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SYNTHESIS AND CYTOTOXICITY OF NOVEL ARTEMISININ ANALOGS

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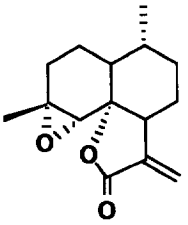
Abstract: A series of artemisinin-related analogs has been synthesized and assayed *in vitro* cytotoxicity. 12-Ethyl-, 12-n-butyl-, homo- and 12-(*N*,*N*-diethylaminomethylbenzoyl)- deoxoartemisinins show a good cytotoxicity against P388 and KB cell lines, respectively. © 1997 Elsevier Science Ltd.

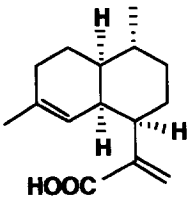
Artemisinin (Qinghaosu) **8**, a sesquiterpene lactone endoperoxide is a first natural trioxane isolated from *Artemisia annua*, L. This compound is of special biological interest because of its outstanding anti-malarial activity,[1] *in vitro* activity against *Pneumocystis carinii*,[2] and *T. gondii*.[3] We reported artemisinin-related sesquiterpene, arteannuin B **1** shows good *in vitro* antineoplastic activity (IC_{50} = 12 μM against L-1210).[4] The first anti-HIV activity of artemisinin-related trioxanes was also reported by our group.[5] Recently a few research groups reported cytotoxicity of artemisinin and its related derivatives.[6] Unique structure bearing endoperoxide could be a trigger for the generation of active oxygen radicals *via* homolytic cleavage of the weak oxygen peroxide bond, which may mediate for the selective and preferable damage to vital cellular structures of the relatively active cancer cells. This concept, coupled with preliminary results prompted us to prepare artemisinin-related compounds and evaluate their *in vitro* cytotoxicity. In this communication, we would like to report, for the first time, the structure-activity relationship of novel deoxoartemisinin analogs.

We designed artemisinin analogs for increasing bioavailability such as stability or water solubility of artemisinin **8**, the lead compound. Since direct introduction of C-C bond at C-12 of artemisinin for the preparation of series of novel analogs may cause destruction of the biologically essential endoperoxide, we applied our photooxygenative cyclization[7,8] of more abundant artemisinic acid **2** as a key step (Scheme 1). A series of novel analogs has been prepared from artemisinic acid as a useful chiral synthon. Reaction of dihydroartemisinyl aldehyde **3**, prepared from artemisinic acid **2** by a known procedure,[7] with ethyl-, n-butyl-, benzyl-, and 1-pentenyl- magnesiumchlorides gave alcohols **4a-d**, respectively (erythro/threo = 4 to 5/1). The aldehyde **3** was also used to prepare homologated alcohol **5**.[7a] Photooxygenative cyclization, as previously mentioned,[8] of alcohols **4a-d** (the mixture of C-12 stereoisomers) and **5** provided analogs **6a-d**,[7b,9] C-C bond introduced at C-12 and homodeoxoartemisinin **7**, respectively. No C-12 epimer of **6a-d** was found. Water-soluble 12-carboxypropyldeoxoartemisinin **6e** (sodium salt) was also prepared from the olefinicdeoxoartemisinin **6d** by direct oxidation ($KMnO_4$) of the terminal olefin in 73 % yield. Treatment of artemisinin **8** with $NaBH_4$ in methanol (0 °C, 1h., 91 % yield) to dihydroartemisinin **9** and subsequent etherification in ethanol under acidic catalysis ($BF_3.Et_2O$) in anhydrous benzene (reflux, 1 h.) gave β-arteether **11** according to the literature.[10] Similarly, coupling of dihydroartemisinin **9** with 12-(*N*, *N*-diethylaminomethyl) benzoic acid (DCC, DMAP, CH_2Cl_2, r. t.) afforded new analog **12**[11] (62 % yield)

Scheme 1



1



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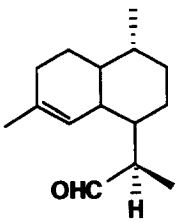

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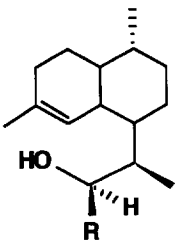
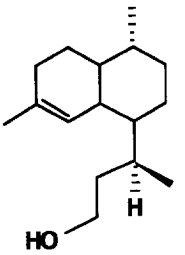


4a: R = ethyl
b: R = n-butyl
c: R = CH_2Ph
d: R = $(CH_2)_3$-CH=CH_2



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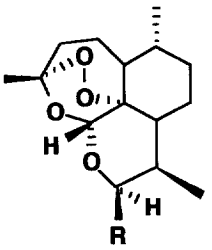
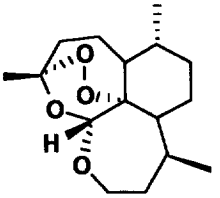
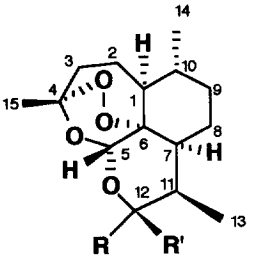


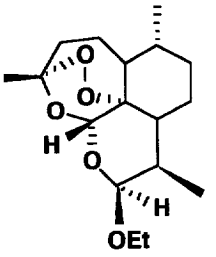
6a: R = ethyl
b: R = n-butyl
c: R = CH_2Ph
d: R = $(CH_2)_3$-CH=CH_2
e: R = $(CH_2)_3$-COOH



7

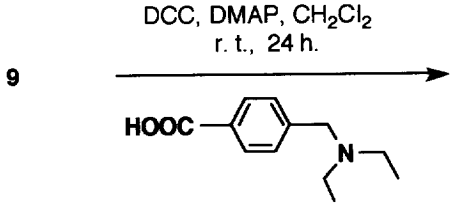


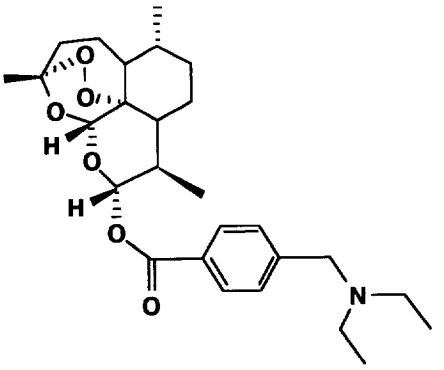
8: R = R' = O
9: R = H, R' = OH
10: R = R' = H



11

Scheme 2





12

(Scheme 2). In this reaction, α-epimer was exclusively obtained ($J_{11,12}$ = 9.8 Hz). This result is consistent with the report that acylation of **9** in alkaline medium led almost exclusively to α-configurated derivatives[13]. β to α Interconversion of configuration at C-12 of **9** was taken place in alkaline solution presumably through an aldehyde-alcohol intermediate. Direct reduction of artemisinin **8** with $NaBH_4$ in the presence of $BF_3.Et_2O$ in THF provided deoxoartemisinin **10** in 75 % yield.[8] Most analogs in this paper possess increased bioavailability in terms of stability or water solubility with retention of biologically essential endoperoxide. Since deoxoartemisinin **10** and (+)-12-n-butyldeoxoartemisinin **6b** lack the carbonyl function and *exo* C-O bond at C-12, they are projected to possess increased stability, thus longer half-life in the body, and they point the way to potential next generation analogs. Furthermore, carboxylic acid was introduced as water-solubilizing functional group into **6e**.

The *in vitro* cytotoxicity of artemisinin and its related trioxanes to the murine and human cancer cells was defined by the microculture tetrazolium assay as described previously.[12] IC_{50} values are presented in Table 1. Deoxoartemisinin **10**,[8] arteether **11**,[10] 12-benzyl- **6c**,[9] 12-(carboxypropyl)- **6e** and 12-(*N*,*N*-diethylaminomethyl)benzoyl- **12** deoxoartemisinins are inactive against P388 while artemisinin **8** and 12-n-butyldeoxoartemisinin **6b**[7b] show a moderate anti-P388 activity. 12-Ethyl analog **6a**[9] and homo-deoxoartemisinin **7** show a modest *in vitro* cytotoxicity against P388. 12-Ethyl analog **6a** is five times more active against P388 cell line than artemisinin **8**, the parent compound. Lipophilicity may play an important role here. Increase in lipophilicity due to the ethyl or methylene group of **6a** and **7** may increase cytotoxicity.[6a,14] All analogs exhibit a moderate cytotoxicity (3 - 32 μg/mL) against KB cell line. 12-(*N*,*N*-diethylaminomethyl)benzoyl- **12** and 12-(n-butyl)- **6b** deoxoartemisinins are four to five times more active than artemisinin **8**. Increased lipophilicity of 12-(n-butyl)deoxoartemisinin **6b** again enhanced the cytotoxicity against KB cells. Compounds **6e**, **7**, **8**, **11**, and **12** are inactive against human ovary carcinoma (SK-OV-3) while compounds **6a**, **6b**, **6c** and **10** shows a moderate activity. In vero cells, cytotoxicity was also determined as shown in Table 1. Compounds **6b**, **6c**, and **12** are two times more toxic to vero cells than

Table 1: *In vitro* cytotoxicities of artemisinin-related trioxanes.[a]

	IC_{50} (μg/mL)			
	P388[b]	KB[c]	SK-OV-3[d]	VERO[e]
8	28	16	-[f]	31
6a	5.5	20	33	30
6b	40	4.5	40	15
6c	-	13	32	15
6e	-	32	-	30
7	8	22	-	30
10	-	24	40	30
11	-	15	-	28
12	-	3	-	15

[a]The cytotoxicity of reference cisplatin was 3.1 μg/mL in these tests. [b]P-388: murine lymphocytic leukemia. [c]KB: human epidermoid carcinoma. [d](SK-OV-3): human ovary carcinoma. [e]VERO: African green monkey kidney cell. [f](-): no activity.

artemisinin. However, **6b** and **12** show more selective cytotoxicity than artemisinin (IC_{50} VERO/KB = 3.3 and 5 for **6b** and **12**, 2 for artemisinin **8**, respectively).

In conclusion, this structure-activity relationship of deoxoartemisinin may be used as a lead for the possible development of new anticancer agents related virtually non-toxic artemisinin (LD_{50} = 4228 mg/Kg orally administered to mice).

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References and Notes:

1. Luo, X-D.; Shen, C-C. *Med. Res. Rev.*, **1987**, *7*, 29.
2. Merali, S. ; Meshnick, S. R. *Antimicro. Agents Chemother.* **1991**, *35*, 1225.
3. Ou-Yang, K.; Krug, E. C.; Marr, J. J.; Berens, R. L. *ibid* **1990**, *34*, 1961.
4. Jung, M.; ElSohly, H. N.; McChesney, J. D. *Planta Medica* **1990**, 56.
5. Jung, M.; Schinazi, R. F. *Bioorg. Med. Chem. Lett.*, **1994**, *4*, 931.
6. a) Woerdenbag, H. J.; Moskal, T. A.; Pras, N.; Maringle, T. M.; ElFeraly, F. S.; Kampinga, H. H.; Konings, A. W. T. *J. of Nat. Prod.* **1993**, *56*, 849.
   b) Zheng, G-Q., *Planta Medica* **1994**, *60*, 54.
   c) Beekman, A. C.; Woerdenbag, H. J.; Kampinga, H. H.; Konings, A. W. T. *Phytotherapy Res.* **1996**, *10*, 140.
7. a) Bustos, D. A.; Jung, M.; ElSohly, H. N.; McChesney, J. D., *Heterocycles*, **1989**, *29*, 2273.
   b) Jung, M.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D., *Synlett*, **1990**, 743.
8. a) Jung, M.; Li, X.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D., *Tetrahedron Lett.*, **1989**, *30*, 5973.
   b) Jung, M.; Li, X.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D.; Milhous, W. K., *J. Med. Chem.*, **1990**, *33*, 1516.
9. Unpublished results, Jung, M.; Bustas, D. A., **1989**.
10. a) Li, Y.; Yu, P. L.; Chen, Y. X.; Li, L. Q.; Gai, Y. Z.; Wang, D. S.; Zheng, Y. P., *Yaoxue Xuebao*, **1981**, *16*, 429.
    b) Brossi, A.; Venugopalan, B.; Gerpe, L. D.; Yeh, H. J. C., Flippen-Anderson, J. L.; Buchs, P.; Luo, L. D.; Milhous, W.; Peters, W., *J. Med. Chem.*, **1988**, *31*, 645.
11. For compound **12**: $[\alpha]_D^{25}$ -45.3° (c 0.05, $CHCl_3$): ^{1}H-NMR ($CDCl_3$, 250 MHz): δ 8.05 (d, J= 8.37 Hz, 2H), 7.42 (d, J= 8.37 Hz, 2H), 6.01 (d, J = 9.81 Hz, 1H, H-12), 5.52 (s, 1H, H-5), 3.61 (s, 2H, benzyl CH_2), 2.74 (m, 1H, H-11), 2.51 (q, J= 11.25, 4.05 Hz, 4H, 2ethyl), 2.38 (ddd, J= 3.75, 5.19, 4.14 Hz, 1H, H-2α), 2.03 (m, 1H, H-2β), 1.42 (s, 3H, 15-CH_3), 1.04 (t, J= 7.14 Hz, 6H, 2CH_3), 0.98 (d, J= 5.94 Hz, 3H, 13-CH_3), 0.92 (d, J= 7.2 Hz, 3H, 14-CH_3). IR ($CHCl_3$): max 3400, 2934, 2876, 1739(C=O), 1612, 1453, 1387, 1267, 1031, 880 (peroxide) cm^{-1}. MS (70 eV): m/e 473 (M^+).
12. Mosmann, T. *J. Immunol. Methods*, **1983**, *65*, 55.
13. a) Shen, C-C.; Zhuang, L. *Med. Res. Rev.*, **1984**, *4*, 58.
    b) Luo, X-D.; Yeh, H. J. C.; Brossi, A. *Helv. Chim. Acta.*, **1984**, *67*, 1515.
14. Kupchan, S. M.; Eakin, M. A.; Thomas, A. M. *J. Med. Chem.*, **1971**, *14*, 1147.

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