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Synthesis and Cytotoxicity Studies of Artemisinin Derivatives Containing Lipophilic Alkyl Carbon Chains

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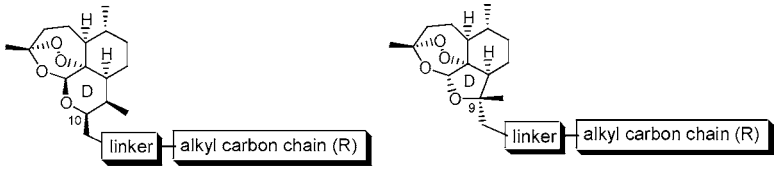
ABSTRACT



linker = amide, ester, alcohol, ketone
R = C_2H_5, C_4H_9, C_6H_{13},C_8H_{17}, $C_{10}H_{21}$, $C_{12}H_{25}$, $C_{14}H_{29}$, $C_{16}H_{33}$, $C_{18}H_{37}$

Cytotoxic artemisinin derivatives have been synthesized by a modular approach of "artemisinin + linker + lipophilic alkyl carbon chain". A strong correlation between the length of the carbon chains and the cytotoxicities against human hepatocellular carcinoma (HepG2) was revealed. Notably, compared with artemisinin (IC_{50} = 97 μM), up to 200-fold more potent cytotoxicity (IC_{50} = 0.46 μM) could be achieved by attachment of a $C_{14}H_{29}$ carbon chain to artemisinin via an amide linker.

Artemisinin (qinghaosu, **1**) is a sesquiterpene lactone endoperoxide isolated from an ancient Chinese herb *Artemisia annua* (Sweet wormwood) (Figure 1).[1,2] Artemisinin and its derivatives are highly effective against multidrug-resistant malaria caused by *Plasmodium falciparum*, and they have been currently used for the clinical treatment of malaria.[3−11] Although the exact mechanism of their antimalarial activities is not clear, substantial experimental evidence indicates that

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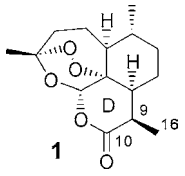


Figure 1. Artemisinin (qinghaosu, **1**).

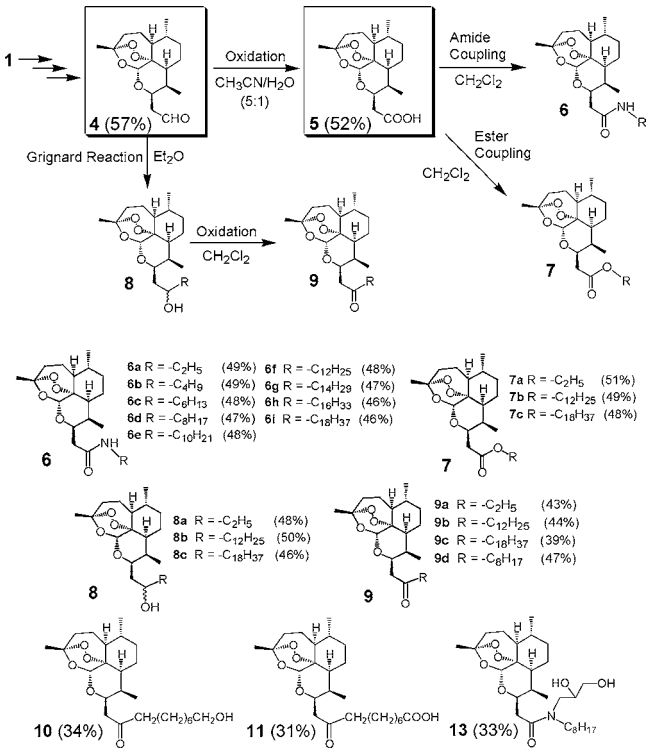
the peroxide moiety of artemisinin and its derivatives is crucial to their antimalarial activities.[3,4,8a,12,13] Given the remarkable success of artemisinin in the treatment of malaria, there is growing interest in exploring the other therapeutic properties of artemisinin.

A search of the literature revealed that cytotoxic artemisinin analogues could be synthesized via chemical modifications of the relatively nontoxic artemisinin at its C_{10}/C_{16} position by covalent attachment of alkyl/aryl groups.[14−18] In addition, by judicious selection of linkers, a variety of dimeric, trimeric, and even tetrameric cytotoxic artemisinin derivatives have been prepared.[19−21] However, given the diverse chemical structures of these cytotoxic artemisinin analogues, identification of the key factors contributing to their cytotoxicities and rational design of new classes of cytotoxic artemisinin analogues is difficult. In this regard, it is of importance to conduct structure−activity relationship (SAR) studies to assist the design and synthesis of new cytotoxic artemisinin derivatives.

Here we report the synthesis and cytotoxicity studies of artemisinin derivatives modified by a modular approach of "artemisinin + linker + lipophilic alkyl carbon chain". Through systematic investigations on these structurally related artemisinin analogues, it was found that the lipophilic carbon chain plays an important role in determining the cytotoxicities of the modified artemisinin analogues.

To maximize the structural diversity of artemisinin analogues and the efficiency of their synthesis, aldehyde **4** and acid **5** derived from **1** were prepared as the key intermediates. Starting from **4** and **5**, amides **6**, esters **7**, alcohols **8**, and ketones **9**, **10**, **11**, and **13** bearing alkyl carbon chains of different length were prepared (Scheme 1, overall yields from **1** were shown in parentheses) (see the Supporting Information for the detailed synthetic schemes).

Scheme 1. Synthesis of Artemisinin Derivatives **6**−**11** and **13**



Apart from the naturally occurring D-six-membered ring artemisinin, a series of D-five-membered ring artemisinin derivatives **19**−**21** were synthesized from key intermediates **17** and **18** (Figure 2).[22]

The *in vitro* cytotoxicity of the artemisinin derivatives against a human hepatocellular carcinoma cell line, HepG2, were conducted by using the MTT assay.[23] The cytotoxicities

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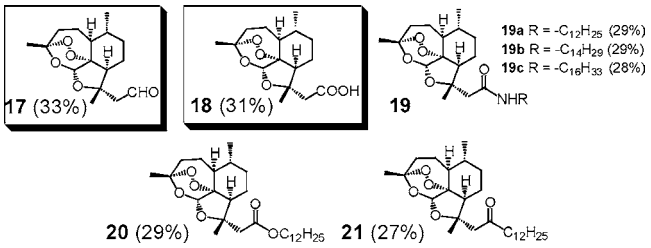
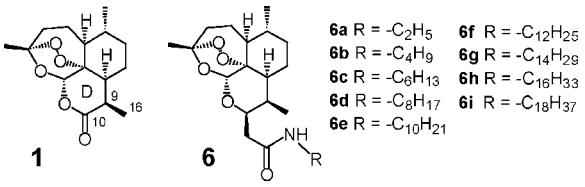


Figure 2. D-Five-membered ring artemisinin derivatives **17**−**21**.

(IC_{50} values) of **1** and amides **6a**−**i** bearing linear alkyl carbon chains of different length are summarized in Table 1.

As a control, unmodified artemisinin **1** was found to be weakly cytotoxic (IC_{50} = 97 μM) aganist a HepG2 cancer

Table 1. Cytotoxicities of **1** and **6a**−**i** against HepG2 Cell Line



compd	**1**	**6a**	**6b**	**6c**	**6d**	**6e**	**6f**	**6g**	**6h**	**6i**
IC_{50} (μM)	97	>100	>100	17.6	9.5	2.8	1.2	0.46	0.79	4.2

cell line. No cytotoxicities (IC_{50} > 100 μM) were obtained for **6a** and **6b** bearing short carbon chains of C_2H_5 and C_4H_9. Interestingly, **6c** with a longer carbon chain of C_6H_{13} exhibited good cytotoxicity with IC_{50} = 17.6 μM. As the length of the carbon chains increased, the cytotoxicities increased gradually from **6d** (C_8H_{17}, IC_{50} = 9.5 μM), **6e** ($C_{10}H_{21}$, IC_{50} = 2.8 μM) to **6f** ($C_{12}H_{25}$, IC_{50} = 1.2 μM). *Notably, the most potent cytotoxicity (IC_{50} = 0.46 μM) was exhibited by* ***6g*** *with a $C_{14}H_{29}$ group.* Further increase in

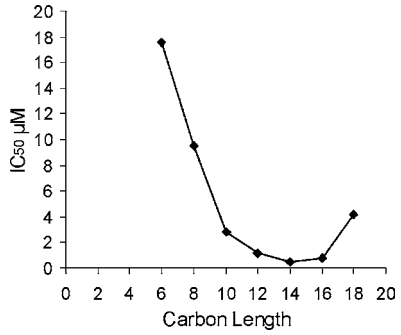
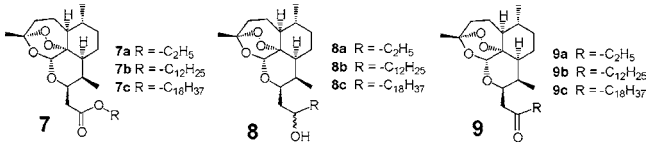


Figure 3. Plot of IC_{50} values vs the carbon chain length for **6c**−**i**.

carbon chain length resulted in a slightly drop of cytotoxicity, i.e., **6h** ($C_{16}H_{33}$, IC_{50} = 0.79 μM) and **6i** ($C_{18}H_{37}$, IC_{50} = 4.2 μM). As summarized in a plot of IC_{50} values versus the length of carbon chains (Figure 3), **6f**−**h** with carbon chains of 12- to 16-carbon exhibited the most potent cytotoxicities while lower cytotoxicities were obtained for analogues bearing shorter or longer carbon chains. *Notably, the cytotoxicity of* ***6g*** *(IC_{50} = 0.46 μM) bearing a $C_{14}H_{29}$ carbon chain is almost 200-fold more potent than that of the unmodified* ***1*** *(IC_{50} = 97 μM).*

Apart from the effect of carbon chain length, we have also examined the effect of linkers on the cytotoxicities. The cytotoxicities of esters **7**, alcohols **8**, and ketones **9** against HepG2 cell line are summarized in Table 2. With a carbon

Table 2. Cytotoxicities of **7**−**9** against HepG2 Cell Line



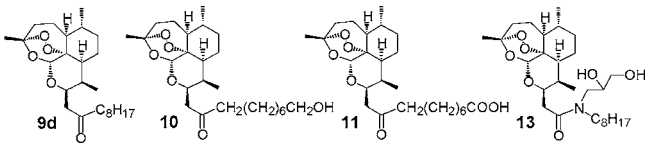
compd (IC_{50}, μM)		
7a (>100)	**7b** (0.72)	**7c** (>100)
α-**8a** (>100)	α-**8b** (2.8)	α-**8c** (>100)
β-**8a** (>100)	β-**8b** (4.4)	β-**8c** (>100)
9a (>100)	**9b** (1.4)	**9c** (>100)

chain of $C_{12}H_{25}$, comparable IC_{50} values were obtained for ester **7b** (0.72 μM), α-alcohol **8b** (2.8 μM), β-alcohol **8b** (4.4 μM), and ketone **9b** (1.4 μM). These IC_{50} values were also similar to that of amide **6f** (1.2 μM). These results indicate that the nature of linkers has no significant effect on their cytotoxicties. Similar cytotoxicities were observed for alcohols α-**8** and β-**8** suggesting that their cytotoxicities were independent of the stereochemistry of the alcohol linker. No cytotoxicity (IC_{50} > 100 μM) was observed for **7a**,**c**, **8a**,**c**, and **9a**,**c** bearing carbon chains of C_2H_5 and $C_{18}H_{37}$.

The above studies indicate that attachment of lipophilic carbon chains significantly enhances the cytotoxicity of artemisinin derivatives. However, an increase in lipophilicity does lead to poor water solubility which is problematical for future development of these compounds as drug candidates. To circumvent this problem, we incorporated polar groups to enhance the water solubility. In this regard, artemisinin analogues **10**, **11**, and **13** incorporated with polar hydroxyl and carboxylic acid groups were prepared and examined. The results are summarized in Table 3.

It is interesting to note that incorporation of polar hydroxyl and carboxylic groups at the terminal ends of the carbon chains remarkably reduces the cytotoxicities. As shown in Table 3, the cytotoxicity of **10** (IC_{50} = 42.3 μM) with a terminal hydroxyl group and **11** (IC_{50} > 100 μM) with a terminal carboxylic acid group was considerably lower than that of their methyl analogue **9d** (IC_{50} = 1.8 μM). These findings indicate that the lipophilic end of the carbon chain is essential for their cytotoxicity. Yet, for **13** with a polar

Table 3. Cytotoxicities of **9d**, **10**, **11**, and **13** against HepG2 Cell Line

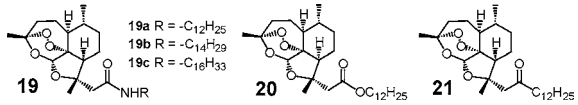


compd	**9d**	**10**	**11**	**13**
IC_{50} (μM)	1.8	42.3	>100	3.5

dihydoxyl group located at the amide linker, a slightly more potent cytotoxicity (IC_{50} = 3.5 μM) than **6d** (IC_{50} = 9.5 μM) was obtained. These findings indicate that the position of polar groups on the carbon chain has a significant effect on the cytotoxicity.

As illustrated in Table 4, potent cytotoxicities (IC_{50} = 0.47−3.7 μM) against HepG2 cell line were observed for

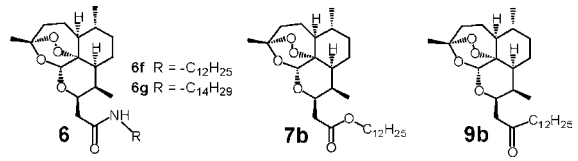
Table 4. Cytotoxicities of **19**−**21** against HepG2 Cell Line



compd	**19a**	**19b**	**19c**	**20**	**21**
IC_{50} (μM)	1.3	0.77	0.74	3.7	0.47

the D-five-membered ring artemisinin analogues **19**−**21**. These cytotoxicities were comparable to their D-six-membered ring artemisinin analogues. These findings indicate that structural differences in the D ring has no significant effects on the cytotoxicities. *Notably, an IC_{50} value of 0.47 μM was obtained for **21** with a $C_{12}H_{25}$ group connected by a ketone linker.*

Table 5. Cytotoxicities of Modified Artemisinins on CCD-19Lu



compd	**6f**	**6g**	**7b**	**9b**
IC_{50}^{normal} (μM)	2.7	2.9	4.1	14.5
IC_{50}^{cancer} (μM)	1.2	0.46	0.72	1.4

To investigate the selectivity between the cancer cell line and normal cells, the cytotoxicities of **6f**, **6g**, **7b**, and **9b** against normal lung fibroblast (CCD-19Lu) were examined. As shown in Table 5, the modified artemisinins displayed a moderate to good selectivity between the cancer and the normal cells. Note that **9b** was around 10-fold less cytotoxic toward normal lung fibroblast cells (IC_{50} = 14.5 μM) than the HepG2 cancer cell line (IC_{50} = 1.4 μM).

To demonstrate the importance of the peroxide functionality on the cytotoxicities, deoxygenated analogue **22** was prepared from **6h** (Figure 4). Compared with the peroxide-

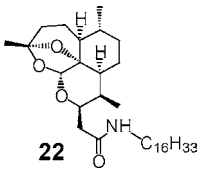


Figure 4. Deoxygenated artemisinin analogue **22**.

containing **6h** (IC_{50} = 0.79 μM), significant loss of cytotoxicity (IC_{50} >100 μM) was observed for the deoxygenated **22**.[24] This result clearly indicated the importance of the endoperoxide moiety to the cytotoxicty.[25]

In summary, we have synthesized and examined the cytotoxicities of a series of artemisinin derivatives bearing lipophilic alkyl carbon chains. There is a strong correlation between the length of the carbon chains and the cytotoxicities of the modified artemisinins. Notably, potent cytotoxicity (IC_{50} = 0.46 μM) against human hepatocellular carcinoma cell line could be achieved by attachment of a $C_{14}H_{29}$ carbon chain to the weakly cytotoxic artemisinin **1** (IC_{50} = 97 μM) via an amide linker. Our results also indicated that polar groups in the carbon chain has a significant effect on the cytotoxicity.[26]

Acknowledgment. This work was supported by The University of Hong Kong (University Development Fund).

Supporting Information Available: Synthesis, compound characterization data, and cytotoxicity studies of artemisinin derivatives. This material is available free of charge via the Internet at http://pubs.acs.org.

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(24) Deoxygenated analogue **22** was synthesized according to literature procedures; see refs 4 and 13.

(25) The importance of endoperoxide moiety on the biological activities of artemisinins has been reported in the literature; see refs 17 and 19b.

(26) For a literature report regarding the relationship between lipophilicity and neurotoxicity of antimalarial artemisinin analogues, see: Bhattacharjee, A. K.; Karle, J. M. *Chem. Res. Toxicol.* **1999**, *12*, 422−428.