Synthesis of Steroidal 1,2,4-Trioxane as Potential Antimalarial Agent

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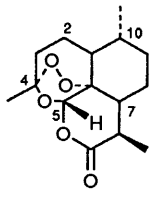
In order to improve the affinity for plasmodial membranes, a new qinghaosu analogue with a combination of the crucial 1,2,4-trioxane moiety and the lipophilic cholesterol skeleton has been synthesized.

Qinghaosu (artemisinin) **1**, extracted from the Chinese herb qinghaosu (*Artemisia annua* L.) is an effective antimalarial drug especially against *P. falciparum* strains responsible for the most severe cases of the disease.[1] Structure–activity studies have



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shown that the 1,2,4-trioxane ring system is a structural requirement for significant antimalarial activity. Since the structure of qinghaosu was identified, a number of its derivatives and analogues containing the 1,2,4-trioxane structure have been synthesized as potential antimalarial agents.[2] So far, however, no qinghaosu analogues have been designed on the basis of the mode of action of qinghaosu on the malarial parasite. It has been proposed that qinghaosu acts primarily on membrane integrity and that its high affinity for plasmodial membranes may be because of its similarity to cholesterol, a compound of which these membranes contain little.[3] On the basis of this reasoning we decided to combine in one compound the crucial 1,2,4-trioxane structure with that of cholesterol in order to see how the introduction of the lipophilic steroidal moiety would affect the antimalarial activity. We expected that the presence of the cholesterol structures would increase the affinity of the compound for plasmodial membranes. Herein, we report the synthesis of this new potential antimalarial agent (see Scheme 1).

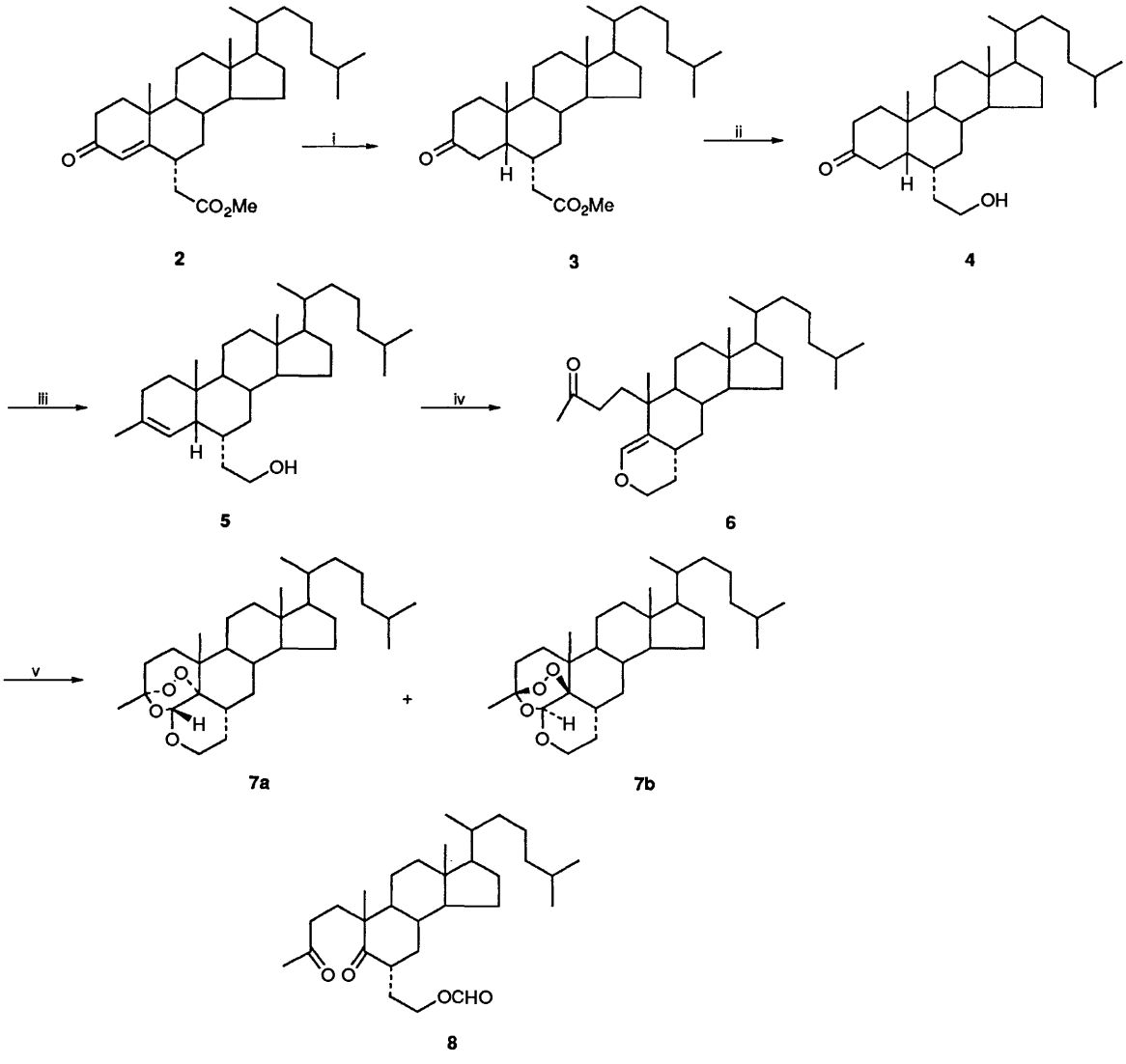
Since, from our earlier synthesis of deoxoqinghaosu, we knew that the 1,2,4-trioxane structure is accessible by photooxidation of a suitable cyclic enol ether, methyl 3-oxocholes-4-en-6β-yl acetate **2** was chosen as our starting material.[4] Hydrogenation of **2** in pyridine with Pd catalyst afforded **3** (80%) with an A/B *cis* configuration. Ketalization of **3** with $CH(OMe)_3$–TsOH, then reduction with lithium aluminium hydride, followed by hydrolysis in the work-up afforded **4** (82%). Treatment of **4** with methylmagnesium iodide provided a mixture of the 3α- and 3β-alcohols which were dehydrated in acetonitrile by $CuSO_4$–SiO_2 to give **5** (89%). Ozonolysis of **5** at −78 °C followed by treatment with *p*-TsOH–toluene yielded the enol ether **6** (36%) after flash column chromatography. Photooxygenation of a CH_2Cl_2 solution of **6** in the presence of Methylene Blue at −78 °C under a bubbling stream of oxygen provided two isomers **7a**† (16%) and **7b**† (20%), and also a by-product **8** (9%). The relative configuration at the new chiral centres C-4, - 5, -6 was unambiguously determined as depicted in **7a** and **7b** by utilization of two-dimensional NOE (NOESY) techniques, respectively. In preliminary testing, compounds **7a** and **7b** were found to be more effective than qinghaosu *in vivo* against *P. berghei* malaria. Further testing of the pharmacological activity of these two compounds is in progress.

Experimental †

Preparation of the Cyclic Enol Ether **6**.—Ozone was bubbled into a solution of compound **5** (0.8 g, 1.9 mmol) in a 1:1 mixture of methanol and methylene dichloride (10 cm^3) at −78 °C until the colour of the reaction mixture turned to blue. After the reaction mixture had been purged of ozone with a stream of nitrogen, zinc dust (300 mg) and acetic acid (0.5 cm^3) were added to it and stirring was continued for 3 h. The solid was filtered off and the filtrate was extracted with ether. The extract was dried (Na_2SO_4) and evaporated and a solution of the residue and PPTS (20 mg) in toluene (50 cm^3) was refluxed for 2 h. Work-up and silica gel column chromatography gave the desired cyclic enol ether **5** (0.30 g, 36%); $[\alpha]_D^{20}$ +20.2‡ (*c* 0.70, chloroform); δ_H(200 MHz, $CDCl_3$) 6.11 (s, 1 H, vinyl proton), 3.70–3.90 (m, 2 H, OCH_2), 2.14 (s, 3 H, Me), 1.02 (s, 3 H, Me), 0.92 (d, 3 H, Me), 0.85 (d, 6 H, 2 Me) and 0.68 (s, 3 H, Me); ν/cm^{-1} 1720, 1643 and 1150–1170; *m/z* 442 (M^+), 427 (M^+ − Me) and 371 (M^+ − $MeCOCH_2Me$) [Found: (HRMS): 442.3801 ($C_{30}H_{50}O_2$). Calc., 442.3811].

Photooxidation of the Cyclic Enol Ether **6**.—Oxygen was passed into a solution of the cyclic enol ether **6** (200 mg, 0.45 mmol) and Methylene Blue (5 mg) in methylene dichloride (50 cm^3) at −78 °C, whilst it was irradiated with a sodium lamp for 1 h. To the reaction mixture was added TMSOTf (10 mm^3) under nitrogen. After addition of triethylamine (1.0 cm^3), the reaction mixture was warmed to room temperature, and concentrated under reduced pressure. Silica gel column chromatography of the oily residue gave **7a** (34 mg, 16%), **7b**† (43 mg, 20%) and a by-product **8** (19 mg, 9%). Selected data for **7a**: m.p. 59–60 °C; $[\alpha]_D^{20}$ +54.0 (*c* 0.38, chloroform); δ_H(600 MHz, $CDCl_3$) 5.02 (s, 1 H, 4-H), 4.13 (d, *J* 12, 1 H, 29α-H), 3.51 (dt, *J* 12, 1.5, 29β-H), 1.42 (s, 3 H, Me), 1.02 (s, 3 H, Me) and 0.66 (s, 3 H, Me); δ_C(600 MHz, $CDCl_3$) 103.57, 94.28, 81.31, 65.17, 56.17, 55.78, 42.69, 42.56, 42.14, 40.06, 39.47, 36.80, 36.10, 35.82, 34.73, 34.41, 31.31, 29.70, 28.31, 27.98, 27.71, 25.77, 23.95, 23.89, 22.80, 22.53, 20.93, 20.73, 18.59 and 12.15; ν/cm^{-1} 1100, 1080, 880 and 840; *m/z* 474 (M^+), 459 (M^+ − CH_3) [Found (HRMS): 474.3646 ($C_{30}H_{50}O_4$). Calc., 474.3709]. For **8b**: m.p. 97–98 °C; $[\alpha]_D^{20}$ −32.3 (*c* 0.3, chloroform); δ_H(600 MHz, $CDCl_3$) 5.35 (s, 1 H, 4-H), 3.94 (dd, *J* 4.8, 12.6, 1 H, 29β-H), 3.81 (t, *J* 12.6, 1 H, 29α-H), 1.42 (s, 3 H, Me), 0.91 (s, 3 H, Me), 0.66 (s, 3 H, Me); δ_C(600 MHz, $CDCl_3$) 103.08, 92.13, 81.69, 60.94, 56.15, 56.06, 51.28, 43.97, 42.44,

† Reaction conditions have not been optimized.
‡ [α_D] Values in units of 10^{-1} deg cm^2 g^{-1}.



Scheme 1 *Reagents and conditions:* i, H_2/Pd; ii, $CH(OMe_3)_3$, TsOH; $LiAlH_4$ Et_2O; $H_3{}^+O$, silica gel; iii, MeMgI, $CuSO_4$-silica gel; iv, O_3, Zn-HOAC; PPTS, Toluene; v, $^1O_2$, Methylene Blue, −78 °C, then TMSOTf

39.80, 39.51, 36.16, 35.74, 35.16, 34.64, 34.14, 33.65, 33.17, 28.18, 28.00, 26.80, 26.18, 24.11, 23.82, 22.78, 22.54, 21.78, 18.63, 14.31 and 11.97; ν/cm^{-1} 1110, 1070, 880 and 830; m/z 475 ($M^+ + 1$), 459 ($M^+ - CH_3$) and 442 ($M^+ - O_2$) [Found (HRMS): 474.3731 ($C_{30}H_{50}O_4$). Calc., 474.3709]. For **8**: $[\alpha]_D^{20}$ +15.1 (*c* 0.69, chloroform), δ_H(200 MHz, $CDCl_3$) 8.00 (s, 1 H, formate), 4.17 (t, 2 H, OCH_2), 2.13 (s, 3 H, Me), 1.10 (s, 3 H, Me), 0.91 (d, 3 H, Me), 0.88 (d, 6 H, 2 Me) and 0.72 (s, 3 H, Me); ν/cm^{-1} 1720 and 1160; m/z 475 ($M^+ + 1$), 459 ($M^+ - Me$) and 404 ($M^+ + 1 - CH_2CH_2COMe$).

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References

1 Qinghaosu Antimalarial Coordinating Research Group, *Chin. Med. J.*, 1979, 92; for comprehensive review, see: D. L. Klayman, *Science*, 1985, **228**, 1049; X. D. Luo, C. C. Shen, *Med. Res. Rev.*, 1987, **7**, 29.

2 S. S. Zaman and R. P. Sharma, *Heterocycles*, 1991, **32**, 1593; A. R. Butler and Y.-L. Wu, *Chem. Soc. Rev.*, 1992, 85.

3 D. S. Ellis, Z. L. Li, H. M. Gu, W. Peters and B. L. Robinson, *Ann. Tropical Med. Parasit.*, 1985, **79**, 367.

4 (*a*) B. Decouvelaere, S. Julia, C. Neuville, S. R. Pathak and G. H. Whitham, *Bull. Soc. Chim. France*, 1965, 227; (b) D. J. Collins, C. M. Horn and V. J. Welker, *Aust. J. Chem.*, 1976, **29**, 2077; (*c*) Y.-J. Rong, M. Cherest, B. Ye and Y.-L. Wu, unpublished.

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