Pergamon

Bioorganic & Medicinal Chemistry Letters 11 (2001) 833–835

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Diaryl Ester Prodrugs of FR900098 with Improved In Vivo Antimalarial Activity

Armin Reichenberg,[a] Jochen Wiesner,[a,b] Claus Weidemeyer,[a] Erhard Dreiseidler,[a] Silke Sanderbrand,[a,b] Boran Altincicek,[a,b] Ewald Beck,[b] Martin Schlitzer[c] and Hassan Jomaa[a,*]

[a]*Jomaa Pharmaka GmbH, Frankfurter Straße 50, D-35392 Gießen, Germany*
[b]*Biochemisches Institut der Universitätsklinik Gießen, Friedrichstraße 24, D-35392 Gießen, Germany*
[c]*Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, D-35032 Marburg, Germany*

Received 27 November 2000; revised 5 January 2001; accepted 31 January 2001

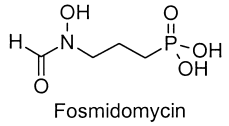
Abstract—The fosmidomycin derivative FR900098 represents an inhibitor of the 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase with potent antimalarial activity. Prodrugs of FR900098 with increased activity after oral administration were obtained by chemical modification of the phosphonate moiety to yield phosphodiaryl esters. One diaryl ester prodrug demonstrated efficacy in mice infected with the rodent malaria parasite *Plasmodium vinckei* comparable to ip drug administration.

Malaria is one of the most serious tropical diseases causing between 1.5 and 2.7 million fatal cases per year. Nearly all fatal cases are caused by *Plasmodium falciparum*, the causative agent of *Malaria tropica*. This is largely due to the widespread emergence of *P. falciparum* strains that are resistant to presently available drugs. As a result, there is an urgent need for new efficient antimalarial agents.[1]

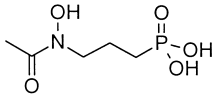
In previous studies we have demonstrated that inhibitors of 1-deoxy-D-xylulose 5-phosphate (DOXP) reductoisomerase exhibit considerable antimalarial activity.[2] The DOXP reductoisomerase converts DOXP into 2-C-methyl-D-erythritol 5-phosphate (MEP) and is an essential enzyme of the MEP pathway providing isopentenyl diphosphate (IPP) for isoprenoid biosynthesis. In addition to malaria parasites, the MEP pathway is present in algae, higher plants, and most bacteria.[3] In mammals, however, IPP is synthesised by the mevalonate pathway while the MEP pathway is absent.

Fosmidomycin and its derivative FR900098 (Fig. 1) are strong and selective inhibitors of the DOXP reductoisomerase[4] and were found to inhibit the growth of multidrug resistant *P. falciparum* strains in vitro.[2] In a murine malaria model, mice infected with *P. vinckei* were cured by both drugs administrated orally (po) or by intraperitoneal (ip) injection.[2] However, the efficacy was lower upon oral administration presumably due to a poor rate of absorption.[5] To increase bioavailability via oral administration, the charged phosphonate moiety of FR900098 was transformed into phosphodiaryl esters expected to be hydrolysed by non-specific plasma esterases.[6] FR900098 was chosen as the lead molecule instead of fosmidomycin since it possesses approximately 2-fold greater antimalarial activity.[2]

Synthesis of the prodrugs **7a**–**c** was accomplished following the sequence shown in Scheme 1.[7] First, Arbusov reaction of triethylphosphite with 1,3-dibromopropane



Fosmidomycin

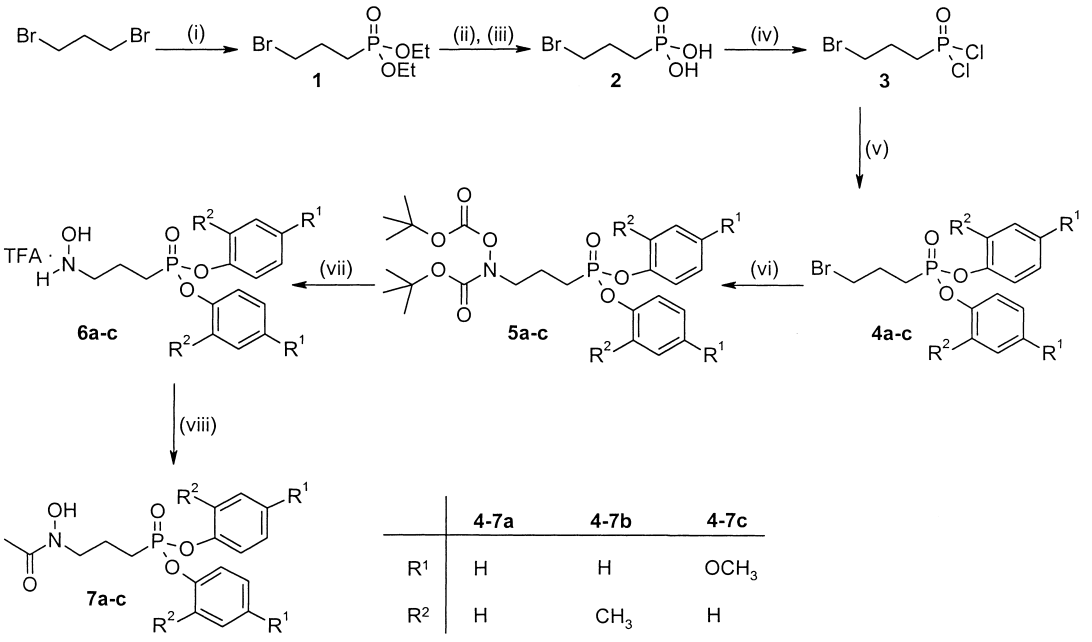


FR900098

Figure 1. Structures of fosmidomycin and FR900098.

*Corresponding author. Tel.: +49-641-9947528; fax: +49-641-9947529; e-mail: kontakt@jomaa-pharmaka.de

0960-894X/01/$ - see front matter
PII: S0960-894X(01)00075-0



Scheme 1. Reagents and conditions: (i) 0.2 equiv triethylphosphite, 30 min, 160 °C; (ii) trimethylbromosilane, 30 min, 0 °C and overnight, rt; (iii) H_2O; (iv) PCl_5, $CHCl_3$, 2.5 h, 70 °C; (v) phenols (see table), pyridin, 1 h, 0 °C and overnight, rt; (vi) BocONHBoc, NaH, DMF, overnight, rt; (vii) trifluoroacetic acid, CH_2Cl_2, 6 h, 0 °C; (viii) triethylamine, acetylchloride, ether, 30 min, 0 °C and 2 h, rt.

provided diethylphosphonate **1**, which was cleaved with trimethylbromosilane to the corresponding phosphonic acid **2**. Chlorination of **2** with PCl_5 and quenching of the intermediate phosphonodichloride **3** with the appropriate phenol resulted in formation of the diphenylphosphonoesters **4a**–**c**. Subsequently, condensation of the bromides **4a**–**c** with the sodium salt of *N*,*O*-bis-(*tert*-Boc)-hydroxylamine gave the fully protected precursers **5a**–**c**. Deprotection with trifluoroacetic acid to trifluoroacetates **6a**–**c** and acylation with acetylchloride led to the desired prodrugs **7a**–**c**.

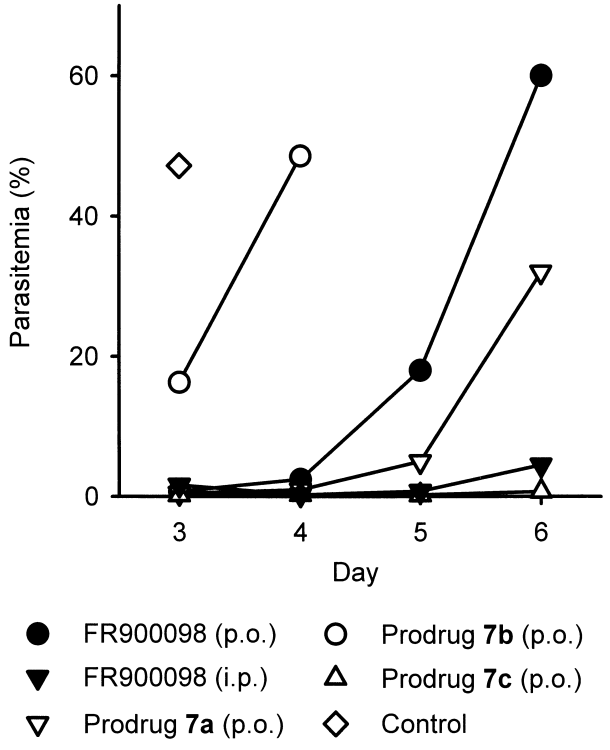


Figure 2. Antimalarial efficacy of FR900098 po and ip and of the prodrugs **7a**–**c**.

The antimalarial activity of the prodrugs was tested in *P. vinckei*-infected mice. On day 0, mice were inoculated by ip injection of 5×10^7 infected erythrocytes from a donor mouse. On days 1 and 2, the mice were treated orally with sub-therapeutic dosages (0.16 mmol/kg) of FR900098 and diaryl ester prodrugs **7a**–**c**.[8] For comparison, one group of mice was treated with an equivalent dosage of FR900098 by ip injection. On the following days, parasitemia was monitored to assess the efficacy of the compounds. In all treated animals, the parasitemia was markedly reduced compared to untreated control animals (Fig. 2). The unsubstituted diphenyl ester **7a** was significantly more active than FR900098 (po). In contrast, the bis-(2-methyl-phenyl) ester **7b** was significantly less active than FR900098 (po). We interpret these results as indicative of an increased hydrolytic stability of the ester due to steric hinderance by the 2-methyl substituent. The most successful derivative of this series was the bis-(4-methoxyphenyl) ester **7c** which was not only much more efficient than FR900098 (po) but proved to be equivalent to FR900098 administrated by ip route.

In summary, prodrugs of FR900098 with significantly increased antimalarial activity were obtained by masking the polar phosphonate moiety as diaryl esters. Establishing detailed SARs with regard to the substitution pattern of the aryl residues remains to be the subject of further investigation. In addition, the prodrugs will be evaluated in non-human primates which possess a more human-like metabolism and can be infected with human malaria parasites.

Acknowledgements

The authors would like to thank Dajana Henschker and Rainer Schmidt for excellent technical assistance and the Institute of Organic Chemistry of the University of Gießen for generous support.

References and Notes

1. Wirth, D. F. *Ann. Rep. Med. Chem.* **1999**, *34*, 349.
2. Jomaa, H.; Wiesner, J.; Sanderbrand, S.; Altincicek, B.; Weidemeyer, C.; Hintz, M.; Turbachova, I.; Eberl, M.; Zeidler, J.; Lichtenthaler, H. K.; Soldati, D.; Beck, E. *Science* **1999**, *285*, 1573.
3. (a) Takahashi, S.; Kuzuyama, T.; Watanabe, H.; Seto, H. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 9879. (b) Rohmer, M. *Nat. Prod. Rep.* **1999**, *16*, 565.
4. (a) Kuzuyama, T.; Shimizu, T.; Takahashi, S.; Seto, H. *Tetrahedron Lett.* **1998**, *39*, 7913. (b) Zeidler, J.; Schwender, J.; Müller, C.; Wiesner, J.; Weidemeyer, C.; Beck, E.; Jomaa, H.; Lichtenthaler, H. K. *Z. Naturforsch.* **1998**, *53C*, 980.
5. Kuemmerle, H.-P.; Murakawa, T.; De Santis, F. *Chemioterapia* **1987**, *6*, 113.
6. Noren, J. O.; Helgstrand, E.; Johansson, N. G.; Misiorny, A.; Stening, G. *J. Med. Chem.* **1983**, *26*, 264.
7. (a) **1** to **5a–c** were synthesised according to established procedures: Hewitt, D. G.; Teese, M. W. *Aust. J. Chem.* **1984**, *37*, 205. (b) Tang, K.-C.; Coward, J. K. *J. Org. Chem.* **1983**, *48*, 5001. (c) Helferich, B.; Curtius, U. *Justus Liebigs Ann. Chem.* **1962**, *655*, 59. (d) Berlin, K. D.; Butler, G. B. *J. Org. Chem.* **1960**, *17*, 2006. (e) Satoh, Y.; Powers, C.; Toledo, L. M.; Kowalski, T. J.; Peters, P. A.; Kimble, E. F. *J. Med. Chem.* **1995**, *38*, 68. Typical experimental procedure for the following steps: 1.02 g (2 mmol) of compound **5a** were dissolved in 15 mL of methylenechloride, cooled to 0 °C and 8.55 g (75 mmol) of trifluoroacetic acid were added dropwise with stirring. Cooling was maintained for a further 6 h. After the solvent and excess trifluoroacetic acid were removed by rotary evaporation, the residue was co-evaporated three times with each 5 mL portion of chloroform and treated twice with 5 mL pentane. The pentane layer was removed and the residue dried in vacuo to leave 0.78 g (1.85 mmol, 93%) of the trifluoroacetate **6a**. After flushing the flask with argon, 20 mL of dry ether was added and cooled to 0 °C. 0.37 g (3.7 mmol) of triethylamine in 5 mL of dry ether were dropped into the flask via a syringe, followed immediately by dropwise addition of 0.15 g (1.85 mmol) of acetyl chloride in 5 mL of dry ether. After stirring for 30 min at 0 °C and 2 h at rt, 30 mL of 1 N HCl, 2×20 mL of brine and dried with Na_2SO_4. Filtration and evaporation of the solvent yield 0.50 g (71%) of the desired prodrug **7a**. ^{1}H NMR ($CDCl_3$/TMS_{int}): δ = 1.93–2.25 (m, 7H, CH_2P, $\underline{CH_2}CH_2P$, CH_3); 2.01 (s, 3H, CH_3); 3.62–3.84 (m, 2H, NCH_2); 7.02–7.22 and 7.23–7.40 (each m, 10H, $H_{arom.}$). ^{13}C NMR ($CDCl_3$/TMS_{int}): δ = 19.6 ($\underline{C}H_2CH_2P$); 20.3 (CH_3); 23.0 (d, J_{C-P} = 142.3 Hz, CH_2P); 47.6 (d, J_{C-P} = 14.9 Hz, NCH_2); 120.5 (d, J_{C-P} = 3.8 Hz, $C_{arom.}$); 125.5, 129.9 ($C_{arom.}$); 150.1 (d, J_{C-P} = 9.1 Hz, C_{ipso}); 172.9 (C=O).
8. FR900098 was dissolved in PBS at 3 mg/mL. Prodrugs **7a–c** were dissolved in DMSO at 150 mg/mL and further diluted in PBS. Parasitemia was monitored by Giemsa-stained blood smears. Mice were euthanised when parasitemia was exceeding app. 40%.