International Journal for Parasitology: Drugs and Drug Resistance 7 (2017) 61–70

Contents lists available at ScienceDirect

International Journal for Parasitology: Drugs and Drug Resistance

journal homepage: www.elsevier.com/locate/ijpddr

Investigating the antiplasmodial activity of primary sulfonamide compounds identified in open source malaria data

Gillian M. Fisher [a], Silvia Bua [b], Sonia Del Prete [b, c], Megan S.J. Arnold [a], Clemente Capasso [c], Claudiu T. Supuran [b], Katherine T. Andrews [a, **, 1], Sally-Ann Poulsen [a, *, 1]

[a] Griffith Institute for Drug Discovery, Griffith University, Queensland, Australia
[b] Neurofarba Department, Sezione di Scienze Farmaceutiche Nutraceutiche, and Laboratorio di Chimica Bioinorganica, Università degli Studi di Firenze, Sesto Fiorentino, Florence, Italy
[c] Istituto di Bioscienze e Biorisorse, CNR, Via Pietro Castellino 111, Napoli, Italy

ARTICLE INFO

Article history:
Received 30 November 2016
Received in revised form
11 January 2017
Accepted 12 January 2017
Available online 13 January 2017

Keywords:
Primary sulfonamide
Plasmodium falciparum
Carbonic anhydrase
Plasmodium knowlesi
TCAMS

ABSTRACT

In the past decade there has been a significant reduction in deaths due to malaria, in part due to the success of the gold standard antimalarial treatment - artemisinin combination therapies (ACTs). However the potential threat of ACT failure and the lack of a broadly effective malaria vaccine are driving efforts to discover new chemical entities (NCEs) to target this disease. The primary sulfonamide (PS) moiety is a component of several clinical drugs, including those for treatment of kidney disease, glaucoma and epilepsy, however this chemotype has not yet been exploited for malaria. In this study 31 PS compounds sourced from the GlaxoSmithKline (GSK) Tres Cantos antimalarial set (TCAMS) were investigated for their ability to selectively inhibit the *in vitro* growth of *Plasmodium falciparum* asexual stage malaria parasites. Of these, 14 compounds were found to have submicromolar activity (IC_{50} 0.16–0.89 μM) and a modest selectivity index (SI) for the parasite versus human cells (SI > 12 to >43). As the PS moiety is known to inhibit carbonic anhydrase (CA) enzymes from many organisms, the PS compounds were assessed for recombinant *P. falciparum* CA (*Pf*CA) mediated inhibition of CO_2 hydration. The *Pf*CA inhibition activity did not correlate with antiplasmodial potency. Furthermore, no significant difference in IC_{50} was observed for *P. falciparum* versus *P. knowlesi* (P > 0.05), a *Plasmodium* species that is not known to contain an annotated *Pf*CA gene. Together these data suggest that the asexual intraerythrocytic stage antiplasmodial activity of the PS compounds examined in this study is likely unrelated to *Pf*CA inhibition.

1. Introduction

Malaria remains one of the world's most important infectious diseases, causing approximately 438,000 deaths in 2015, mainly African children under the age of five (WHO, 2015). While it is possible that the first generation RTS,S malaria vaccine will be employed in some regions in the future, the World Health Organization (WHO) remains cautious and recommends that other malaria prevention and treatment strategies continue, including the development of new drugs (WHO, 2016). This recommendation is driven by the threat of malaria parasite resistance or reduced clinical efficacy emerging to all current antimalarial drugs including the gold standard artemisinin combination therapies (ACTs) (WHO, 2015; Fairhurst and Dondorp, 2016). Added to this, the majority of agents in the current antimalarial drug development portfolio are based on known antimalarial pharmacophores (Wells et al., 2015), which may compromise their widespread use due to potential issues of cross resistance. With few antimalarial chemical classes (e.g. spiroindoline, imidazolepiperazine and triazolopyrimidine chemotypes) presently under advanced development (MMV, 2016) there is an urgent need to ensure that the antimalarial drug discovery pipeline is primed with new chemical entities, ideally those with novel modes of action to avoid cross-resistance to existing drugs. The primary sulfonamide (PS) chemotype is not currently

* Corresponding author.
** Corresponding author.
E-mail addresses: K.Andrews@griffith.edu.au (K.T. Andrews), s.poulsen@griffith.edu.au (S.-A. Poulsen).
[1] These authors contributed equally.

http://dx.doi.org/10.1016/j.ijpddr.2017.01.003

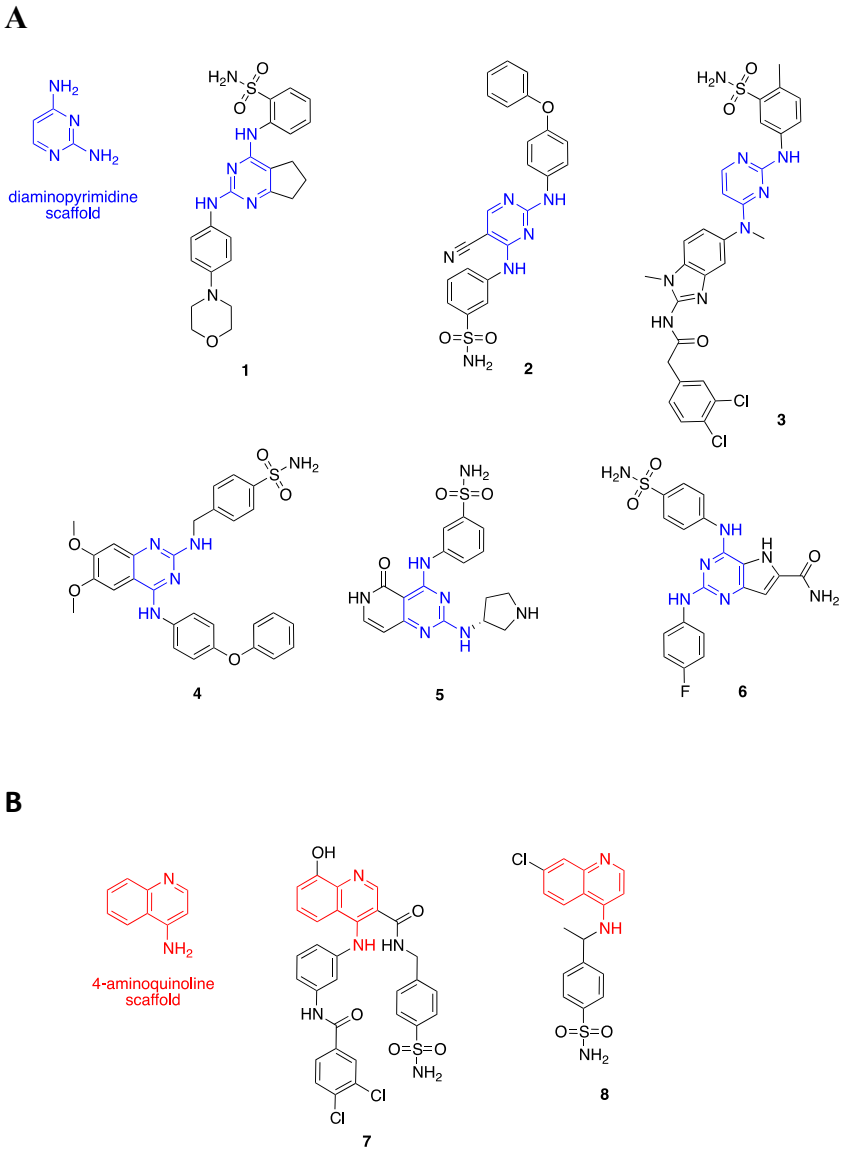

Fig. 1. Structures of TCAMS PS compounds with an antimalarial pharmacophore. Diaminopyrimidine based compounds highlighted in blue; 4-aminoquinoline based compounds highlighted in red. Compound **4** and **6** were tested as the formate salt, compounds **3**, **5**, **7** were tested as the trifluoracetate salt. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

used for malaria prevention or treatment, but has a proven track record for treatment of other diseases, including glaucoma, renal disorders and epilepsy (Poulsen, 2010; Supuran, 2010; Pastorek and Pastorekova, 2015; Supuran and Winum, 2015).

In previous work we identified several PS glycosides with moderate *in vitro* antiplasmodial activity (50% growth inhibitory concentration (IC_{50}) ~1 μM) and selectivity for the parasite versus human cells (Selectivity Index (SI) > 40) (Andrews et al., 2013). Published work from another group identified a thioureido benzenesulfonamide with similar *in vitro* activity against *P. falciparum* (IC_{50} ~1 μM) and *in vivo* activity against *P. berghei* in a mouse malaria model (ID_{50} 10 mg/kg) (Krungkrai et al., 2008). Additional evidence that PS compounds have antimalarial potential comes from high throughput screening of a GlaxoSmithKline (GSK) library of ~2,000,000 compounds. The results of the GSK screen led to compilation of the Tres Cantos antimalarial set (TCAMS), with data made publicly available as a resource for antimalarial lead identification and basic research into the "druggable" genome of *P. falciparum* through deposition in the open access European Bioinformatics Institute ChEMBL Neglected Tropical Disease archive. The TCAMS dataset contains ~13,500 compounds that inhibit the *in vitro* growth of drug-sensitive (3D7) and multi-drug resistant (Dd2) *P. falciparum* parasites (≥80% and ≥50% at 2 μM, respectively) (Gamo et al., 2010). Following a substructure search of this open source malaria data, we identified 31 PS compounds (Figs. 1 and 2) that were subsequently provided by GSK and investigated in this study.

PS compounds are known to inhibit carbonic anhydrase (CA) enzyme activity in many organisms (Supuran, 2008). CA enzymes maintain an important physiological equilibrium: the hydration of carbon dioxide to bicarbonate anion and a proton: $H_2O + CO_2 \leftrightarrows HCO_3^- + H^+$ and are responsible for HCO_3^- and pH homeostasis, including within erythrocytes. Malaria parasite CA inhibitors were first suggested as a potential new class of antimalarials in 1998 (Sein and Aikawa, 1998) and later the esterase activity of *P. falciparum* CA (*Pf*CA; PlasmoDB (Aurrecoechea et al., 2009) gene