Available online at www.sciencedirect.com
ScienceDirect
Experimental Parasitology 118 (2008) 487–497
Experimental Parasitology
www.elsevier.com/locate/yexpr

Antimalarial quinolones: Synthesis, potency, and mechanistic studies

Rolf W. Winter [a,b], Jane X. Kelly [a,b], Martin J. Smilkstein [a], Rozalia Dodean [b], David Hinrichs [a], Michael K. Riscoe [a,b,*]

[a] *Biomedical Laboratory R&D Service, Mail Code: RD-33, Experimental Chemotherapy Laboratory, VA Medical Center, 3710 SW U.S. Veterans Hospital Road, Portland, OR 97239, USA*
[b] *Department of Chemistry, Portland State University, Portland, OR 97207, USA*

Received 31 January 2007; received in revised form 19 October 2007; accepted 30 October 2007
Available online 7 November 2007

Abstract

In the present article we examine the antiplasmodial activities of novel quinolone derivatives bearing extended alkyl or alkoxy side chains terminated by a trifluoromethyl group. In the series under investigation, the IC_{50} values ranged from 1.2 to ≈30 nM against chloroquine-sensitive and multidrug-resistant *Plasmodium falciparum* strains. Modest to significant cross-resistance was noted in evaluation of these haloalkyl- and haloalkoxyquinolones for activity against the atovaquone-resistant clinical isolate Tm90-C2B, indicating that a primary target for some of these compounds is the parasite cytochrome bc_1 complex. Additional evidence to support this biochemical mechanism includes the use of oxygen biosensor plate technology to show that the quinolone derivatives block oxygen consumption by parasitized red blood cells in a fashion similar to atovaquone in side-by-side experiments. Atovaquone is extremely potent and is the only drug in clinical use that targets the *Plasmodium* bc_1 complex, but rapid emergence of resistance to it in both mono- and combination therapy is evident and therefore additional drugs are needed to target the cytochrome bc_1 complex which are active against atovaquone-resistant parasites. Our study of a number of halogenated alkyl and alkoxy 4(1*H*)-quinolones highlights the potential for development of ''endochin-like quinolones'' (ELQ), bearing an extended trifluoroalkyl moiety at the 3-position, that exhibit selective antiplasmodial effects in the low nanomolar range and inhibitory activity against chloroquine and atovaquone-resistant parasites. Further studies of halogenated alkyl- and alkoxy-quinolones may lead to the development of safe and effective therapeutics for use in treatment or prevention of malaria and other parasitic diseases.
Published by Elsevier Inc.

Index Descriptors and Abbreviations: *Plasmodium falciparum*; Malaria; Quinolone; Acridone; Chemotherapy; Cytochrome *b*; Drug resistance; Mode of action; Synergy; Endochin

1. Introduction

Malaria is a potentially fatal tropical disease that is spread by mosquitoes from person to person and caused by protozoan parasites of the genus *Plasmodium*. *Plasmodium falciparum* causes cerebral malaria, the most severe form of the infection, and it is responsible for most of the estimated 1 million deaths attributed to malaria annually (Greenwood et al., 2005; Snow et al., 2005). Pregnant women and young children are most likely to succumb to cerebral malaria and it is in treatment of these two vulnerable populations that the antimalarial armamentarium offers few options (Ashley et al., 2006; Greenwood, 2006; Winstanley and Ward, 2006). The primary drugs for treatment of malaria have been the quinolines chloroquine (CQ), quinine (QN), and mefloquine and the antifolate combination of pyrimethamine and sulfadoxine. The usefulness of these drugs has greatly diminished due to the spread of drug-resistant strains of both *P. falciparum* and *Plasmodium vivax* (White, 2004; White et al., 1999)

* Corresponding author. Address: Biomedical Laboratory R&D Service, Mail Code: RD-33, Experimental Chemotherapy Laboratory, VA Medical Center, 3710 SW U.S. Veterans Hospital Road, Portland, OR 97239, USA. Fax: +1 503 7211804.
E-mail address: riscoem@ohsu.edu (M.K. Riscoe).

0014-4894/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.exppara.2007.10.016

throughout malarious regions of the world. Consequently, therapeutic options for treatment of malaria are dwindling and replacement drugs, the endoperoxide artesunate (Ashley and White, 2005; Edwards and Biagini, 2006; Meshnick, 2002) [including its formulation with other drugs, i.e., "artemisinin combination therapies"] and the atovaquone–proguanil combination known as Malarone® (Boggild et al., 2007), offer a rather thin wall of protection against a total collapse in malaria chemotherapy. As a result there is an urgent need for developing new and safe drugs for treating or preventing malaria.

We recently described the structural optimization of acridones using antimalarial activity and lymphocyte cytotoxicity as criteria in calculating a value known as the *in vitro therapeutic index* that was used to guide further improvements (Winter et al., 2006). A unique and defining feature of acridones optimized for antimalarial potency is the presence of an extended alkyl or alkoxy side chain that is terminated by one or more trifluoromethyl (CF_3) groups. While it may be assumed that such modification would enhance *in vivo* efficacy by blocking or hindering catabolism of the side chain by host P450 enzymes, the striking enhancement of *in vitro* potency suggests that the CF_3 groups are important in the antiplasmodial mode of action of the acridone constructs.

Our aim in the present study was to extend the structure-activity profiling of haloalkoxy/alkyl-bearing acridone molecules to include other aromatic ketones, in an effort to find a basic core structure that retains selective and potent activity against *Plasmodium* parasites. Herein we focus our studies on quinolones and present results on the antiplasmodial mechanism of these compounds.

2. Materials and methods

2.1. Materials

Unless otherwise stated all chemicals and reagents were from Sigma–Aldrich Chemical Company in St. Louis, MO (USA). SYBR Green I dye, used for determination of antiplasmodial IC_{50} values, was purchased from Molecular Probes (now Invitrogen, Eugene, OR, USA). RPMI-1640, gentamicin, and Albumax II® were purchased from Gibco (now Invitrogen, Carlsbad, CA, USA). Blood, a source for red cells used to culture the parasite, was purchased from Lampire Biologicals (Pipersville, PA, USA). White blood cells were removed by centrifugation followed by removal of the buffy coat and uppermost red blood cells. 96-well Oxygen biosensor plates were purchased from BD Biosciences (San Jose, CA, USA). 7-Methoxy-3-ethoxycarbonyl-4(1*H*)-quinolone and 7-methoxy-4(1*H*)-quinolone were prepared by the method of Lauer et al. (Lauer et al., 1946). 3-Heptyl-7-methoxy-2-methyl-4(1*H*)-quinolone (Endochin) and the hexyl congener, as well as 7-methoxy-2-methy1-4(1*H*)-quinolone, were synthesized by the method of Salzer et al. (Salzer et al., 1948). The synthesis of selected 4(1*H*)-quinolones is described below. Each of the quinolone derivatives was characterized by ^1H (500 MHz) NMR and high-resolution mass spectrometry to ensure identity and purity prior to use in this study.

2.2. General and specific approaches to synthesis of selected quinolone derivatives

2.2.1. Synthesis of 3-ethoxycarbonyl-7-(6,6,6-trifluorohexyloxy)-4(1H)-quinolone (compound 6)

The procedure [Gould–Jacobs quinoline reaction (Gould and Jacobs, 1939)] required initial synthesis of *m*-(6,6,6-trifluorohexyloxy)-aniline, obtained by heating *m*-nitrophenol (3.45 g), ethanol (50 ml), KOH (1.5 g), and $CF_3(CH_2)_5Br$ (Oakwood, Columbia, SC, USA) for 3 days, followed by cooling, filtering, and concentrating. The residue, an oil, had $M^+ = 277$ (26%), ^1H NMR spectrum (500 MHz, $CDCl_3$): ring system: $\delta_2 = 7.71$ ppm, t, $J = 2.3$ Hz, 1H; $\delta_4 = 7.81$, d-d-d, $J_{24} = 2.20$, $J_{45} = 8.24$, $J_{46} = 0.92$ Hz, 1H; $\delta_5 = 7.42$, t, $J = 2.2$ Hz, 1H; $\delta_6 = 7.22$, d-d-d, $J_{26} = 2.2$, $J_{56} = 8.2$, $J_{46} = 0.92$ Hz, 1H. Substituents: $\delta_{OCH_2} = 4.05$, t, $J = 6.26$ Hz, 3H; $\delta_{CH_2(\beta-\delta)} = 1.60$, m, 2H; 1.66, m, 2H; 1.86, m, 2H; $\delta_{CH_2CF_3} = 2.2$, m, 2H.

The crude *m*-(6,6,6-trifluorohexyloxy)-nitrobenzene (derived from the previous step) was heated with 100 ml of $HCl_{conc.}$ + 28.5 g $SnCl_2$ for 1 h; the color turned to pale yellow in about 30 min. After 1 h, the volume was reduced to about 50 ml by boiling off hydrochloric acid. After cooling, water (300 ml) and NaOH (40 g NaOH + 130 ml water) were added. Once the mixture had cooled again, it was extracted with ethyl acetate (3 × 150 ml) and the combined extracts were then washed with 50 ml of water. Drying (Na_2SO_4) and removal of solvent gave 5.31 g of crude *m*-(6,6,6-trifluorohexyloxy)-aniline as a pale yellow oil. A sample was purified by chromatography but the bulk was used in the condensation step with ethoxymethylenemalonic ester directly. $M^+ = 247$ (28%), ^1H NMR spectrum (500 MHz, $CDCl_3$): ring system: $\delta_2 = 6.22$ ppm, t, $J = 2.3$ Hz, 1H; $\delta_4 = 6.30$, d-d-d, $J_{24} = 2.3$, $J_{45} = 8.14$, $J_{46} = 0.76$ Hz, 1H; $\delta_5 = 6.27$, t, $J = 8.0$ Hz, 1H; $\delta_6 = 6.27$, d-d-d, $J_{26} = 2.15$, $J_{56} = 8.0$, $J_{46} = 0.9$ Hz, 1H. Substituents: $\delta_{OCH_2} = 4.14$, t, $J = 6.26$ Hz; 3H; $\delta_{CH_2(\beta-\delta)} = 1.53$, m, 2H; 1.62, m, 2H; 1.77, m, 2H; $\delta_{CH_2CF_3} = 2.1$, m, 2H; $\delta_{NH_2} = 3.65$, s, br, 2H.

The method employed in the condensation reaction to prepare 3-ethoxycarbonyl-7-(6,6,6-trifluorohexyloxy)-4(1*H*)-quinolone was based on the methods of Lauer et al. (Lauer et al., 1946) except that the *m*-alkoxy-aniline used was *m*-(6,6,6-trifluorohexyloxy)-aniline. Crude *m*-(6,6,6-trifluorohexyloxy)-aniline (5.12 g) and 4.65 g of diethyl ethoxymethylenemalonate (1 eq.) were mixed and set aside for 1 h; warming was noticed. Subsequently, the mixture was heated at 100 °C for 3 h, after which time all volatile components were removed under vacuum (0.02 atm., 70 °C), weight of residue = 8.43 g. This product showed the correct ^1H NMR spectrum for the expected product diethyl *m*-(6,6,6-trifluorohexyloxy-anilino)-methylene-malonate and was used without further characterization. It was dissolved in 15 ml of Dowtherm A (see note below)