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REPORT

Novel synthetic inhibitors of the microbial M1 aminopeptidases ePepN and PfA-M1

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ABSTRACT

Infections by bacteria resistant to conventional antibiotics are a worldwide health problem. Likewise, the parasite causal agent of malaria, main tropical parasitic disease, has developed resistance to the traditional drugs. In this sense, aminopeptidases PepN, from the bacterium *Escherichia coli*, and PfA-M1, from the *Plasmodium falciparum* parasite, are new targets in these sicknesses. The objective of this work was the identification of synthetic inhibitors of ePepN and PfA-M1, with potentialities as anti-bacterial and anti-malarial agents. Using two multicomponent Ugi-reactions, two libraries of 33 bestatin- and actinonin-based peptidomimetics were synthesized and evaluated against PfA-M1. In addition, 22 tetrazole-peptidomimetics were synthesized and evaluated against ePepN. Three tetrazoles (YTE003, YTE007, YTE008) were identified as potent and selective ePepN inhibitors, regarding porcine M1 aminopeptidase (pAPN). YTE003 shows *in vitro* anti-bacterial activity, which supports its potential as leader compound in the field of anti-bacterial agents. Toward PfA-M1, compound KBE009 was identified as a potent inhibitor, with *in vitro* anti-malarial activity, that does not inhibit pAPN at concentrations of therapeutic relevance, and it is not cytotoxic up to 200 μmol/L against the human HUVEC cell line. It was found that KBE009 inhibits aminopeptidase activity in the whole and isolated parasite in the same order of magnitude as its *in vitro* anti-malarial activity. These properties lead into considering the peptidomimetic a promising molecule as a lead compound against malaria.

Keywords: anti-bacterial and anti-malarial agents, microbial M1 aminopeptidases, synthetic aminopeptidase inhibitors, Ugi-multicomponent reaction

RESUMEN

Nuevos inhibidores de metaloaminopeptidasas M1 microbianas obtenidos mediante síntesis química. Las infecciones por bacterias resistentes a los antibióticos convencionales constituyen un problema de salud mundial. Igualmente, el parásito causante de la malaria, principal enfermedad parasitaria tropical, ha desarrollado resistencia a los medicamentos tradicionales. En este sentido, las aminopeptidasas ePepN, de la bacteria *Escherichia coli*, y PfA-M1, del parásito *Plasmodium falciparum*, constituyen nuevos blancos en estas enfermedades. El objetivo de este trabajo fue la identificación de inhibidores sintéticos de ePepN y PfA-M1, con potencialidades como agentes antibacterianos y antimaláricos. Mediante dos reacciones multicomponentes de Ugi se sintetizaron dos bibliotecas de 33 peptidomiméticos basados en la bestatina y la actinonina, y se evaluaron frente a PfA-M1. Además, se sintetizaron 22 peptidomiméticos tetrazoles, los cuales se evaluaron frente a ePepN. Frente a esta enzima, se identificaron 3 tetrazoles (YTE003, YTE007 y YTE008) como inhibidores potentes y selectivos de ePepN, en comparación con la aminopeptidasa M1 porcina (APNp). El YTE003 presenta actividad antibacteriana *in vitro*, lo que refuerza sus potencialidades como compuesto líder en el campo de los agentes antibacterianos. Frente a PfA-M1, se identificó el compuesto KBE009 como un inhibidor potente, con actividad antimalárica *in vitro*, que no inhibe a la APNp a concentraciones de relevancia terapéutica, y no es citotóxico hasta 200 μmol/L frente a la línea de células humanas HUVEC. Se comprobó que el KBE009 inhibe la actividad aminopeptidasa en el parásito íntegro y aislado en el mismo orden de magnitud que su actividad antimalárica *in vitro*. Estas propiedades permiten considerar al peptidomimético una molécula promisoria como compuesto líder contra la malaria.

Palabras clave: agentes antibacterianos y antimaláricos, aminopeptidasas M1 microbianas, inhibidores sintéticos de aminopeptidasas, reacción de Ugi multicomponente celular

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Introduction

The resistance of human-pathogenic bacteria to conventional antibiotics is one of the main health problems [1]. On the other hand, malaria is the main parasitic human disease in tropical regions, its more lethal ethiological agent is the protozoan *Plasmodium falciparum* [2]. Therefore, the development of new, potent and selective antibacterial and antimalarial agents against novel molecular targets is an urgent need [3].

In this context, proteolysis is one fundamental physiological process mediating microbial survival, growth and development [4]. Therefore, bacterial and parasitic proteases have been focused as potential

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targets for microbial inhibition [5, 6]. Aminopeptidases (AP) are among the most studied proteases, particularly metallo-AP of the M1 family (APN) [7]. Two microbial APNs have been spotted as good inhibition targets: ePepN from *Escherichia coli* and the PfA-M1 from *P. falciparum* [6, 8].

So far, the most potent inhibitors of these two APNs reported are synthetic peptides or peptidomimetics [9]. Metallo-APs are typically inhibited by bestatin, a natural pseudopeptide which is an analogue of the transition state of the catalytic mechanisms of these enzymes, and also by actinonin [10, 11]. However, a key disadvantage of these molecules for therapeutics comprises their low selectivity to inhibit metallo-APs since they also inhibit human enzymes [12]. For this reason, bestatin and actinonin have been used as models to design new peptidic compounds of higher activity and selectivity indexes against microbial metallo-APs [13]. In this line, tetrazole compounds have shown activity against protozoa and bacteria [14].

In the way to generate new compounds, muticomponent reactions condense three or more molecules either sequentially or simultaneously to generate a product which integrates most atoms from the starting materials. Such reactions are highly advantageous, for instance, to generate a highly diverse and complex structures, and, thereby, molecular combinatorial libraries [15]. Hence, in this work, both enzymes were obtained by recombinant procedures. Besides, it is described the generation of two libraries of 33 peptidomimetics based on the structure of bestatin and actinonin and obtained through multicomponent reactions, and 22 tetrazole peptidomimetics. Starting from those libraries, new potent and selective inhibitors of ePepN and PfA-M1 APNs were identified as leader structures, to generate novel compounds as antibacterial and antimalarial agents.

Materials and methods

Bestatin-based N-alkylated peptidomimetics were syhthesized by the Ugi-4-components reaction, and from actinonin by the five centers Ugi-4-components reaction, as reported by Méndez *et al*. [16] and González-Baceiro *et al*. [17]. Tetrazole-peptidomimetics were synthesized through the Ugi-4-azide-components reaction and APNp was obtained as described by Mendez *et al*. [18].

For this, the *pfam1* gene was designed, optimized, synthesized and cloned into an *E. coli* expression vector as described [19]. Furthermore, PfA-M1 was expressed, purified and identified by Western blot and proteomic procedures [19].

Subsequently, the ePepN was expressed, purified, identified by proteomic procedures and its kinetics characterized [16, 20]. The bestatin inhibition kinetics was done according to Varela *et al*. [21].

The PfA-M1 kinetics was characterized by studying the effect of Zn^{2+} on the enzymatic activity and its inhibition profile determined [22]. Optimal pH and kinetic parameters were established as described by González-Baceiro *et al*. [19, 22].

Screening experiments were done for assessing the 22 synthetic tetrazole peptidomimetics against ePepN [18] and the 33 bestatin-based synthetic N-alkylated peptidomimetics and actinonin-based compounds against PfA-M1 [17]. Tetrazole peptidomimetics and the KBE009 peptidomimetic were further compared against APNp for its inhibition selectivity against ePepN [18] and PfA-M1 [17], respectively. Kinetic characterization of ePepN inhibition by YTE008 was performed according to [18].

The *in silico* molecular docking and dynamic simulations were done for the YTE008-ePepN [18] and KBE009-PfA-M1 [17]. Kinetics assays were conducted with fluorogenic substrates, intact and red blood cells-isolated *P. falciparum* 3D7, and the bestatin-based and synthetic peptidomimetics (including KBE009) [17]. Moreover, tehrazole peptidomimetics were tested for antibacterial activity against *E. coli in vitro* [18].

The 33 bestatin-based synthetic N-alkylated peptidomimetics and the actinonin-based compounds were screened for the inhibition of *P. falciparum* 3D7 and FcB1 [17]. Furthermore, tetrazole compounds, the bestatin-based synthetic N-alkylated peptidomimetics and actinonin-like compounds were tested for hemolytic activity [17, 18]. The former were also tested for cytotoxic activity [18], as KBE009 cytotoxicity in HUVEC cells [17]. Lastly, bestatin-based and synthetic peptidomimetics, including KBE009 were assayed for growth inhibition *in vitro* against a transgenic *P. falciparum* strain overproducing PfA-M1[23].

Results and discussion

Synthesis of bestatin-, actinonin- and tetrazole-based peptidomimetics

The first part of this work describes the use of the Ugi-4-components (Ugi-4C) reaction to generate bestatin-based N-alkylated peptidomimetics (Figure 1A). The design was oriented to preserve the bestatin amino terminus, which is responsible for its interaction with several glutamate residues in the ePepN and PfA-M1 active sites. It also allowed the introduction of hydrophobic side chains able to interact with the S1 and S1' subsites in these enzymes [24, 25]. The synthesized compounds are tripeptides Phe–N(aa)–Leu in which aa. stands for the amino acid part added during the Ugi-4C reaction. These are new compounds, which could also lead to new inhibition modes. The Boc-phenyl-alanine and leucine methyl ester were used as acidic and amine components, respectively. The multicomponent nature of this process supports the introduction of new diverse elements from different carbonyl and isonitrile components. Additionally, the Ugi-4C reaction supports obtaining N-substituted peptides, of metabolic stability and membrane permeability higher than their analogous peptide structures [26].

In a second approach, the five centers Ugi-4-components (Ugi-5C-4C) reaction was applied to design and obtain actinonin-based compounds (Figure 1B). So far, most approaches used for obtaining analogues of this natural product are based on introducing the hydroxamic acid or its reversed variant's functional group, potent bidentate ligands of the metal ion of the therapeutic targets of interest [15]. It is possible to deduce that this property is the one determining the low inhibitory selectivity reported for most of the constituents of this family of compounds [11]. Therefore, the approach used in this work comprised the

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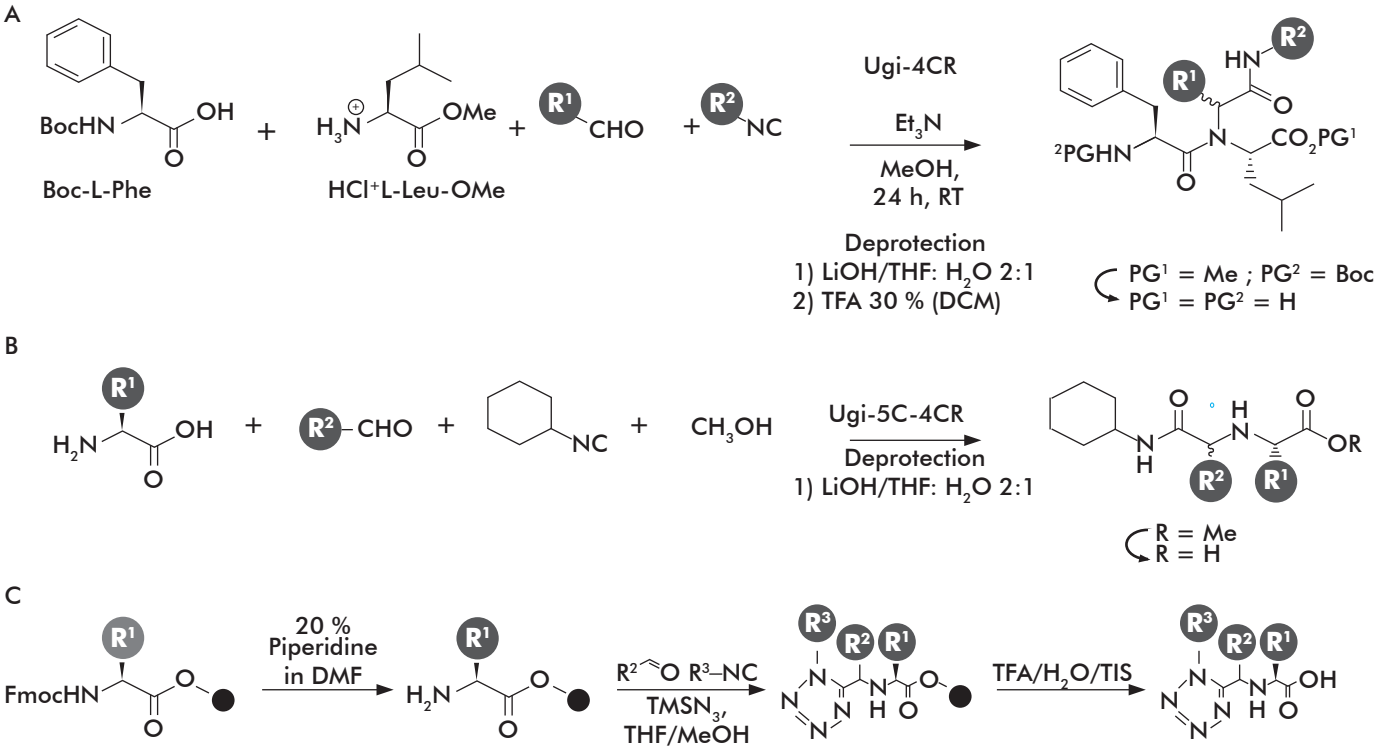

Figure 1. Synthesis of bestatin and actinonin analogs and tetrazole peptidomimetics. A) Synthesis of bestatin analogs through Ugi-4 components reactions (Ugi-4CR). B) Synthesis of actinonin analogs through Ugi 5 centers-4 components reactions (Ugi-5C-4CR). C) Synthesis of tetrazole peptidomimetics through the Ugi-azide 4 components reaction in solid phase. The solid circle in C) stands for the inert solid phase support. DMF: di-methyl formamide. TFA: Trifluoroacetic acid. TMSN: Trimethylsilyl azide. THF: Tetrahydrofurane.TIS: Tri-isopropylsilane.

substitution of this residue by a carboxylic acid. This reaction supports the generation of pseudopeptides, on which a secondary amide introduction from the cyclohexyl isonitrile can be designed. This compound can be selected as fixed component to reproduce the steric-electronic properties of the L-propinol residue in the natural inhibitor and different substituents from the other components (*e.g.* α amino acid and carbonyl compound). Hence, L-Leu, L-Ile, L-Phe and L-Trp were the selected amino acids, able to support the introduction of voluminous hydrophobic residues. Such residues could, thereby, interact as previously described with diverse hydrophobic residues of the active site in the therapeutic targets of interest.

Furthermore, we report the solid phase, multicomponent synthesis of tetrazole peptidomimetics through the Ugi-azide-4-components (Ugi-azide-4C) reaction with amino acids bound to a resin as solid phase (Figure 1C). It was supported by the reported metal chelating capacity of tetrazoles, including their documented antimicrobial activity against pathogenic bacteria and protozoa [14]. We hypothesized that tetrazoles, properly functionalized (with functional and voluminous hydrophobic groups able to bind to the active center of metallo-AP) could inhibit ePepN through Zn^{2+} metal ion chelation. This method was selected because it provides an efficient access to 1,5 di-substituted tetrazoles with three different sites for diversity generation arising from the amine, carbonyl and isocyanide components. Amino acids with hydrophobic side chains were selected, such as phenylalanine, benzyl-protected tyrosine, tryptophan and valine. Aldehyde components allowed the addition of substituents like 2-furyl, *p*-methoxyphenyl, 3-pyridyl and 2-imidazolyl.

Identification of potent and selective inhibitors of ePepN with antibacterial activity *in vitro*

The r-ePepN enzyme (r-ePepN) was over-expressed in *E. coli* BL21 Gold (DE3) at shaker scale, starting from a plasmid DNA genetic construct. Expression was induced by adding 0.5 mmol/L IPTG at the late exponential bacterial growth phase, and incubated at 30 °C for 14 h. This procedure provided a volumetric yield of 50 mg/L of active and soluble protein in the bacterial cytosol. The protein was purified by ion exchange chromatography using a Q-Sepharose Fast Flow matrix, to 85 % of purity. The purified recombinant ePepN (r-ePepN) was identified through trypsin digestion of the respective SDS-PAGE gel band. The resulting 16 peptides were sequenced by Liquid Chromatography coupled to Tandem Mass Spectrometry (LC-MS/MS). They corresponded to and spanned up to 25 % of the database-deposited sequence of ePepN.

The values of the apparent Michaelis-Menten constant ($K_M$ap) for r-ePepN against the Ala- and Leu-pNA substrates matched those reported for this enzyme [27]. It was experimentally determined that bestatin displays a reversible non-competitive ($\alpha > 1$) or mixed type of inhibition against r-ePepN. Three out of the 22 tetrazole peptidomimetics screened against r-ePepN (YTE003, YTE007, YTE008) were identified, inhibiting the enzyme with IC_{50} values lower than

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2 µmol/L (Figure 2). Besides, these three compounds were highly selective for the inhibition of the bacterial enzyme as compared to the orthologue porcine enzyme (APNp). Of them, YTE008 is a non-competitive inhibitor ($\alpha < 1$) of ePepN and YTE003 displays antibacterial activity *in vitro* ($IC_{50} < 50$ µmol/L at 3 and 6 h of exposure; $IC_{50} < 100$ µmol/L at 20 h (Figure 2). This last tetrazole shows potentialities for obtaining a new leader molecule to develop antibacterial agents.

Moreover, it was successfully predicted that YTE008 bears a probable binding mode to the ePepN active site through hydrophobic interactions, without contact with the Zn^{2+} cation (Figure 3). This was determined by *in silico* molecular docking and molecular dynamics' simulations. The YTE008 inhibitor does not interact with any residue located in the substrate binding site, thereby, allowing the simultaneous binding of the inhibitor and the substrate. Otherwise, it interacts with a critical catalytic tyrosine residue, such interaction consistent with the non-competitive binding mode.

None of the three compounds (YTE003, YTE007 or YTE008) was neither a potent hemolytic agent (YTE003 and YTE007 40 % hemolysis, YTE008 15 % hemolysis after 20 h, all of them at 100 µmol/L) nor cytotoxic (no decrease of cell viability in P3X63Ag murine myeloma cells after 20 h incubation with YTE008, IC_{50} 100 µmol/L for YTE003 at 6 and 20 h, and for YTE007 at 20 h).

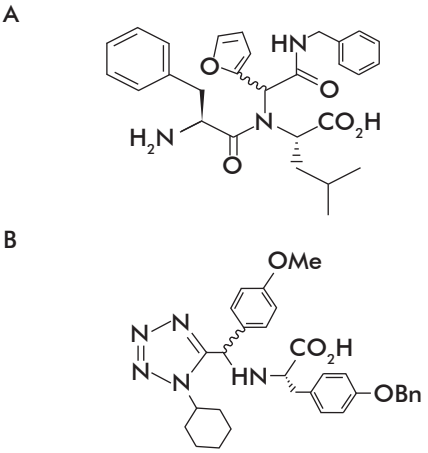

Figure 2. Bestatin and tetrazole analogs displaying inhibitory activity against microbial metallo-aminopeptidases and potential antimalarial and antibacterial activity, respectively. A) KBE009. PfAM1 (IC_{50} = 0.8 µmol/L). *P. falciparum* 3D7 (IC_{50} = 18 µmol/L), similar to bestatin (IC_{50} = 21 µmol/L). pAPN does not show inhibition at therapeutic concentrations. Non-cytotoxic for HUVEC cells. B) YTE003. ePepN (IC_{50} = 1.4 µmol/L). pAPN (IC_{50} = 1580 µmol/L). *E. coli* at 3 and 6 h exposure ($IC_{50} < 50$ µmol/L); 40 % of hemolysis at 100 µmol/L after 20 h. IC_{50} = 100 µmol/L at 6 and 20 h exposure in P3X63Ag cells.

Identification of potent and selective PfA-M1 inhibitors with anti-malarial activity *in vitro*

A variant of the PfA-M1 gene fused to a His tag was designed, which was optimized for gene expression in *E. coli*. Consequently, the Codon Adaptation Index in the *E. coli* host was increased from 0.65 to 0.96 in the modified gene, together with the G/C content from 26.36 to 39.28 %. Then, the optimized *PfA-M1* gene was synthesized and cloned into the plasmid vector for protein expression in *E. coli* (GeneArt, Germany).

The recombinant PfA-M1 enzyme (rPfA-M1) was then expressed into the BL21 *E. coli* strain at shaker scale, its expression induced by adding 1 mmol/L IPTG at the late exponential bacterial growth phase followed by incubation at 37 °C for 18 h. Up to 100 mg/L volumetric yield of active and soluble protein was obtained at the bacterial cytosol. The induction method used provided a higher expression level (rPfA-M1 represents 19 % of total proteins in the soluble fraction) and volumetric yield than the addition of 0.1 mmol/L IPTG at the start of the bacterial culture or the autoinduction with 0.2 % lactose. It was established 18 h as the optimal time for induction and two ultrasound cycles as optimal for the extraction of the rPfA-M1 enzyme from bacterial cells.

The recombinant enzyme was further purified by Immobilized Metal Ion Affinity Chromatography (IMAC) by using Co^{2+} as divalent metal cation. This cation supports the purification of a protein at a higher purity than using Ni^{2+} and Cu^{2+}. Besides, a 50 mmol/L imidazole concentration was selected as optimal to elute the adsorbed recombinant protein from the matrix. The rPfA-M1 was then purified from the soluble fraction of bacterial protein extract by IMAC, resulting in a final yield of 24 mg/L and 97 % purity. Both the purified rPfA-M1 and its degradation products were identified by Western Blot with an anti-His tag antibody. rPfA-M1 and its main degradation product were further identified by LC-MS/MS from tryptic peptides derived from both protein bands

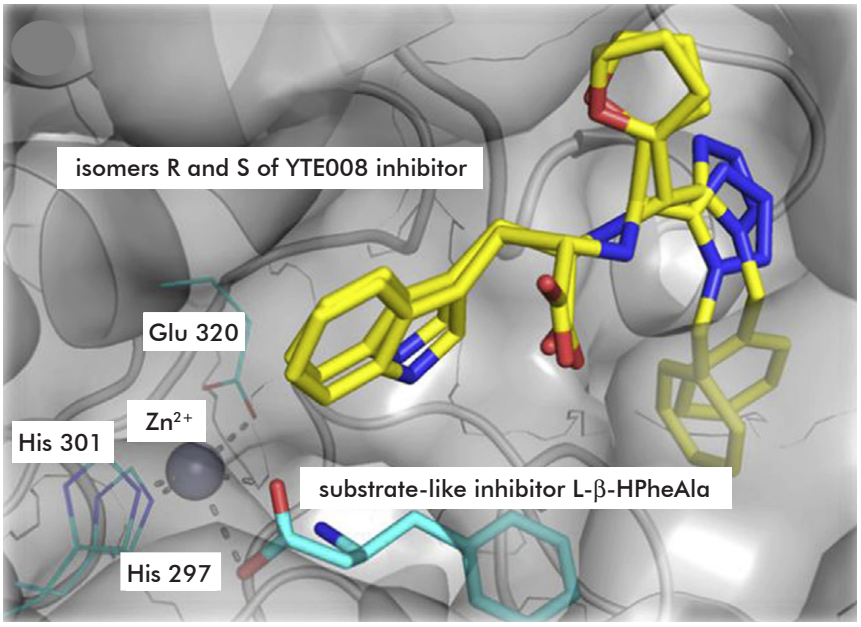

Figure 3. Predicted binding mode for the ePepN-YTE008 complex. Overlapped representation of the modelled complexes of isomers R and S of the inhibitor in a surface diagram of the active site of ePepN. YTE008 and the subtrate-like inhibitor L-β-HPheAla are shown as sticks. Color codes: carbon atoms in yellow (YTE008), and in light blue (L-β-HPheAla), oxygen in red, nitrogen in dark blue. The Zn^{2+} atom is depicted as a sphere in gray.

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extracted from SDS-PAGE gel. All the peptides sequenced corresponded to the PfA-M1 sequence deposited at the genetic sequence database, with 69 and 25 % coverage of rPfA-M1 and the major degradation product, respectively. None of the sequenced peptides were from the endogenous *E. coli* APN sequence, ePepN. This indicated that this last protein was not a significant contaminant in the purified rPfA-M1 fraction, despite the similarity in molecular size between both proteins.

The purified rPfA-M1 protein was not activated by $ZnCl_2$ at the 10 nmol/L-1 µmol/L concentration range, only inhibited at cation concentrations above 1 µmol/L. This further indicated that Zn^{2+} addition was not required for the enzyme activity assays. The optimal pH was set to 7.2-7.4 for the AP activity of the rPfA-M1 enzyme against the Leu-pNA chromogenic substrate and the fluorogenic substrates Ala-, Leu-, Arg- and Met-AMC. This value was the one reported for the native PfA-M1 enzyme [28].

Then, the kinetic parameters of the rPfA-M1 enzyme were established with chromogenic substrates Ala-, Leu-, Arg- and Lys-pNA. The AP showed substrate specificity for neutral and basic amino acids at the amino terminus, as previously reported [28].

Similarly, kinetics parameters were determined for the rPfA-M1 enzyme with the fluorogenic substrates Ala-, Leu-, Arg- and Met-AMC. The K_Map values [29] obtained were close and the substrate specificity similar [28], to those previously reported by other groups for the native PfA-M1. It was corroborated that the rPfA-M1 was insensitive to PMSF, E64 and pepstatin A, which are typical inhibitors of serine, cysteine and aspartic acid proteases, respectively. Meanwhile, the recombinant enzyme was inhibited by amastatin, a metallo-AP inhibitor, by 1,10-phenantroline, a metallo protease-inhibitor and bestatin, a basic and neutral metallo-AP inhibitor [10].

The 33 synthetic peptidomimetics were initially screened against the rPfA-M1 and erythrocyte cultures of *P. falciparum* 3D7, this last sensitive to chloroquine. Of them, five were identified as inhibiting the enzyme and the *in vitro* growth of the parasite at least 45 % at 20 and 25 µmol/L, respectively. Similar results were obtained against the chloroquine-resistant parasite strain FcB1. None of the five peptidomimetics was able to promote the loss of the erythrocytes' cellular integrity at 25 µmol/L. One of them, identified as KBE009 (Figure 2), was shown as a potent inhibitor of PfA-M1 at submicromolar concentrations (IC_{50} = 0.8 µmol/L) in dose-effect studies. This compound also displayed an antimalarial activity (IC_{50} = 18 µmol/L) as potent as that reported for bestatin (IC_{50} = 21 µmol/L) against the *P. falciparum* 3D7 strain. Notably, KBE009 showed the same reported potency for its antimalarial activity against the FcB1 parasite strain (IC_{50} = 16 µmol/L). It was proven by the same procedure that this peptidomimetic does not inhibit the pAPN at relevant therapeutic concentrations. Besides, KBE009 is non-cytotoxic up to 200 µmol/L in the HUVEC human cell line. Consequently, a new molecule is provided, belonging to the small group of known PfA-M1 inhibitors, showing potentialities to generate leader structures against malaria.

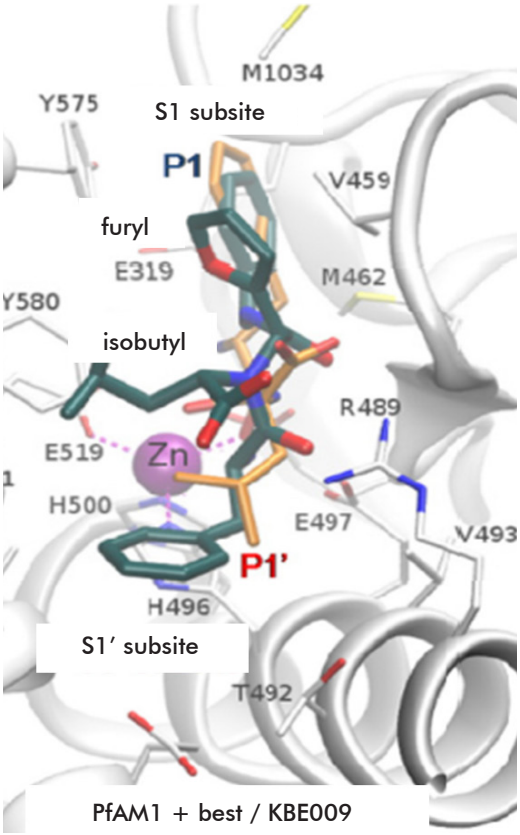

Figure 4. *In silico* molecular docking simulation for binding of KBE009 and bestatin to the active site of PfA-M1. Only a diasteromer of KBE009 is shown (dark green representation), both isomers bind similarly. Bestatin (best) is shown in orange. Coordination binding of Zn^{2+} is shown as discontinuous lines.

Then, it was predicted by *in silico* molecular docking simulations that the binding mode of KBE009 to PfA-M1 was through hydrophobic interactions and by geometric complementarities between the inhibitor and the active site of the AP, with no coordination with the Zn^{2+} ion of the enzyme (Figure 4). Such hydrophobic interactions are similar to those described by other groups for inhibitors of this AP [13]. Moreover, the selective inhibition of rPfA-M1 could be explained by the lower geometric complementarity and the few hydrophobic interactions between KBE009 and the active site of pAPN. Noteworthy, this novel interaction type differs from that previously reported for the rest of the known potent and selective inhibitors of this APN [25].

Then, a kinetic assay was validated with the fluorogenic substrates Ala- and Leu-AMC with both intact and erythrocyte culture-isolated *P. falciparum* 3D7 as primary source of native PfA-M1 and using bestatin as enzyme inhibitor. Bestatin inhibits the endogenous AP activity against both substrates with a potency equivalent to that shown against rPfA-M1 (IC_{50} < 0.31 µmol/L), expressed as interval. Similar potency values for inhibition of neutral metallo-type AP activity have been reported, using parasite extracts [30]. Otherwise, the endogenous AP activity, measured with the Arg-AMC substrate both in the intact and cell culture-isolated

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parasite, was inhibited only 50 % at 160 µmol/L bestatin. It was corroborated that KBE009 inhibits the AP activity in intact and isolated parasite against the Ala- and Leu-AMC substrates, with IC_{50} values of 82 and 27 µmol/L, respectively. These values were at the same order of magnitude of the IC_{50} for the KBE009 antimalarial activity *in vitro* (18 µmol/L). The endogenous AP activity was also inhibited by the AL005, YAN005 and KBE021 peptidomimetics showing antimalarial activity. They were not chosen due to their low selectivity for the inhibition of rPfA-M1, but used as positive controls in the experiment at 25 µmol/L.

Consistently, the KBE003 compound, which is a weak inhibitor of rPfA-M1 and a potent antimalarial compound, was employed at 200 µmol/L as negative control, not inhibiting the endogenous PfA-M1-type AP activity against the Ala-AMC substrate.

It was observed that the resistance of a transgenic strain of *P. falciparum* 3D7 over-expressing the *PfA-M1* gene against the anti-malarial effect of KBE009 was approximately 42-fold the resistance of the wild *P. falciparum* 3D7 strain. Such differential resistance was more than 88-, 6-, 37- and 17-fold the control compounds bestatin, AL005, YAN005 and KBE021, respectively. The same procedure has been previously used by other authors to establish that the PfA-M1 is a target of the bestatin's anti-malarial effect, and of another compound [31]. These evidences indicated that the *in vitro* anti-malarial effect of KBE009 and its inhibition of the native PfA-M1 are related. This supports the classification of this peptidomimetic as one of the few PfA-M1 inhibitors showing an anti-malarial activity mechanism of action experimentally related to the inhibition of this AP.

Conclusions

In summary, two microbial APNs were obtained recombinant, ePepN and PfA-M1, with kinetic properties similar to those of the native enzymes, able to be used as targets for inhibitors' identification. Using the Ugi multicomponent reactions, 33 peptidomimetics were synthesized, based on bestatin and actinonin, as well as 22 tetrazole peptidomimetics, all challenged against both APNs. Consequently, two new compounds were identified, one against the r-ePepN and *E. coli* cultures, and the other against rPfA-M1 and *P. falciparum* cultures. These two compounds represent potential leader structures for the development of novel and effective antibacterial and antimalarial therapeutic agents.

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Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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