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Short communication

A new derivative detected in accelerated ageing of artesunate-amodiaquine fixed dose combination tablets

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ABSTRACT

An unknown impurity detected in small amounts during the heat treatment of artesunate-amodiaquine bilayer tablets was purified by semipreparative HPLC and identified by MS and NMR as the tetrahydrofuranyl acetate-rearranged derivative of anhydrodihydroartemisinin. When anhydrodihydroartemisinin was treated with a Fe^{II} salt in acetonitrile–water solution, the same product was generated, together with an isomeric 2-deoxy-4α-hydroxy-anhydrodihydroartemisinin derivative, as expected from the usual homolytic radical opening of the endoperoxide bond previously described for other artemisinin derivatives.

1. Introduction

Artesunate-amodiaquine fixed dose combination is a new antimalarial drug formulation associating in the same galenic form (bilayer tablet) two active ingredients: artesunate and amodiaquine [1]. Artesunate (**1**, Fig. 1) is the hemisuccinic ester of dihydroartemisinin (**2**), a molecule resulting from the reduction of artemisinin (**3**), itself extracted from *Artemisia annua* L., a herbal plant utilized in traditional Chinese medicine (*Qinghao*) [2].

In artesunate-amodiaquine fixed dose combination bilayer tablets issued from pilot lots manufactured by Sanofi, held in ICH zone IV storage conditions during 6 months, 40 °C and 75% RH, several products deriving from artesunate [4] have already been observed above the threshold of identification (>0.3%) in some lots, in addition to already known degradation products such as dihydroartemisinine (**2**), artemisinine (**3**), and 9,10-anhydrodihydroartemisinin (**4**). In previous studies, one of these HPLC-detected impurity named A has tentatively been identified as a mixture of 2-deoxy-dehydrodihydroartemisinin (MW = 250), and a cyclohexanone derived product (MW = 220) [Sanofi, private communication]. Another HPLC-detected impurity named B was present, generally below the threshold of identification (≤0.3%) and could not be isolated in sufficient amount to be identified. However this impurity was established as a relevant degradation product and identification was considered as useful.

During complementary stability studies carried out at higher temperatures (50, 60 and 70 °C/75% RH), beside several other unknown impurities, several of the former degradation products were formed in higher amounts (Fig. 2), offering an opportunity for isolation and identification of impurity B. Table 1 summarizes the temperature stability results based on the studies performed by Bertin-Pharma (Private communication, Artigues, May 2011).

The aim of the present work was to isolate and chemically identify one of these impurities, sometimes appearing after one to several weeks of heating at 50 °C or higher in a 0.3–1% range, and designated as "impurity B"

2. Experimental

2.1. Chemicals

Artesunate and dihydroartemisinin were obtained from Sanofi. All other reagents and solvents were of the highest purity available. Aged artesunate-amodiaquine tablets have been obtained from Bertin-Pharma Laboratories (Artigues, France)

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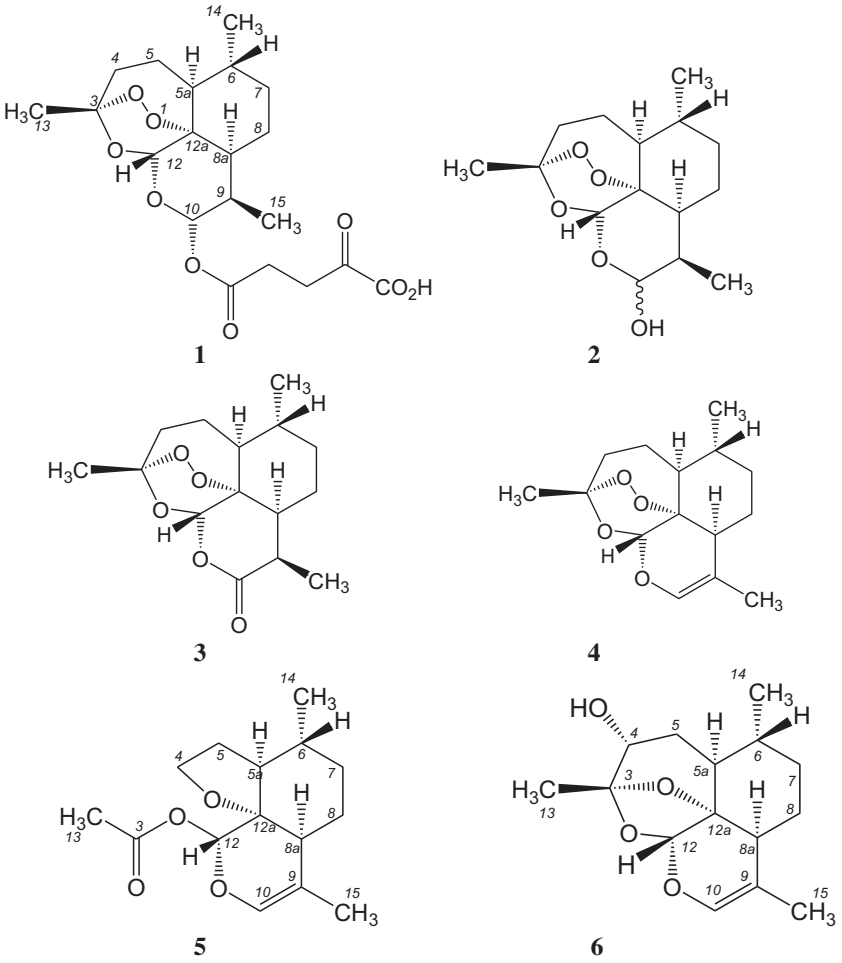

Fig. 1. Artesunate (1) and related compounds.

2.2. Materials and methods

^{1}H, ^{13}C, and 2D NMR experiments were acquired at 300 K (unless indicated) on a Bruker Avance 500 MHz spectrometer in $CDCl_3$ using a 5 mm probe with the usual 1D and 2D sequences (COSY, HSQC, HMBC, TOCSY, NOESY). The ^{1}H and ^{13}C chemical shift values were reported on the δ scale in ppm relative to $CDCl_3$ (7.28 ppm and 77.10 ppm respectively). All spectra were recorded without sample spinning.

Optical rotations were recorded at 589 nm using a PerkinElmer 341 polarimeter using a 10 cm/1 ml cell.

The separation and quantification of compounds was carried out using an Agilent model 1100 liquid chromatograph interfaced with an Agilent DAD detector (200–400 nm) and MS detection.

Analytical HPLC separations on tablet extracts were performed on YMC ODS-AQ Column (250 × 4.6 mm) at 40 °C in isocratic mode, solvent 10 mM phosphate buffer pH 3.8- acetonitrile (47:53), 0.8 ml/min and detection at 205 nm (Fig. 2). Coupled

Table 1
Evolution of degradation products in amodiaquine-artesunate combination tablets at different temperatures (75% RH). Percent amounts of impurities and unknown products based on peak areas (relative to total products).

	Artemisinin (**3**) % area	Dihydro-artemisinin (**2**) % area	Glycan (**4**) % area	Impurities		Unknown products % area
				A % area	B % area	
Initial	<*ldc*[a]	0.075 ± 0.005	<*ldc*	<*ldc*	0.10 ± 0.05	<*ldc*
50 °C						
7 days	<*ldc*	0.095 ± 0.005	0.020 ± 0.005	0.06 ± 0.02	0.245 ± 0.005	0.30 ± 0.05
14 days	<*ldc*	0.175 ± 0.005	<*ldc*	0.10 ± 0.05	0.33 ± 0.03	0.27
30 days	0.07 ± 0.01	0.245 ± 0.015	<*ldc*	0.12 ± 0.04	0.40 ± 0.05	0.15 ± 0.01
60 °C						
7 days	<*ldc*	0.17 ± 0.02	<*ldc*	0.14 ± 0.03	0.40 ± 0.04	0.40 ± 0.02
14 days	0.08 ± 0.08	0.51	0.03 ± 0.03	0.35	0.60 ± 0.015	0.35 ± 0.05
30 days	0.30 ± 0.15	1.3 ± 0.2	0.125 ± 0.015	1.13 ± 0.12	0.81 ± 0.08	0.39 ± 0.01
70 °C						
7 days	0.32 ± 0.01	0.51 ± 0.06	0.17 ± 0.02	0.95 ± 0.01	0.725 ± 0.025	1.40 ± 0.1

[a] *ldc*: lower detectable concentration.