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Design, Synthesis and Antimalarial Activity of Novel, Quinoline-Based, Zinc Metallo-Aminopeptidase Inhibitors

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Abstract—PfA-M1, a neutral zinc aminopeptidase of Plasmodium falciparum, is a new potential target for the discovery of antimalarials. The design and synthesis of a library of 45 quinoline-based inhibitors of PfA-M1 is reported. The best inhibitor displays an IC50 of 854 nM. The antimalarial activity on a CQ-resistant strain and the specificity towards mammalian aminopeptidase N are also discussed.

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Introduction

Malaria, caused by the parasite Plasmodium falciparum, is still prevalent and lethal in many countries. Chloroquine (CQ), exerting its antimalarial activity by inhibiting haemozoin formation in the food vacuole of the parasite, has been the standard drug for many decades. Unfortunately, the spread of resistant P. falciparum to this molecule has created an urgent need to develop new antimalarial treatments. In a project to find new quinoline-based antimalarials that would not induce resistance, unlike CQ, we have designed several compounds displaying a piperazine linker. Several of these compounds were active on the CQ-resistant strain FcB1.

The recent publication of the genome of the parasite opens the opportunities of a better understanding of the biology of the parasite. This will hopefully result in the discovery of new specific targets to tackle with drugs. Proteases expressed in the erythrocytic stage of P. falciparum, for example, can be considered as interesting targets for the design of antimalarials.

In particular, PfA-M1, a neutral zinc-aminopeptidase (M1 family), inhibited by the classical aminopeptidase inhibitor bestatin, has been proposed:

(1) to hydrolyze the small peptides generated in the food vacuole, during hemoglobin digestion by acidic endopeptidases (aspartyl, cysteine and metallo-endopeptidases), into amino-acids at the level of the cytoplasm, and:
(2) to play a role in the erythrocyte re-invasion by the parasite. PfA-M1 shares a maximal homology in the active site region (44%) with two human proteases: the human aminopeptidase-N and leukotriene A4 hydrolase. It is the only aminopeptidase of P. falciparum that has been purified and biochemically characterized. This enables the design and testing of potential inhibitors. Such compounds would help understanding the role of this enzyme and validate it as a therapeutic target.

In a project aiming at decreasing the potential for resistance occurrence of our quinoline-based antimalarials, we have designed dual inhibitors that would inhibit both the haemozoin formation and one of the proteases potentially involved in globin digestion. Such a strategy has been developed by Avery for the design of malarial cysteine proteases inhibitors based on mefloquine and chloroquine. The screening of several of our in-house quinoline-based compounds allowed us to identify compound 1 as an interesting inhibitor of PfA-M1, that served as a starting point for analoguing (Fig. 1).

We report here the design and parallel synthesis of a library of 45 non-peptide analogues of 1, and its biological
evaluation on PfA-M1. Preliminary results on the anti-
malarial activity on FcB1 strain of the most active enzyme
inhibitors, and preliminary specificity data on mammalian
aminopeptidase N, are presented and discussed.

Chemistry

PfA-M1 is a zinc-metalloprotease, of the M1 family,
preferably cleaving basic, hydrophobic, as well as ar-
omatic amino-acids.\textsuperscript{8} Zn-chelating groups are critical for
the inhibition of such enzymes.\textsuperscript{11} To fulfill this function,
we incorporated at least a carboxylic acid or an
hydroxamate group in our molecules. In order to mimic
the side chain of a Leucine residue, an isobutyl group
(Ibu), analogue of the methyl-cyclopropyl moiety in 1,
was also added in our potential inhibitors.

The 45 analogues (5 amines \times 9 carboxylic acids) have
been synthesized at a 5-\textmu mol scale, in parallel, accord-
ing to the two-step procedure described in Scheme 1.
The first step consists in the formation of the amide
bond using PyBrop as the activator of the carboxylic
acid followed by deprotection of the Boc and tBu
groups of amines, hydroxamates and carboxylic acids.

The synthesis of this full combinatorial library required
specific protected amines and carboxylic acids pre-
cursors to be synthesized prior to the coupling step.

Amines 3-6 (Fig. 2) all derived from compound 2 pre-
viously obtained from 4,7-dichloro-quinoline and 1,4-
bis-(3-aminopropyl)-piperazine by nucleophilic aro-
matic substitution.\textsuperscript{12} They were synthesized either by

\begin{align*}
\text{Qu} & \text{NH} \text{R}_1 + \text{HO} \text{OCO} \text{R}_2 \quad \text{a} \rightarrow \quad \text{Qu} \text{N} \text{R}_1 \text{R}_2 \quad \text{b} \rightarrow \quad \text{deprotected compound}
\end{align*}

Scheme 1. (a) 1.1 equiv PyBrop, 2 equiv DIEA in DCM, overnight, rt;
(b) TFA/DCM (20/80), 72 h, rt.

reductive amination of an aldehyde or N-alkylation
with the appropriate halide (Scheme 2).

As for the carboxylic acid precursors, we synthesized
malonic derivatives (24-28) according to the procedure
described in Scheme 3. These moieties have been pre-
viously described in inhibitors of matrix-metallopro-
teases (MMPs).\textsuperscript{13} We also added to these in-house
precursors, some commercially available N-protected
amino-acids Lys, Leu, Phe, Asp (29-32, Fig. 3).
Analytical control

Each crude product was tested for purity using HPLC and identity using MALDI-TOF. In all cases, the purity exceeded 80% and the mass spectrum was consistent with the anticipated product structure.

Biological Assays

The analogues and the deprotected precursors were screened for their ability to inhibit PfA-M1 at 10 μM. Crude products displaying an inhibition percentage above 50% were selected for re-synthesis and IC_{50} determination on fully purified and controlled samples. The selected compounds were then tested for their ability to inhibit FcB1 parasite growth. The specificity against other aminopeptidases was evaluated in a model of mammalian aminopeptidase-N from porcine kidney.

Structure–activity relationships on PfA-M1

Out of the 45 compounds tested at 10 μM, three compounds (33–35, Fig. 4), displayed a percentage of inhibition above threshold. Interestingly, they all derived from the same amine 6. After re-synthesis and testing, they displayed IC_{50}s from 0.85 to 1.54 μM on PfA-M1, that is, a 10-25 times increase in potency comparing to compound 1 (Table 1).

The presence of a Zn-chelating group is essential for the activity. Compound 33, the closest analogue of 1, bearing an hydroxamate is 10 times more active on the enzyme. In contrast, amine 6 is inactive. The hydroxamate group is more efficient than the carboxylic acid group. Carboxylic acids analogues of 33 and 34 were inactive. The isobutyl group is essential for activity, since the analogues of 33–35 without this moiety (derivatives of 2) were inactive.

The relative positions of the Zn-chelating group and the isobutyl group are critical for the activity. For example, compounds 36–37 (Fig. 5) are inactive while displaying the same groups in a different 3D arrangement as respectively in 34 and 33. None of the analogues derived from the amino-acids (37 for instance) supposed to be recognized by the enzyme, was active, suggesting that the Zn-chelating group should be supported by the carboxylic acid precursor.

The central position in malonic derivatives seems tolerant since it can accommodate with basic (NH₂) or hydrophobic (isobutyl) groups. Nevertheless, the best analogue, compound 34, is not substituted in that position. In particular, this suggests that an amino-group is not essential for the recognition by the enzyme although it is an aminopeptidase.

Antimalarial activity and specificity

The three PfA-M1 inhibitors are sub-micromolar inhibitors of the parasite growth. Their antimalarial activity is due to the inhibition of both PfA-M1 and haem detoxification. Interestingly, reference compound 38, displaying a COOH moiety at the same position as the hydroxamate OH in compound 34 (and a cyclopropylmethyl group instead of an isobutyl group), is weakly active on PfA-M1, and less active than 34 on parasite growth inhibition. Specificity for compounds 33–35, as expressed with the ratio of the IC_{50} on the two targets, varies from 0.9 to 30.5. The size of the substitu-ant on the malonic methylene appears to be critical.

Conclusion

We have successfully designed non-peptidic inhibitors of PfA-M1, an aminopeptidase of P. falciparum. The quinoline moiety allows these compounds also to be inhibitors of haem detoxification, making these compounds potentially dual inhibitors of hemoglobin digestion. Yet the relative part of each mode of action needs to be determined. The three selected inhibitors 33–35 are active against CQ-resistant strain, thus validating their ability to cross membranes. The activity on PfA-M1, and the specificity against other aminopeptidases such as mammalian aminopeptidase-N need to be improved. Such issues are under investigation and results will be reported in due course. Antimalarial activities and rough SARs emerging from this study,
have encouraged us to pursue the optimization of PfA-M1 inhibitors. Especially, we are now focusing on inhibitors devoided of the quinoline moiety.

References and Notes

7. Swissport accession number of PfA-M1: O96935.
14. Native PfA-M1 was purified according to the procedure described in ref 9, and diluted 10 times in Tris–HCl buffer (25 mM; pH 7.4) before use. The assays were set up in 96-well plates. The compounds were tested at a final concentration of 0.3 mM in Tris–HCl buffer (25 mM; pH 7.4) before use. The assays were set up in 96-well plates and proceeded like for PfA-M1 with the same substrate Leu-pNA (Km = 0.13 mM). The Z’ factor of the test was 0.80 allowing activities to be determined with a single point with a 95% confidence. The reference inhibitor was bestatin.
15. (3TFA): NMR (CD3OD) 1H δ ppm 0.80–0.84 (m; 1H); 1.10–1.26 (m; 5H); 1.27–1.30 (m; 6H); 2.75–3.58 (m; 14H); 5.76 (d; J = 7.1 Hz; 1H); 7.76 (d; J = 9.0 Hz; J = 1.1 Hz; 1H); 7.95 (s; 1H); 8.50–8.56 (m; 2H); 9.30 (br s; 1H; 2NH+); 9.73 (br s; 0.5H; NHO); MALDI-TOF: 534.2 (M+H+).
16. (2TFA): NMR (CD3OD) 1H δ ppm 0.80–0.84 (m; 1H); 1.10–1.26 (m; 5H); 1.27–1.30 (m; 6H); 2.75–3.58 (m; 14H); 5.76 (d; J = 7.1 Hz; 1H); 7.76 (d; J = 9.0 Hz; J = 1.1 Hz; 1H); 7.95 (s; 1H); 8.50–8.56 (m; 2H); 9.30 (br s; 1H; 2NH+); 9.73 (br s; 0.5H; NHO); MALDI-TOF: 534.2 (M+H+).
17. (2TFA): NMR (CD3OD) 1H δ ppm 0.80–0.84 (m; 1H); 1.10–1.26 (m; 5H); 1.27–1.30 (m; 6H); 2.75–3.58 (m; 14H); 5.76 (d; J = 7.1 Hz; 1H); 7.76 (d; J = 9.0 Hz; J = 1.1 Hz; 1H); 7.95 (s; 1H); 8.50–8.56 (m; 2H); 9.30 (br s; 1H; 2NH+); 9.73 (br s; 0.5H; NHO); MALDI-TOF: 534.2 (M+H+).
18. In-vitro inhibition of haem polymerization induced by lipids, unpublished data (see ref 3 for protocol).