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Exploring the scope of new arylamino alcohol derivatives: Synthesis, antimalarial evaluation, toxicological studies, and target exploration

Miguel Quiliano [a, b], Adela Mendoza [a], Kim Y. Fong [c], Adriana Pabón [d], Nathan E. Goldfarb [e, 1], Isabelle Fabing [f], Ariane Vettorazzi [g], Adela López de Cerain [g], Ben M. Dunn [e], Giovanny Garavito [h], David W. Wright [c], Eric Deharo [i], Silvia Pérez-Silanes [a, b], Ignacio Aldana [a, b], Silvia Galiano [a, b, *]

[a] Department of Organic and Pharmaceutical Chemistry, Faculty of Pharmacy and Nutrition, University of Navarra, Pamplona, 31008, Spain
[b] Institute of Tropical Health (ISTUN), University of Navarra, Pamplona, 31008, Spain
[c] Department of Chemistry, Vanderbilt University, Station B 351822, Nashville, TN 37235, USA
[d] Grupo Malaria, Universidad de Antioquía, Medellín, Colombia
[e] Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, FL, USA
[f] Laboratoire de Synthèse et Physicochimie de Molécules d'Intérêt Biologique SPCMIB – UMR5068, CNRS - Université Paul Sabatier, 118, route de Narbonne, 31062, Toulouse Cedex 09, France
[g] Department of Pharmacology and Toxicology, Faculty of Pharmacy and Nutrition, University of Navarra, Pamplona, 31008, Spain
[h] Universidad Nacional de Colombia, Sede Bogotá, Facultad de Ciencias, Departamento de Farmacia (DFUNC), Grupo de investigación FaMeTra (Farmacología de la Medicina tradicional y popular), Carrera 30 45-03, Bogotá D.C., Colombia
[i] UMR 152 PHARMA-DEV, Université Toulouse, IRD, UPS, 31062, Toulouse, France

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ABSTRACT

Synthesis of new 1-aryl-3-substituted propanol derivatives followed by structure-activity relationship, *in silico* drug-likeness, cytotoxicity, genotoxicity, *in silico* metabolism, *in silico* pharmacophore modeling, and *in vivo* studies led to the identification of compounds **22** and **23** with significant *in vitro* antiplasmodial activity against drug sensitive (D6 $IC_{50} \leq 0.19$ μM) and multidrug resistant (FCR-3 $IC_{50} \leq 0.40$ μM and C235 $IC_{50} \leq 0.28$ μM) strains of *Plasmodium falciparum*. Adequate selectivity index and absence of genotoxicity was also observed. Notably, compound **22** displays excellent parasitemia reduction (98 ± 1%), and complete cure with all treated mice surviving through the entire period with no signs of toxicity. One important factor is the agreement between *in vitro* potency and *in vivo* studies. Target exploration was performed; this chemotype series exhibits an alternative antimalarial mechanism.

1. Introduction

Malaria, a major tropical disease for which no long-term sustainable treatment is available, continues to affect large parts of the world. According to the latest World Malaria Report, 3.2 billion people in 96 countries are at risk of being infected. In 2015 alone, 214 million cases were reported globally resulting in an estimated 438,000 deaths mainly consisting of African children and pregnant women (WHO, 2015). This life-threatening disease is caused by *Plasmodium* species with *Plasmodium falciparum* (*P. falciparum*) being the most deadly. Currently, first-line therapy includes artemisinin-based combination therapies (ACT) (Delves et al., 2012; Wells et al., 2015); however, in recent years parasite resistance against artemisinin and its derivatives has emerged and spread along the Cambodia-Thailand border (Ashley et al., 2014; Tun et al., 2015). To address this challenge, new antimalarial entities are needed. Although recent efforts in antimalarial drug discovery have focused on new targets, nonclassical chemical scaffolds, and vaccines (Biamonte et al., 2013; Barnett and Guy, 2014; Wells et al., 2015), extensive studies on classical antimalarial chemotypes or drug repurposing are needed due to the high cost and time required

* Corresponding author. Departament of Organic and Pharmaceutical Chemistry, Faculty of Pharmacy and Nutrition, Institute of Tropical Health (ISTUN), University of Navarra, Irunlarrea 1, Pamplona, 31008, Navarra, Spain.
E-mail address: sgaliano@unav.es (S. Galiano).
[1] Present address: Department of Pharmaceutical and Biomedical Sciences, College of Pharmacy, California Health Sciences University, Clovis, CA, USA.

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in the drug discovery and development process (Andrews et al., 2014; Biamonte, 2014).

One classic antimalarial chemotype present in potent antimalarial drugs, including quinine (1), mefloquine (2), lumefantrine (3), and halofantrine (4), is the arylamino alcohol (ß-amino or γ-amino alcohol moiety). The structural requirements for antiplasmodial activity include the presence of an aromatic and amino alcohol portion linked by a carbon chain of two or three atoms in length (Bhattacharjee and Karle, 1996) (Fig. 1). Based on this antiplasmodial pharmacophore, antimalarial amino alcohols continue to attract the interest of various research groups due to their high biological activity and ADMET values. Representative studies of the ß-amino alcohol moiety include the works of Guy et al. who performed the optimization of propafenone analogues (Lowes et al., 2011, 2012) as a product of high throughput screening (Weisman et al., 2006), and Smith and Chibale et al. who developed totarol (Clarkson et al., 2003; Tacon et al., 2012) and chalcone (Hans et al., 2010), respectively, as natural product-like hybrid derivatives. In addition, mefloquine and 4-aminoquinoline derivatives are valid synthetic approaches explored by Milner et al. (2010) and Kobarfard et al. (2012), respectively, among other studies (Robin et al., 2007; D'hooghe et al., 2011). However, there are only a few examples of the γ-amino alcohol moiety reported in the last thirteen years (D'hooghe et al., 2009; Perez-Silanes et al., 2009; Mendoza et al., 2011; Quiliano and Aldana, 2013). D'hooghe et al. (2009) reported a wide range of antiplasmodial activity against the chloroquine sensitive *P. falciparum* strain D10; however, submicromolar values were not reached (6 μM ≤ IC_{50} ≤ 175 μM). Thus, further studies on the unexplored γ-moiety as a source of new antimalarial drugs are needed.

Our research on γ-amino alcohols explored the structure-activity relationship (SAR) of 1-aryl-3-susbtituted propanol derivatives (APD) with promising results (Perez-Silanes et al., 2009; Mendoza et al., 2011; Quiliano and Aldana, 2013). Initial SAR studies showed that all of the aryl-ketone derivatives were inactive against 3D7, NF54, and FCR-3 strains of *P. falciparum* (Perez-Silanes et al., 2009; Mendoza et al., 2011). In contrast, APD were active against 3D7 (0.19 ≤ IC_{50} ≤ 0.38 μM) (Perez-Silanes et al., 2009), NF54 (1.3 ≤ IC_{50} ≤ 8 μM) (Mendoza et al., 2011), and FCR-3 (0.5 ≤ IC_{50} ≤ 10 μM) (Mendoza et al., 2011) strains of *P. falciparum*. However, low parasitemia reduction (≤65%) was observed in the *in vivo Plasmodium berghei* (*P. berghei*) mouse model. Interestingly, linker reduction in one carbon atom between the alcohol and amine portion (γ-to ß-amino alcohol) in APD reduced the activity by half. Previous, *in silico* studies proposed plasmepsin II (PM2) as a putative target for APD (Mendoza et al., 2011), but has yet to be confirmed experimentally. Nonetheless, more studies with APD are necessary to be validated as potential antimalarial hits (MMV, 2008).

Thus, exploration and development of APD as antimalarials requires (1) expanding SAR studies, (2) generating additional analogues with high potency against both chloroquine sensitive and multidrug resistant strains of *P. falciparum*, (3) improving parasitemia reduction in the *P. berghei* mouse model, (4) establishing a safe toxicological profile, and (5) exploring biological targets in *P. falciparum*.

In this study, we expanded the chemical scope in the antimalarial framework by preparing new APD for testing their antiplasmodial activity *in vitro* against *P. falciparum* (sensitive and resistant strains) and *in vivo* against *P. berghei*. Chiral separation and enantiomeric testing were also conducted. Further, cytotoxicity and genotoxicity studies were performed on active compounds and potential metabolites. Since target validation is a crucial step in the drug discovery process, APD compounds were evaluated for their ability to inhibit both the PM2 enzyme and the hemozoin formation pathway. Finally, pharmacophore modeling studies were performed on APD.

2. Material and methods

2.1. Synthesis of APD compounds

The methods used for synthesizing the final compounds (**14**–**26**) are presented in Schemes 1 and 2. The synthetic method has been published previously (Perez-Silanes et al., 2009; Mendoza et al., 2011). The starting arylamines 2-nitro-4-trifluoromethyl phenyl piperazine, 4-(4-fluorophenyl)-1,2,3,6-tetrahydropyridine and 4-trifluoromethyl phenyl piperazine were commercially

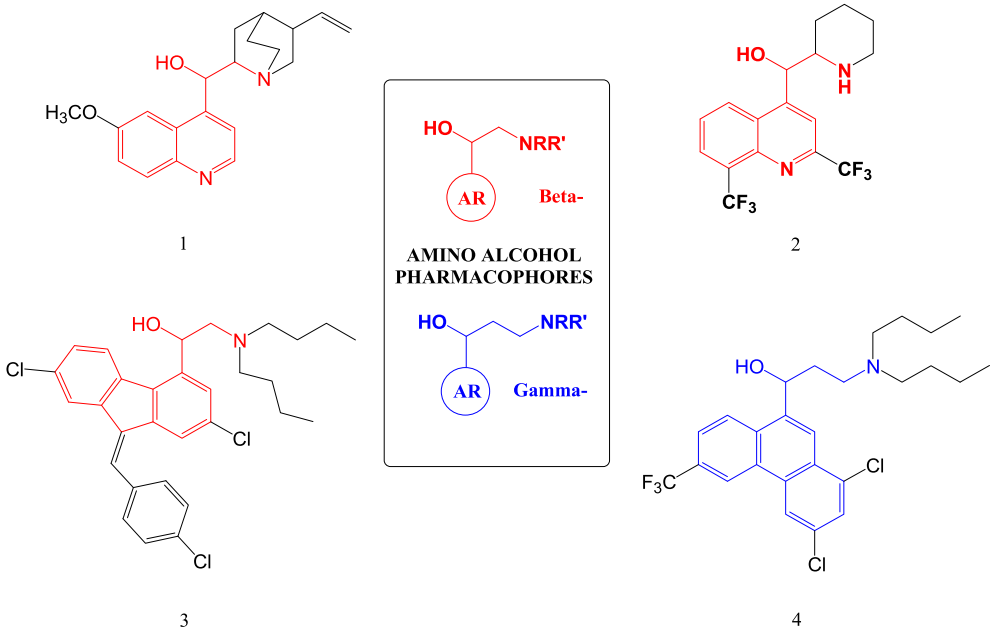

Fig. 1. Antimalarial drugs with amino alcohol moiety as a pharmacophore: (1) quinine; (2) mefloquine; (3) lumefantrine; (4) halofantrine.