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Evaluation of spiropiperidine hydantoins as a novel class of antimalarial agents

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ABSTRACT

Given the rise of parasite resistance to all currently used antimalarial drugs, the identification of novel chemotypes with unique mechanisms of action is of paramount importance. Since *Plasmodium* expresses a number of aspartic proteases necessary for its survival, we have mined antimalarial datasets for drug-like aspartic protease inhibitors. This effort led to the identification of spiropiperidine hydantoins, bearing similarity to known inhibitors of the human aspartic protease β-secretase (BACE), as new leads for antimalarial drug discovery. Spiropiperidine hydantoins have a dynamic structure–activity relationship profile with positions identified as being tolerant of a variety of substitution patterns as well as a key piperidine *N*-benzyl phenol pharmacophore. Lead compounds **4e** (CWHM-123) and **12k** (CWHM-505) are potent antimalarials with IC_{50} values against *Plasmodium falciparum* 3D7 of 0.310 μM and 0.099 μM, respectively, and the former features equivalent potency on the chloroquine-resistant Dd2 strain. Remarkably, these compounds do not inhibit human aspartic proteases BACE, cathepsins D and E, or *Plasmodium* plasmepsins II and IV despite their similarity to known BACE inhibitors. Although the current leads suffer from poor metabolic stability, they do fit into a drug-like chemical property space and provide a new class of potent antimalarial agents for further study.

1. Introduction

Malaria is caused by the parasite *Plasmodium*. In 2013, there were approximately 198 million cases of malaria leading to ~584,000 deaths, being particularly deadly to young children in sub-Saharan Africa.[1] *Plasmodium falciparum*, the most lethal species, has developed varying degrees of resistance to all currently used antimalarial drugs.[2–5] Approaches to combat parasite resistance include combination of antimalarial drugs as standard treatment regimens, as well as identification of new antimalarial drugs with unique mechanisms of action that can be combined with existing antimalarial drugs.

Plasmodium expresses a number of aspartic proteases necessary for its survival, including essential aspartic proteases plasmepsin V (PMV or PM-5) and signal peptide peptidase (*Pf*SPP).[6–11] While a number of potent peptidomimetic inhibitors of *Plasmodium* aspartic proteases have been identified,[7,12–14] we have focused on repurposing classes of drug-like aspartic protease inhibitors developed by the pharmaceutical industry for human aspartic proteases such as β-secretase (BACE)[15,16] or renin.[17]

Abbreviations: PM, plasmepsin; SPP, signal peptide peptidase; HIV, human immunodeficiency virus; SAR, structure–activity relationships; BACE, beta-site APP cleaving enzyme 1 or beta-secretase; TCAMS, Tres Cantos Antimalarial Set; RBC, red blood cells; CatD, cathepsin D; CatE, cathepsin E; MLM, mouse liver microsomes; RLM, rat liver microsomes; HLM, human liver microsomes; PK, pharmacokinetics.

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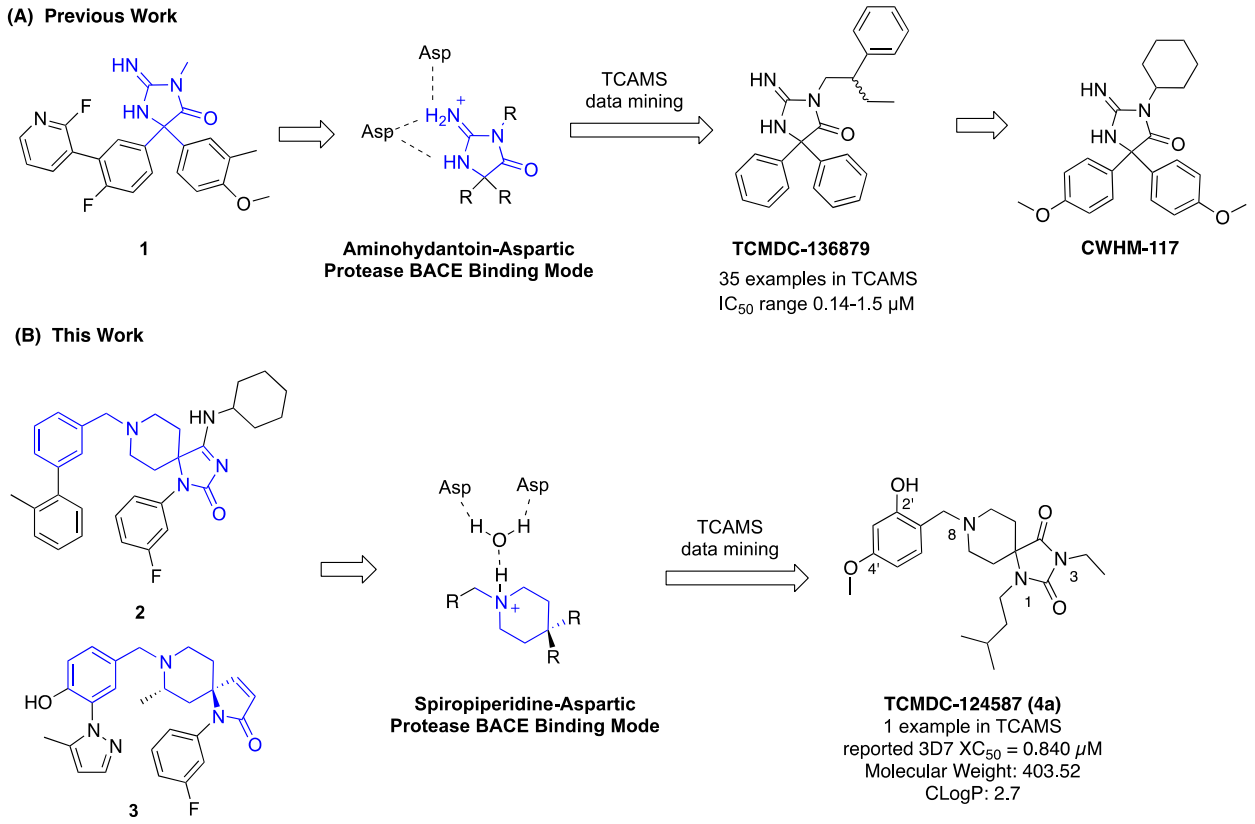

Figure 1. Strategy to identify drug-like aspartic protease inhibitors as novel antimalarials.

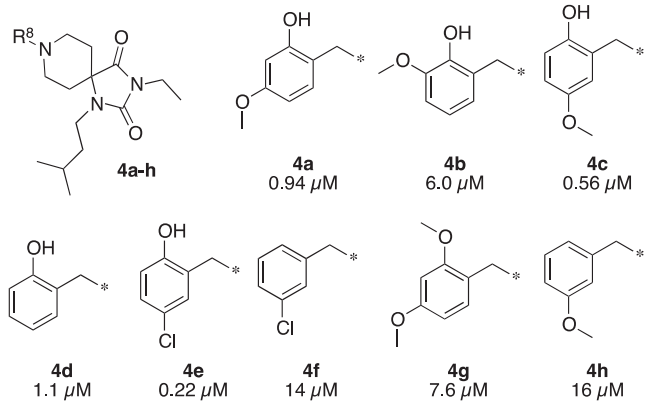

Figure 2. Preliminary R^8 structure–activity relationships. Reported potencies are IC_{50} values in *P. falciparum* 3D7 infected erythrocytes.

We have hypothesized that maintaining core structural motifs known to bind the aspartate residues in the active site may allow identification and optimization of novel classes of antimalarial compounds. Accordingly, we mined the Tres Cantos Anti-Malarial dataset (TCAMS) representing thousands of compounds[18] for drug-like aspartic protease inhibitors. For example, we recently reported our identification and initial optimization of aminohydantoins as novel antimalarial compounds with selectivity for *Plasmodium* and in vivo antimalarial efficacy (e.g., CWHM-117) originating from BACE inhibitor **1** and database hit TCMDC-136879 (Fig. 1a).[19]

Spiropiperidine-containing compounds such as **2** and **3** have been reported as non-peptidomimetic BACE inhibitors[16,20–22] and represent a novel scaffold for development of new antimalarial aspartic protease inhibitors (Fig. 1b). The reported x-ray crystal structure of **2** (3FKT)[16] demonstrates the mechanism by which the protonated piperidine nitrogen forms a salt bridge with a water molecule in the active site. Similarly, other related piperidine and pyrrolidine BACE, renin and HIV protease inhibitor crystal structures demonstrate similar binding modes,[17,23] leading us to hypothesize that the spiropiperidine scaffold may be an appropriate core for mining antimalarial phenotypic screening databases. Substructure-based searching of the TCAMS revealed a single hit, TCMDC-124587 (**4a**), with a reported XC_{50} of 0.840 μM. Given its modest molecular weight, favorable *C*Log*P*, and submicromolar antimalarial potency, an effort to validate this hit and evaluate the potential of this class of spiropiperidines as antimalarials was initiated.

2. Results and discussion

2.1. Validation of hit and initial SAR

Searches of commercially available compound databases revealed that TCMDC-124587 and closely-related analogs could be purchased from ChemBridge. Most commercially-available compounds were derivatized at the R^8 position. Two iterations of sets of six spiropiperidines each, including TCMDC-124587, were purchased and evaluated for inhibition of parasite growth in *P. falciparum* 3D7-infected red blood cells. Key structure–activity relationships are shown in Figure 2. Of foremost importance, **4a** was found to have similar 3D7 potency (IC_{50} = 0.940 μM) as reported in the screening dataset. Substituent position was found to be important. For example, moving the methoxy group from the 4′- to the 3′- or 5′-positions resulted in 6-fold loss or 2-fold improvement in potency, respectively (**4b**,**c**). While deletion of the methoxy group (**4d**) did not have a significant impact on