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Synthesis and *in vivo* Antimalarial Activity of 12α-Trifluoromethyl-Hydroartemisinin

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Summary: 12α-Trifluoromethyl-hydroartemisinin **4** was selectively prepared from the reaction of artemisinin **1** with $TMSCF_3$ in good yields. The α configuration of the CF_3 substituent was established by NMR experiments. Compound **4** exhibits a better antimalarial activity against *Plasmodium vinckei* in mice, than artemisinin. Copyright © 1996 Elsevier Science Ltd

Artemisinin **1**, an active principle of the Chinese medicinal plant *Artemisia annua* Linn, is used in the treatment of patients with cerebral malaria as well as those infected with drug-resistant strains of *Plasmodium falciparum*.[1,2] Unfortunately, its poor solubility in both water and oil has hampered its further development. The search for more potent analogs of artemisinin with better therapeutic index and bioavailability, and good solubility has become the prime target of many laboratories in the world.[3] The overwhelming majority of artemisinin derivatives synthesized to date are esters, ethers, carbonates or urethane derivatives of the hydroxyl group of dihydroartemisinin.[3] These compounds, especially ether derivatives **2** such as β-arteether and β-artemether had generally higher potency[2] than artemisinin **1** but had short half-lives in plasma.[4] These other derivatives are supposed to be pro-drugs of dihydroartemisinin **3**, since they are readily converted into **3** by enzymes present in rat liver.[4,5]

The substitution of hydrogen atoms by fluorine in bioactive compounds can induce great changes in molecular properties, like those of solubility and lipophilicity, and can prevent metabolic oxidations such as hydroxylation.[6] Thus, several mono- and polyfluorinated artemisinin derivatives were prepared[7,8] by modification of the ether group of the dihydroartemisinin ether **2**. The *in vitro* antimalarial activities of these known fluorinated derivatives are in most cases equal to or greater than their non-fluorinated analogs or precursors.[8]

In other respects, only few examples of 12β-alkyldeoxyartemisinin derivatives are reported in the literature.[9,10] These compounds are prepared from artemisinin by replacing a carbon-oxygen bond at C-12 position by a carbon-carbon bond. However, fluorinated artemisinin derivatives in

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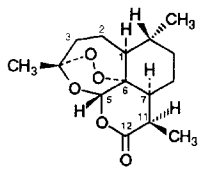
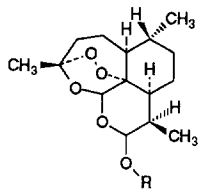
which a fluoroalkyl group is directly attached to the C-12 carbon have never been synthesized. In this paper, we report the stereoselective synthesis and the antimalarial activity of the 12α-trifluoromethyl-hydroartemisinin **4**. The presence of the electron withdrawing trifluoromethyl group the C-12 position could increase the stability of the hemiketal towards ring opening and might, therefore, increase the plasma half-life of the compound.

The usefulness of $TMSCF_3$ (the Ruppert's reagent),[11,12] as a CF_3 anion equivalent for nucleophilic trifluoromethylation of carbonyl derivatives was largely illustrated in the preparation of bioactive compounds.[13] Although the carbonyl groups of esters are unreactive towards $TMSCF_3$, γ- and δ-lactone are known to react and give trifluoromethyl adducts.[14]



1



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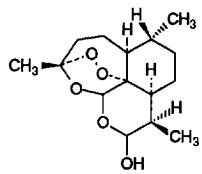

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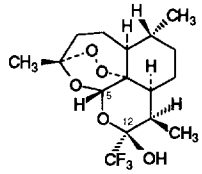


4

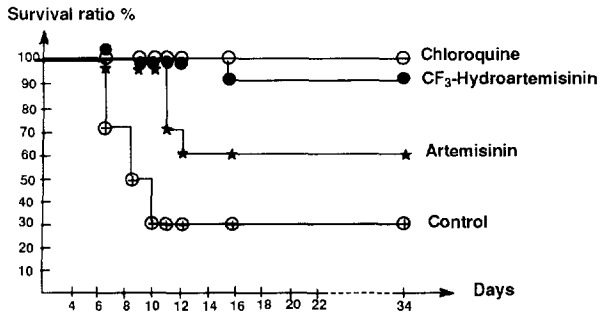
Artemisinin **1** was treated with 1.1 equivalent of $TMSCF_3$ in the presence of 1.1 equivalent. of a solution of tetrabutylammonium fluoride in THF at room temperature. Surprisingly, no reaction occurred with these standard conditions. However 12α-trifluoromethyl-hydroartemesinin **4** was obtained in good yield (87 %) by performing the reaction using 1.1 equivalent of trihydrate tetrabutylammonium fluoride (TBAF, $3H_2O$) as catalyst at room temperature for 25 h. The trifluoromethylation reaction proceeded with high selectivity: one stereoisomer was obtained exclusively.[15] The usual stereochemical assignment of C-12, based on the coupling constant $J_{11,12}$ is not possible in this case. Its configuration was deduced from other NMR experiments: the presence of a homonuclear NOE between protons O-H-12 and axial H-5 (3 %) indicated their spatial proximity and hence, the axial β-position of the hydroxyl group. Furthermore in hetero $\{^{19}F\}$,H, NOE experiments an enhancement was observed for both H-11 (6 %) and CH_3-11 (3 %) signals, confirming the equatorial position of the CF_3 group, in *trans* relationship with the CH_3 group. From this data stereochemistry at the carbon C-12 is assigned to be *epi* (β-OH).

The antimalarial activity of the compound **4** was studied in mice infected with *Plasmodium vinckei petteri* (strain 279 BY),[16] known for its usual sensitivity to antimalarial drugs and for its synchron development in mice.[17] Effect of treatments was observed by life span prolongation of mice and are represented in the fig. 1. The control group showed 70 % mortality at day 10. The group treated with artemisinin exhibited 40 % of mortality, at day 11. Only one mouse among mice treated with compound **4** died and much later than mice of other groups (day 16).[18]

The lower death rate as well the delayed start of mortality for the group treated with artemisinin illustrates the activity of the drug. However its short plasmatic half-life entailed a redevelopment of parasitism at day 11.[19] The interesting activity of compound **4**, higher than that of artemisinin, could probably be the result of a better pharmacokinetic profile. Additional biological

data regarding compound **4**, such as course of parasitemia and identification of the sensitive stage, have to be collected now.

Figure 1: Antimalarial activity of compound **4**.



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15. A complete assignment of the ^{1}H and ^{13}C chemical shifts in **4** was made by HMQC, HMBC and COSY experiments. **12α-Trifluoromethyl-hydroartemisinin**: mp 117-118 °C (AcOEt-Petrol Eth.); $[\alpha]^{25}_{D}$ + 69.5 ° (c = 2, CH_2Cl_2); ^{1}H NMR ($CDCl_3$) δ 0.94 (m, 1H, H-9_{ax}), 0.97 (d, $J_{H\text{-}14,H10}$= 6.1 Hz, 3H, Me-14), 1.07 (d, $J_{H13\text{-}H11}$= 7.2 Hz, 3H, Me-13), 1.30 (m, 1H, H-10), 1.32 (m, 1H, H-1), 1.42 (s, 3H, Me-15), 1.49 (dddd, $J_{H\text{-}2ax,H\text{-}2eq}$= 13.7 Hz, $J_{H\text{-}2ax,H\text{-}3ax}$= 13.3 Hz, $J_{H\text{-}2ax,H1ax}$= 11.3 Hz, $J_{H\text{-}2ax,H3eq}$= 13.7 Hz, 1H, H-2_{ax}), 1.55 (dt, $J_{H\text{-}7,H\text{-}8ax}$= 12.6 Hz, $J_{H\text{-}7,H\text{-}8eq}$= $J_{H\text{-}7,H\text{-}11}$= 5.3 Hz, 1H, H-7), 1.7 (dq, $J_{H\text{-}9eq,H\text{-}9ax}$= 13.3 Hz, $J_{H\text{-}9eq,H\text{-}8ax}$= $J_{H\text{-}9eq,H\text{-}8eq}$= $J_{H\text{-}9eq,H\text{-}10}$= 3.4 Hz, 1H, H-$9_{eq}$), 1.81 (dddd, $J_{H\text{-}8ax,H\text{-}8eq}$= 14.1 Hz, $J_{H\text{-}8ax,H\text{-}9ax}$= 13.3 Hz, $J_{H\text{-}8ax,H\text{-}7}$= 12.8 Hz, $J_{H\text{-}8ax,H\text{-}9eq}$= 3.5 Hz, 1H, H-$8_{ax}$), 1.85 (m, 1H, H-$8_{eq}$), 1.91 (dddd, $J_{H\text{-}2eq,H\text{-}2ax}$= 13.7 Hz, $J_{H\text{-}2eq,H\text{-}1ax}$= 6.6 Hz, $J_{H\text{-}2eq,H\text{-}3ax}$= 4.07 Hz, $J_{H\text{-}2eq,H\text{-}3eq}$= 2.9 Hz, 1H, H-$2_{eq}$), 2.05 (ddd, $J_{H\text{-}3eq,H\text{-}3ax}$= 14.6 Hz, $J_{H\text{-}3eq,H\text{-}2eq}$= 5.0 Hz, $J_{H\text{-}3eq,H\text{-}2ax}$= 2.9 Hz, 1H, H-$3_{eq}$), 2.4 (ddd, $J_{H\text{-}3ax,H\text{-}3eq}$= 14.6 Hz, $J_{H\text{-}3ax,H\text{-}2ax}$= 13.3 Hz, $J_{H\text{-}3ax,H\text{-}2eq}$= 4.07 Hz, 1H, H-$3_{ax}$), 2.77 (d, $J_{OH,H\text{-}11}$= 2.2 Hz, 1H, OH), 2.84 (qdd, $J_{H\text{-}11ax,H\text{-}13}$= 7.15 Hz, $J_{H\text{-}11ax,H\text{-}7}$= 5.3 Hz, $J_{H\text{-}11ax,OH}$= 2.2 Hz, 1H, H-11_{ax}), 5.55 (s, 1H, H-5); ^{13}C NMR ($CDCl_3$) δ 12.4 (C-13), 20.1 (C-14), 23.1 (C-8), 24.6 (C-2), 25.5 (C-15), 28.2 (C-11), 34.4 (C-9), 36.05 (C-3), 37.4 (C-10), 45.8 (C-7), 51.74 (C-1), 79.9 (C-6), 88.8 (C-5), 96.9 (q, $^{2}J_{C\text{-}F}$= 31.0 Hz, C-12), 104.37 (C-4), 122.6 (q, $^{1}J_{C\text{-}F}$= 282 Hz, CF_3); ^{19}F NMR ($CDCl_3$, $CFCl_3$) δ -84.5 (q, $J_{H\text{-}F}$= 1.5 Hz, CF_3); Anal. Calc. for $C_{16}H_{23}F_3O_5$: C, 54.54 %, H, 6.58; Found: C, 54.55 %; H, 6.59.
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18. Treatments (artemisinin, chloroquine sulfate, compound **4**) were performed during 4 days, one day after the infection, by intraperitoneal route. The drugs were given once a day at 0.0355 mmole. Kg^{-1} concentration as a suspension, in an aqueous solution of carboxymethyl cellulose (1 %). The control group received only carboxymethyl cellulose excipient at 0.1 %.
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