Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1003–1006

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

STABILITY OF ACETAL AND NON ACETAL-TYPE ANALOGS OF ARTEMISININ IN SIMULATED STOMACH ACID

Mankil Jung* and Seokjoon Lee

Department of Chemistry, Yonsei University, Seoul 120-749, Korea

Received 10 February 1998; accepted 20 March 1998

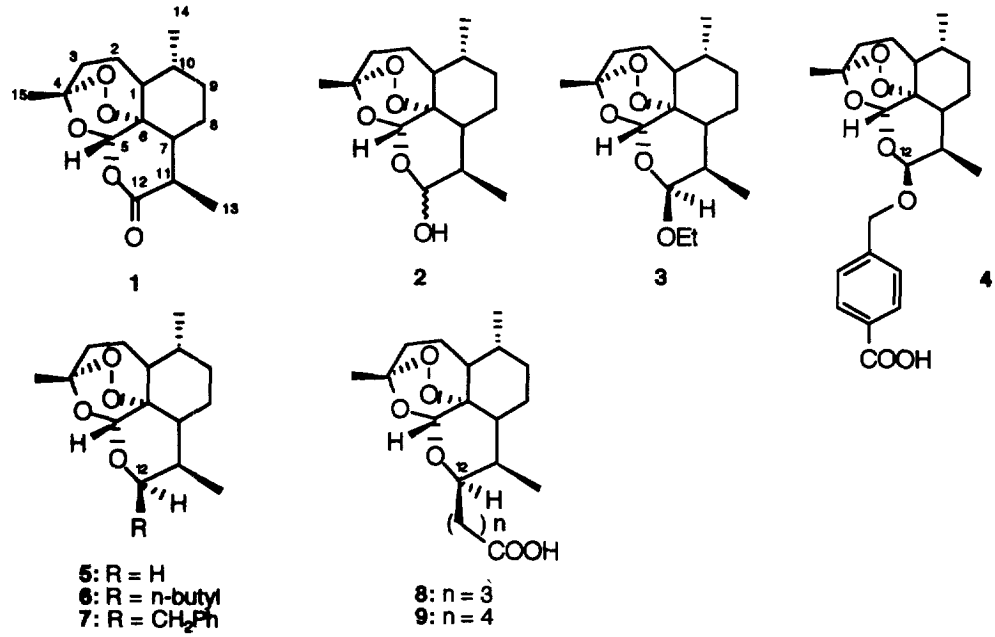
Abstract: A series of non acetal-type analogs of artemisinin containing C-C bond at position-12 have been found to be 15 - 22 times more stable than acetal(C-O)-type prodrugs of artemisinin in simulated stomach acid.

Malaria is one of the number one infectious diseases causing 2 million people deaths every year in the world today. Because of the excellant antimalarial activity against drug-resistant strains of *Plasmodium falciparum* and clinical safety, artemisinin (qinghaosu) **1** has recently gained tremendous interests from many bioorganic and medicinal chemists and pharmacologists for the synthesis, biological activities, mechanisms of drug action and particularly bioavailability. The stability of drugs are gaining more importance every day for the drug industry. In addition to potent antimalarial activity, an ideal artemisinin-related drug candidate should possess (1) an external C-C bond at position-12 for increased chemical stability, thus providing a longer half-life in the body, and (2) high water-solubilizing groups such as carboxylates.[1] Artemisinin is surprisingly stable in neutral solvent heated up to 150 °C.[2] Being a hemiacetal, dihydroartemisinin is chemically more vulnerable than its parent compound, artemisinin, a lactone itself. Artemether, arteether **3**, artesunate, dihydroartemisinin **2** and artelinic acid **4**, which are acetal-type prodrugs under clinical use or trials, respectively, are susceptable to moisture and acidic conditions.[2] From literature data[2] on other arteether and artesunate analogs, it would appear that any derivative at carbon-12 of the type $-OCH_2CH_2R$ and $-O(C{=}O)CH_2R$ may not have sufficient stability in aqueous solution to be clinically useful in an intravenous injectable dosage form. Therefore, we are still in urgent need of an acid-stable and water-soluble product from the series for oral and parenteral application. In bioavailability studies of orally administered arteether, for example, one should consider the effect of gastric pH and emptying time in the design of the study. In the simulated stomach acid (pH=2.0, 37 °C), Baker *et al.*[3] reported that arteether was found to be unstable and easily undergoes hydrolysis, so that sufficient quantities of arteeher would be expected to be lost during the typical time of residence within the stomach before it reach the small intestines, where it would be absorbed. If the pH were drop to 1.0, giving a half-life somewhere in the range of 50 min, one might expect that 25 - 75 % of the arteether might decompose during this time. During the course of the search of more chemically stable and high water-soluble analog, we replaced a C-O bond at C-12 into C-C bond for the purpose of preventing metabolic generation of hemiacetal, which rapidly decomposes in the stomach.[1] There have been no reports on the stability comparison of acetal and non acetal-type prodrugs under acidic conditions. In this communication, we would like to report the first successful demonstration of extraordinarily increased stability of ideal acid-stable, water-soluble and highly active non acetal-type prodrugs in the simulated stomach acid (pH=2.0, 37 °C).

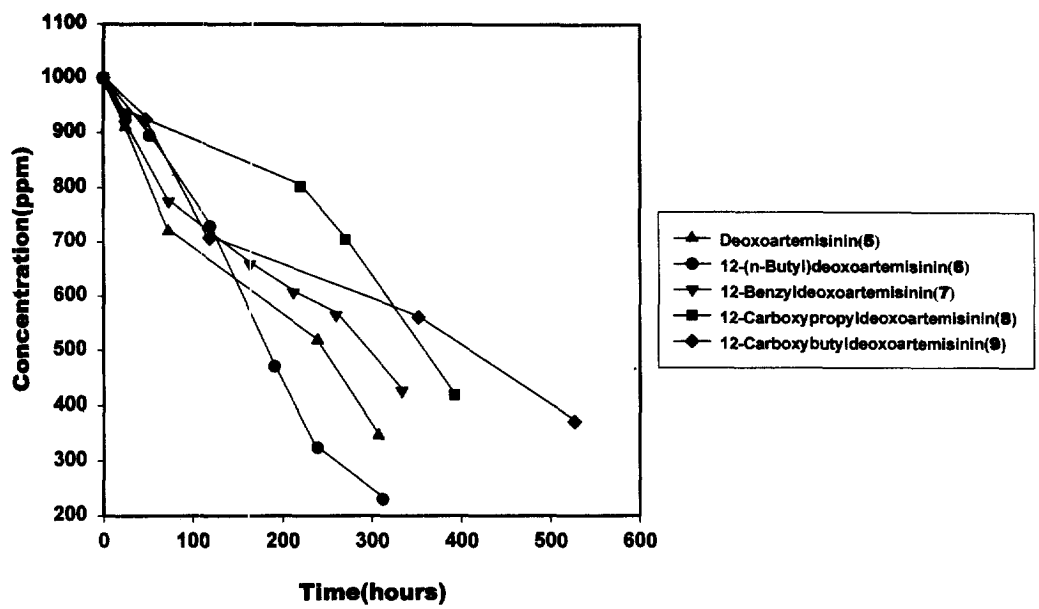
In order to examine the effect of molecular structure on the stability of artemisinin analogs in simulated stomach acid, a series of acetal (C-O)-type **2-4** and non acetal (C-C)-type prodrugs **5-9** (Figure 1) have been

0960-894X/98/$19.00
PII: S0960-894X(98)00160-7

prepared either from artemisinin 1[4-6] or artemisinic acid.[6-8] To measure the stability of acetal (C-O)-type **2-4** and non acetal (C-C)-type **5-9** prodrugs in simulated stomach acid, the disappearance of prodrugs (1 mg/mL)



(Fig. 1) Acetal (C-O)-type (**2-4**) and non acetal (C-C)-type (**5-9**) prodrugs of artemisinin



(Fig. 2) Kinetics of the hydrolysis and decomposition of non acetal (C-C)-type prodrugs in 0.01 N HCl, pH=2.0, 37 °C

in 0.01 N HCl, pH 2.0 at 37 °C was followed by HPLC analysis.[9] All prodrugs tested were found to disappear with pseudo-first order kinetics, with half-life of 10.98 to 23.5 hours for acetal-type prodrugs and of 165.12 to 386.88 hours for non acetal-type prodrugs (Figure 2). Preliminary HPLC studies of the acidic decomposition of acetal (C-O)-type **1-4** and non acetal (C-C)-type **5-9** prodrugs in simulated stomach acid had been shown in Table 1. At pH = 2.0 in water at 37 °C, all prodrugs **2**, **3**, and **4** retaining the acetal at 12-position had an *in vitro* half-life of between 10.98 to 17.19 hours.

Table 1: Stability of Acetal (C-O)-type and Non Acetal (C-C)-type Prodrugs of Artemisinin in Acidic solution (1 mg/mL, 0.01 N HCl, pH=2.0, 37 °C)

Acetal(C-O)-type compound	half-life (hours)
Artemisinin (**1**)	23.50
Dihydroartemisinin (**2**)	17.19
Arteether (**3**)	10.98
Artelinic Acid (**4**)	13.11

Non Acetal(C-C)-type compound	half-life (hours)
Deoxoartemisinin (**5**)	213.36
12-(n-Butyl)-deoxoartemisinin (**6**)	165.12
12-Benzyldeoxoartemisinin (**7**)	285.60
12-(Carboxypropyl)-deoxo-artemisinin (**8**)	386.88
12-(Carboxybutyl)-deoxo-artemisinin (**9**)	374.40

Dihydroartemisinin **2** has longer half-life than arteether **3** and artelinic acid **4**. Thus it becomes clear that aqueous solution of arteether **3** (or of other analogs **1**, **2**, and **4** retaining the acetal at 12-position) would not have enough stability to be used as aqueous solution intended for intravenous administration. In accordance with our proposal, all of non acetal-type analogs, **5** and **6-9**, retaining C-C bond at 12-position show extraordinarily increased acid stability with a half-life of between 165.12 to 386.88 hours. Table 1 shows that the half-life of deoxoartemisinin **5** (213.36 hr) was 19 times longer than arteether **3** (10.98 hr) under aqueous conditions at pH = 2.0 at 37 °C. It was also found that the half-life deoxoartemisinin **8** is 27 times longer than that of arteether **3** under neutral (pH = 7.0) aqueous solution at room temperature. Thus, one could make a crude estimate that deoxoartemisinin **8** would have a half-life of 704 days at pH 7.0 at room temperature by extrapolation of the kinetics to 25 °C using the Arrhenius equation. Interestingly, carboxyalkyl groups at 12-position of **8** and **9** drastically prolonged half-life. At the same time, carboxyl groups of these prodrugs are useful for the increase of water-solubility. Unique structure bearing C-C bond at C-12 position played a key role in remarkable prolonging half-life of the non acetal-type prodrugs. In general, it was found that the half-life of non acetal-type analogs (165.12 to 386.88 hours) was 15-22 times

longer than acetal-type analogs (10.98 to 23.50 hours) under simulated stomach acid condition (pH = 2.0 at 37 °C). Thus, non acetal-type prodrugs **5**-**7** have sufficient stability in simulated stomach acid for oral administration. **8** and **9** have sufficient stability and good water-solubility in aqueous solution to have the shelf-life that would be need for intravenous administration. *It is noteworthy to mention that in vitro antimalarial activities of non acetal-type prodrugs* **5**-**9** *against P. falciparum are comparable to eight to nine times more active than those of acetal-type prodrugs* **1**-**4**. Non acetal-type deoxoartemisinin **5** (IC_{50} = 0.15 ng/mL) and benzyldeoxoartemisinin **7** (IC_{50} < 0.17 ng/mL) show 8-fold increased *in vitro* and superior *in vivo* antimalarial activity as compared to artemisinin **1** (IC_{50} = 1.21 ng/mL).[6, 10] n-Butyldeoxoartemisinin **6**[1] (IC_{50} = 1.15 ng/mL) and carboxyalkyldeoxoartemisinins **8**, **9** (IC_{50} = 1.30 and 1.28 ng/mL, respectively)[7] show comparable activities to those of acetal-type artemisinin and artelinic acid **4** (IC_{50} = 1.38 ng/mL).[5] Non acetal-type compounds **5** and **7** show comparable *in vitro* activity to that of arteether **3** (IC_{50} = 0.16 ng/mL)[6] and nine times *in vitro* activity to that of acetal-type artelinic acid **4**.[5]

In conclusion, we unambiguoesly demonstrated, for the first time, extraordinary increase (15 - 22 times) of stability toward acid of non acetal (C-C)-type prodrugs of artemisinin as expected. Non acetal (C-C)-type prodrugs, in particular, compounds **5**, **6** and **7** deserve further evaluation as drug candidates for oral administration without formulation problem, while compounds **8** and **9** both for oral and intravenous administration because of their high acid stability, good water solubility and comparable or higher antimalarial activity than those of acetal (C-O)-type prodrugs.

Acknowledgments: The authors wish to acknowledge the financial support from the Korea Research Foundation (001-D00287) made in the program year of 1997. We thank Dr. Yong Nam Kim at OromTech, Inc. for helpful discussions and technical assistance.

References and Notes:

1. Jung, M.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D., *Synlett*, **1990**, 743.
2. Luo, X-D.; Shen, C-C., *Med. Res. Rev.*, **1987**, *7*, 29.
3. Baker, J. K.; McChesney, J. D.; Chi, H. T., *Pharm. Res.*, **1993**, *10*, 662.
4. Li, Y.; Yu, P. L.; Chen, Y.X.; Gai, Y. Z.; Wang, D. S.; Zheng, Y. P., *Yaoxue Xuebao*, **1981**, *16*, 429.
5. Lin, A. J.; Klayman, D. L.; Milhous, W. K., *J. Med. Chem.*, **1987**, *30*, 2147.
6. Jung, M.; Li, X.; Bustos, D. A.; ElSohly, H. N.; McChesney, J. D.; Milhous, W. K., *J. Med. Chem.*, **1990**, *33*, 1516.
7. Jung, M.; Freitas, A. C. C.; McChesney, J. D.; ElSohly, H. N., *Heterocycles*, **1994**, *39*, 23.
8. Jung, M.; Bustos, D. A.; ElSohly, H. N.; McChesney, J.D., *Bioorg. & Med. Chem. Lett.*, **1991**, *1*, 741.
9. Sample Preparation and Measurement of Stability - A 100 μl portion of a 1.0 mg artemisinin prodrug stock solution (in acetonitrile) was added to 1.0 mL of a freshly prepared 0.01 N HCl aqueous solution (preheated to 37 °C). The resulting mixture was sealed to prevent water evaporization and maintained at 37 °C in a water bath. Samples (**1**- **4**) of the reaction mixture were taken at time intervals of 0, 200, 400, 600, 800, 1000, 1200, 1400 min. and non acetal-type samples (**5**, **6**, **7**, **8**, **9**) at 2, 4, 6, 8, 10, 12, 14 days. The samples were stored in dry ice-acetone bath and analyzed (in triplicate) as quickly as possible. HPLC with UV detector (Waters 486 tunable absorbance detector, Millipore) was used to identify the decomposition of the prodrugs and quantitation. All prodrugs were detected with wavelength of 210 nm except 250 nm for aromatic compounds **4** and **7**. The column was Nova-Pak^R C-18 (4 μm particle size, 15.-3.9 cm length) column used with a mobile phase (1.0 mL/min) which was completely deoxygenated by flowing argon gas. Two mobile phase systems were used, where system 1 (for the assay of n-butyldeoxoartemisinin (t_r = 5.53 min.) were consisted of 0.1 M ammonium acetate with 80 % acetonitrile in water. System 2, used for 12-carboxypropyldeoxoartemisinin (t_r = 2.62 min.) consisted of 0.1 M ammonium acetate with 30 % acetonitrile in water. Two internal standards were used: arteether (t_r = 3.45 min.) for HPLC system 1 and artelinic acid (t_r = 4.43 min.) for system 2.
10. Jung, M.; Lee, S., *Heterocycles*, **1997**, *45*, 1055.