peters, W. *Parasitol. Today*, **1996**, *12*, 79.
[28] Meshnick, S.R. *Med. Trop. (Mars)*, **1998**, *58*(3 Suppl.), 13.
[29] Beekman. A. C.; Barentsen, A. R. W.; Woerdenbag, H. J.; Uden, W. V.; Pras, N.; Konings, A. W. T.; El-Feraly, F. S.; Galal, A. M.; Wikström, H. V. *J. Nat. Prod.*, **1997**, *60*, 325.
[30] Jung, M. *Bioorg. Med. Chem. Lett.*, **1997**, *7*, 1091.
[31] Posner, G. H.; Ploypradith, P.; Parker, M. H.; O'Dowd, H.; Woo, S.-H.; Northrop, J.; Krasavin, M.; Dolan, P.; Kensler, T. W.; Xie, S.; Shapiro, T. A. *J. Med. Chem.*, **1999**, *42*, 4275.
[32] Li, Y.; Shan, F.; Wu, J.-M.; Wu, G.-S.; Ding, J.; Xiao, D.; Yang, W.-Y.; Atassi, G.; Léonce, S.; Caignard, D.-H.; Renard, P. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 5.
[33] Posner, G. H.; Northrop, J.; Paik, I.-H.; Borstnik, K.; Dolan, P.; Kensler, T. W.; Xie, S.; Shapiro, T. A. *Bioorg. Med. Chem.*, **2002**, *10*, 227.
[34] Efferth, T.; Benakis, A.; Romero, M.R.; Tomicic, M.; Rauh, R.; Steinbach, D.; Häfer, R.; Stamminger, T.; Oesch, F.; Kaina, B.; Marschall, M. *Free Radic. Biol. Med.*, **2004**, *37*, 998.
[35] Lai, H.; Singh, N.P. *Cancer Lett.*, **1995**, *91*, 41.
[36] Singh, N.P.; Lai, H. *Life Sci.*, **2001**, *70*, 49.
[37] Andrews, N.C. *Nat. Rev. Genet.*, **2000**, 1, 208.
[38] Reizenstein, P. *Med. Oncol. Tumor Pharmacother.*, **1991**, *8*, 229.
[39] Lai, H.; Sasaki, T.; Singh, N.P.; Messay, A. *Life Sci.*, **2005**, *76*, 1267.
[40] Kim, S.J.; Kim, M.S.; Lee, J.W.; Lee, C.H.; Yoo, H.; Shin, S.H.; Park, M.J.; Lee, S.H. *J. Cancer Res. Clin. Oncol.*, **2006**, *132*, 129.
[41] Sadava, D.; Phillips, T.; Lin, C.; Kane, S.E. *Cancer Lett.*, **2002**, *179*, 151.

[42] Jeyadevan, J. P.; Bray, P. G.; Chadwick, J.; Mercer, A. E.; Byrne, A.; Ward, S. A.; Park, B. K.; Williams, D. P.; Cosstick, R.; Davies, J.; Higson, A. P.; Irving, E.; Posner, G. H.; O'Neill, P. M. *J. Med. Chem.,* **2004**, *47*, 1290.
[43] Posner, G. H.; McRiner, A.J.; Paik, I.-H.; Sur, S.; Borstnik, K.; Xie, S.; Shapiro, T.A.; Alagbala, A.; Foster, B. *J. Med. Chem.,* **2004**, *47*, 1299.
[44] Liu, Y.; Wong, V. K.-W.; Ko, B. C.-B.; Wong, M.-K.; Che, C.-M. *Org. Lett.,* **2005**, *7*, 1561.
[45] Paik, I.-H.; Xie, S.; Shapiro, T.A.; Labonte, T.; Narducci Sarjeant, A.A.; Baege, A.C.; Posner, G.H. *J. Med. Chem.*, **2006**, *49*, 2731.
[46] Folkman, J. *New Engl. J. Med.*, **1971**, *285*, 1182.
[47] Arnold, F.; West, D. C. *Pharmacol. Ther.*, **1991**, *52*, 407.
[48] Hyder, S. M.; Stancel, G. M. *Mol. Endocrinol.*, **1999**, *13*, 806.
[49] Hanahan, D. *Nat. Med.*, **1998**, *5*, 1359.
[50] Klein, R.; Klein, B. E.; Moss, S. E. *Diabetes Metab. Rev.*, **1989**, *5*, 559.
[51] Koch, A. E. *Arthritis Rheum.*, **1998**, *41*, 951.
[52] Chen, H.-H.; Zhou, H.-J.; Fang, X. *Pharmcol. Res.*, **2003**, *48*, 231.
[53] Chen, H.-H.; Zhou, H.-J.; Wu, G.D.; Lou, X.-E. *Pharmacology*, **2004**, *71*, 1.
[54] Chen, H.-H.; You, L.-L.; Shang-bin, L. *Cancer Lett.*, **2004**, *211*, 163.
[55] Dell'Eva, R.; Pfeffer, U.; Vene, R.; Anfosso, L.; Forlani, A.; Albini, A.; Efferth, T. *Biochem. Pharmacol.*, **2004**, *68*, 2359.
[56] Cornali, E.; Zietz, C.; Benelli, R.; Weninger, W.; Masiello, L.; Breier, G.; Tschachler, E.; Albini, A.; Sturzl, M. *Am. J. Pathol.*, **1996**, *149*, 1851.
[57] Wu, G.-D.; Zhou, H.-J.; Wu, X.-H. *Vasc. Pharmacol.*, **2005**, *41*, 205.
[58] Lee, J.; Zhou, H.-J.; Wu, X.-H. *Cancer Chemother. Pharmacol.*, **2006**, *57*, 213.
[59] Lee, S.; Oh, S. *Tetrahedron Lett.*, **2002**, *43*, 2891.
[60] Oh, S.; Jeong, I.H.; Shin, W.S.; Lee, S. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 3665.
[61] Oh, S.; Jeong, I.H.; Ahn, C.M.; Shin, W.S.; Lee, S. *Bioorg. Med. Chem.*, **2004**, *12*, 3783.
[62] Oh, S.; Jeong, I.H.; Lee, S. *J. Org. Chem.*, **2004**, *69*, 984
[63] Oh, S.; Jeong, I.H.; Shin, W.S.; Lee, S. *Bioorg. Med. Chem. Lett.*, **2004**, *13*, 3683.
[64] Jung, M.; Tak, J.; Chung, W.Y.; Park, K.K. *Bioorg. Med. Chem. Lett.*, **2006**, *16*, 1227.
[65] Qian, R. Sh.; Li, Zh. L.; Yu, J. L.; Ma, D. J. *J. Trad. Chin. Med.*, **1981**, *22*, 63.
[66] Shen, M.; Ge, H. L.; He, Y. X.; Song, Q. L.; Zhang, H. Z. *Sci. Sin., Ser. B (Engl. Ed.)*, **1984**, *27*, 398.
[67] Huang, G. J.; Zhao, Y. *J. Trad. Chin. Med.*, **1983**, *3*, 171.
[68] Li, X. Y.; Liang, H. Zh. *Acta Pharm. Sin.*, **1986**, *7*, 471.
[69] Noori, S.; Naderi, G.A.; Hassan, Z.M.; Habibi, Z.; Bathaie, S.Z.; Hashemi, S.M. *Int. Immunopharmacol.*, **2004**, *4*, 1301.
[70] Yang, Z.S.; Zhou, W.L.; Sui, Y.; Wang, J.X.; Wu, J.M.; Zhou, Y.; Zhang, Y.; He, P.L.; Han, J.Y.; Tang, W.; Li, Y.; Zuo, J.P. *J. Med. Chem.,* **2005**, *48*, 4608.
[71] Zhou, W.L.; Wu, J.M.; Wu, Q.L.; Wang, J.X.; Zhou, Y.; Zhou, R.; He, P.L.; Li, X.Y.; Yang, Y.F.; Zhang, Y.; Li, Y.; Zuo, J.P. *Acta Pharmacol. Sin.*, **2005**, *26*, 1352.
[72] Tiercy, J.M.; Villard, J.; Roosnek, E. *Transpl. Immunol.*, **2002**, *10*, 215.
[73] Goes, N.; Chandraker, A. *Curr. Opin. Nephrol. Hypertens.*, **2000**, 9, 683.
[74] Mitchell, T. G.; Perfect, J. R. *Clin. Microbiol. Rev.*, **1995**, *8*, 515-548.
[75] Morello, A. J.; Mizer, H. E.; Wilson, M. E.; Granato, P. A. *Microbiology in Patient Care*; McGraw-Hill: New York, **1998**; 323.
[76] Galal, A.M.; Ross, S.A.; Jacob, M.; ElSohly, M.A. *J. Nat. Prod.*, **2005**, *68*, 1274.
[77] Romero, M.R.; Efferth, T.; Serrano, M.A.; Castano, B.; Macias, R.I.; Briz, O.; Marin, J.J. *Antiviral Res*., **2005**, *68*, 75.
[78] Sells, M.A.; Chen, M.L.; Acs, G. *Proc. Natl. Acad. Sci. USA,* **1987**, *84*, 1005.
[79] Schalm, S.W.; de Man, R.A.; Heijtink, R.A.; Niesters, H.G.M. *J. Hepatol.*, **1995**, *22*(Suppl.), 52.
[80] Efferth, T., Marschall, M., Wang, X., Huong, S.M., Hauber, I., Olbrich, A.; Kronschnabl, M.; Stamminger, T.; Huang, E.S. *J. Mol. Med.*, **2002**, *80*, 233.

Received: 08 September, 2006 Revised: 12 January, 2007 Accepted: 26 January, 2007