Oligopeptidase B and B2: comparative modelling and virtual screening as searching tools for new antileishmanial compounds

ANA CAROLINA R. SODERO[1]*, ANA CAROLINA G. O. DOS SANTOS[1], JULIANA F. R. E MELLO[1], JÉSSICA B. DE JESUS[1], ALESSANDRA M. T. DE SOUZA[1], MARIA ISABEL C. RODRIGUES[2], SALVATORE G. DE SIMONE[2], CARLOS R. RODRIGUES[1] *and* HERBERT L. DE MATOS GUEDES[3,4]*

[1] *Laboratório de Modelagem Molecular e QSAR (MODMOLQSAR), Departamento de Fármacos e Medicamentos, Universidade Federal do Rio de Janeiro (UFRJ), Av. Carlos Chagas Filho, 373, 21941-599, Brazil*
[2] *Centro de Desenvolvimento Tecnológico em Saúde (CDTS)/Instituto Nacional de Ciência e Tecnologia de Inovação em Doenças de Populações Negligenciadas (INCT-IDPN), 21045-900 Rio de Janeiro, RJ, Brazil*
[3] *Grupo de Imunologia e Vacinologia, Laboratório de Inflamação, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro, RJ, Brazil*
[4] *Núcleo Multidisciplinar de Pesquisa UFRJ – Xerém em Biologia (NUMPEX-BIO), Polo Avançado de Xerém – Universidade Federal do Rio de Janeiro, 25245-390 Duque de Caxias, RJ, Brazil*

(*Received 1 July 2016; revised 19 October 2016; accepted 19 October 2016; first published online 29 December 2016*)

SUMMARY

Leishmaniasis are diseases caused by parasites of the genus *Leishmania* and transmitted to humans by the bite of infected insects of the subfamily Phlebotominae. Current drug therapy shows high toxicity and severe adverse effects. Recently, two oligopeptidases (OPBs) were identified in *Leishmania amazonensis*, namely oligopeptidase B (OPB) and oligopeptidase B2 (OPB2). These OPBs could be ideal targets, since both enzymes are expressed in all parasite lifecycle and were not identified in human. This work aimed to identify possible dual inhibitors of OPB and OPB2 from *L. amazonensis*. The three-dimensional structures of both enzymes were built by comparative modelling and used to perform a virtual screening of ZINC database by DOCK Blaster server. It is the first time that OPB models from *L. amazonensis* are used to virtual screening approach. Four hundred compounds were identified as possible inhibitors to each enzyme. The top scored compounds were submitted to refinement by AutoDock program. The best results suggest that compounds interact with important residues, as Tyr490, Glu612 and Arg655 (OPB numbers). The identified compounds showed better results than antipain and drugs currently used against leishmaniasis when ADMET *in silico* were performed. These compounds could be explored in order to find dual inhibitors of OPB and OPB2 from *L. amazonensis*.

Key words: Leishmaniasis, oligopeptidase, virtual screening, docking.

INTRODUCTION

The leishmaniasis are parasitic diseases with severe morbidity and mortality rates. According to World Health Organization, an estimated 1·3 million new cases and over 20 000 deaths occur annually (WHO, 2015). The leishmaniasis are caused by parasites of the genus *Leishmania* and the family *Trypanosomatidae*, which are transmitted to humans by the bite of infected insects of the subfamily Phlebotominae (Killick-Kendrick, 1999; WHO, 2015). Different etiological agents can cause leishmaniasis, but *Leishmania amazonensis* is the main agent of the anergic diffuse cutaneous leishmaniasis (Grimaldi & McMahan-Pratt, 1991; Santos *et al.* 2008).

Currently, the chemotherapy shows several adverse effects associated with these drugs, such as high toxicity, leading to treatment withdrawal and parasitic resistance. Thus, the development of new therapeutic agents against leishmaniasis, with lower adverse effects and toxicity, is an urgent priority. A widely used strategy in the drug discovery and development process is the identification of new therapeutic targets (Libusova *et al.* 2004; Padmanabhan *et al.* 2005; Reguera *et al.* 2005; Genestra *et al.* 2006; de Matos Guedes *et al.* 2007; de Matos Guedes *et al.* 2008; Kaur *et al.* 2012; Vermelho *et al.* 2014). The proteases from parasites have emerged as promising chemotherapeutic targets. The genome sequencing of *Leishmania major* allowed the identification of two oligopeptidases (OPBs) (Ivens *et al.* 2005). OPB has been previously known in trypanosomatids (Caler *et al.* 1998; Morty *et al.* 1999). The OPB from *L. amazonensis* were first cloned and sequenced by de Matos Guedes *et al.* (2007). Oligopeptidase B2

* Corresponding authors: Laboratório de Modelagem Molecular e QSAR (MODMOLQSAR), Departamento de Fármacos e Medicamentos, Universidade Federal do Rio de Janeiro (UFRJ), Av. Carlos Chagas Filho, 373, 21941-599, Brazil. E-mail: acrsodero@pharma.ufrj.br and Grupo de Imunologia e Vacinologia, Laboratório de Inflamação, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, 21941-902 Rio de Janeiro, RJ, Brazil. E-mail: herbert@biof.ufrj.br

doi:10.1017/S0031182016002237

(OPB2, also called OPB-like) was for the first time reported in 2008 using the genome sequence of *L. major* and cloned and sequenced from *L. amazonensis* (de Matos Guedes *et al*. 2008). These works also revealed the catalytic triad and the subsites S1 and S2. In addition, OPBs can be ideal targets to new therapeutic approaches, since both enzymes are expressed in all parasite lifecycle and there are no homologues identified in humans (de Matos Guedes *et al*. 2007; de Matos Guedes *et al*. 2008).

The VHTS (virtual high-throughput screening) has become a powerful tool for lead discovery. It is an *in silico* approach that can be used to search small molecules that possibly interact with a molecular target (Cerqueira *et al*. 2015). Hence, this work aims to identify dual inhibitors of OPB and OPB2 from *L. amazonensis* by *in silico* approach. Additionally, it intends to deeply understand their molecular inhibitory mechanisms, supporting the rational design of new antileishmanial drugs.

MATERIALS AND METHODS

Comparative modelling

The aminoacids sequences of OPB and OPB2 were obtained from UniProtKB database (access code A7XAB0 and A8QXT1, respectively) (UniProt, 2012). Template structure was obtained using the standard options of BLASTP server (Altschul *et al*. 1997) against the Protein Data Bank (Berman *et al*. 2000). The template selection considered the best results for identity, similarity and gaps.

T-Coffee server (Notredame, 2010) was used to the alignment step and the three-dimensional (3D) model was constructed using the Modeller program version 9.10 (Sali & Blundell, 1993). The quality of the models was evaluated using PROCHECK (Laskowski *et al*. 1993), PROVE (Pontius *et al*. 1996) and VERIFY-3D (Bowie *et al*. 1991; Luthy *et al*. 1992) programs.

Due to the lack of information about the 3D structure of the binding site from *L. amazonensis* OPBs, the binding sites aminoacids were defined based on the antipain inhibitor complexed with OPB from *L. major* (PDB code 2XE4) (McLuskey *et al*. 2010). Thereby, the residues within 5 Å distant of any atom of antipain were considered as the binding site. It shows a diameter larger than 20 Å, with 36 residues to OPB2 and 35 residues to OPB, which includes the catalytic triad (Fig. S1).

Virtual screening

To search for novel compounds with possible inhibitory activity against *L. amazonensis* OPB and OPB2, a virtual screening approach was employed using DOCK Blaster server (Irwin *et al*. 2009). DOCK Blaster is a fully automated docking system that performs the search of compounds that fits in the provided protein binding site in ZINC database (Irwin & Shoichet, 2005). Subset 12 (clean-fragments) containing 1 611 889 entries was selected. Thus, score calculations for each compound could allow a large-scale molecular docking study.

The top-ranked compounds predicted to fit each enzyme were selected to perform a refinement step using AutoDock4·2 program (Morris *et al*. 1998, 2009).

Table 1. Lamarckian Genetic Algorithm values used to find the best parameters set to molecular docking

Parameters	Values
Initial population	50, 80, 100, 150
Number of energy assessments	2 500 000, 5 000 000
Mutation rate	0·02, 0·20, 0·30
Crossover rate	0·50, 0·80, 0·90
Elitism	1, 10
Numbers of runs	20, 25, 30, 40, 50, 100, 150

Molecular docking

In order to establish a protocol in AutoDock program, the 3D structure of antipain (compound 1) was re-docked into the co-crystallized OPB from *L. major*. Antipain was considered partially flexible, with the backbone rigid. Lamarckian Genetic Algorithm was used and parameters such as initial population, number of energy assessments, mutation rate, crossover rate, elitism and numbers of runs were modified (Table 1). The best parameters set were used to perform the molecular docking of the top-ranked compounds from DOCK Blaster.

The DockBlaster binding poses were used as starting structures for docking simulations at AutoDock. Visual inspection of the generated complexes was performed using AutoDockTools (Morris *et al*. 2009) and PyMOL (The PyMOL Molecular Graphics System, Version 1.4.1 Schrödinger, LLC.) programs.

Cross-docking of the top-ranked compounds

The cross-docking approach was employed to compare the binding affinities of compounds to identify ligands that can synergistically target both enzymes. The best compound obtained by AutoDock at OPB was used as starting structure for docking into OPB2 binding site by AutoDock program. The reverse was also performed: the best compound obtained by AutoDock to OPB2 was used as starting structure to docking into OPB-binding site.

In silico physicochemical and toxicological analyses

The ADMET assessment of the four best compounds was performed using ADMET Predictor™

https://doi.org/10.1017/S0031182016002237 Published online by Cambridge University Press

Table 2. Comparison of identity, similarity and gaps between primary structures of the *L. amazonensis* OPB and OPB2 with *L. major* OPB

Enzymes	OPB (%)	OPB2 (%)
Identity	90	23
Similarity	40	40
Gaps	10	10

(Simulation Plus Inc., Lancaster, CA), an approach based on the degree of concordance among the individual QSAR networks in an ensemble model. Drugs currently used against leishmaniasis were also calculated and compared with the selected compounds. The Lipinski's Rule of 5, toxicological risks and ADMET risk were evaluated. ADMET risk is a score was parameterized based on predicted properties that represent potential obstacles to a compound being successfully developed. ADMET risk provide a range between 0 and 24, which the greater the number, the higher probability to have pharmacokinetic and toxicological problems.

RESULTS

Comparative modelling

Comparison of OPB and OPB2 sequences showed 31% identity. The enzymes have 731 and 905 amino acids, respectively, being 114 amino acids of OPB2 C-terminal extension. This unusual C-terminal extension was observed in several proteins of parasites, such as cysteine protease (Mottram *et al*. 1989), 3-mercaptopyruvate sulphutransferase of *Trypanosomatidae* spp. (Williams *et al*. 2003) and aspartic protease of *Schistosoma* spp. (Wong *et al*. 1997). However, the C-terminal sequence of OPB2 has no homologous template at PDB. Since it is not associated with enzyme activity (de Matos Guedes *et al*. 2008; Polgar, 2002), we did not build its structure.

The accuracy of the models depends on the sequence identity between template and target sequences, where higher identity gives better results. Herein, the comparative modelling was carried out using the 3D structure of OPB from *L. major* as template (PDB code 2XE4, 1·65 Å resolution) for both enzymes, where the identity, similarity and gaps are showed at Table 2. According to Blast results, the alignment between OPB and OPB2 from *L. amazonensis* with OPB from *L. major* covers 98 and 81% of total sequences, respectively (Fig. S3 and S4, Supporting Information).

The alignment obtained from T-COFFEE was provided as input in MODELLER9v10 to generate the 3D model of OPB and OPB2 (Fig. 1). According to the Ramachandran plot, the OPB model showed 92·3% residues in the allowed regions and only 0·3% residues in the disallowed regions. Similar results were observed for OPB2 model, which showed more than 90% residues in the allowed regions and 0·7% in the disallowed regions (Fig. S2, Supporting Information). Verify 3D results of OPB and OPB2 presented 98·32 and 72·81% residues with compatible 1D–3D scores >0·2, respectively. The models were considered useful by validation results.

Analysis of OPB and OPB2 molecular models

The models and the template structures were superimposed to evaluate the structural similarities. The catalytic triad of the three enzymes was conserved in a similar conformation (Fig. 1, S3 and S4).

Through the superposition of OPB and OPB2 3D structure models, it was observed that the secondary and tertiary structures were conserved, with a root-mean-square deviation (RMSD) of 0·67 Å. The comparison between predicted models suggests they have structural resemblance. The enzymes consist of two domains: the catalytic and the β-propeller domains (de Matos Guedes *et al*. 2007). The catalytic domain of both enzymes presented 10 β strands surrounded by 13α-helix at OPB and 12α-helix at OPB2. The β-propeller domain of the OPB model has 14 pairs of β strands distorted and arranged radially around the central tunnel of the enzyme, while OPB2 model has 13 pairs of β strands in the same arrangement. Few differences in the number of loops were observed.

The analysis of the molecular electrostatic potential map of OPB and OPB2 *L. amazonensis* surfaces showed different charges distribution profile (Fig. S5, Supporting Information). The OPB showed more negative regions in the surface, while OPB2 showed more positive regions as similarly described to prolyl oligopeptidase family (Polgar, 2002; de Matos Guedes *et al*. 2007). However, the binding site surface of OPB2 from *L. amazonensis* showed more negative regions than the OPB. The negative regions of OPB2 binding site are due mainly to residues Glu539, Glu606, Glu659 and Asp702. Nevertheless, Glu539 and Glu606 residues are substituted by Ile492 and Ser560 at OPB-binding site, respectively, which contribute to a less negative pocket. These residues could be related to enzymes specificities.

The structure obtained by X-ray diffraction of *L. major* OPB in complex with the antipain was used to provide detailed information about the enzyme-binding pocket (McLuskey *et al*. 2010). For this reason, the *L. amazonensis* OPB- and OPB2-binding sites were selected based on *L. major* OPB structure. To analyse the catalytic environment, residues about 5 Å from antipain inhibitor, including the catalytic triad residues were selected as the binding site to run the DOCK Blaster calculation.

https://doi.org/10.1017/S0031182016002237 Published online by Cambridge University Press

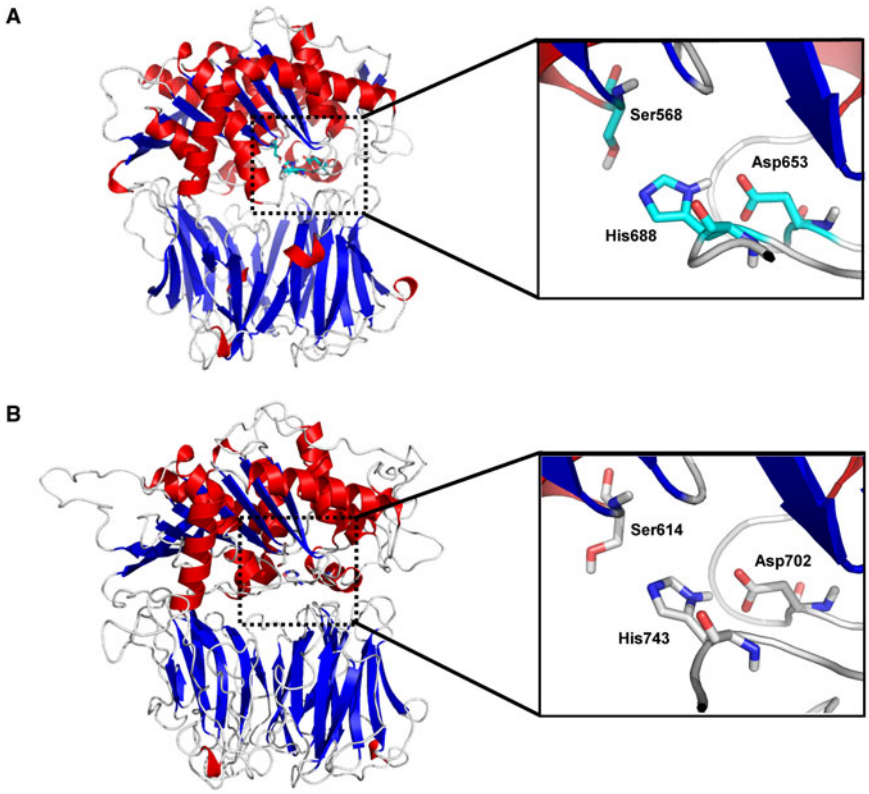


Fig. 1. Cartoon diagrams of *L. amazonensis* (A) OPB and (B) OPB2 models showing the zoomed view of the catalytic triads in stick (Ser568, Asp653, His688 and Ser614, Asp702 and His743, respectively).

Virtual screening and molecular docking into binding sites of OPB and OPB2 from L. amazonensis

The virtual screening on *L. amazonensis* OPB and OPB2 was performed in two steps. Initially, the search of possible inhibitors for each enzyme by Virtual Screening was performed with DOCK Blaster server, followed by data refinement by AutoDock program. Analysed compounds are described at Table 3.

Initially, the subset 12 (clean-fragments), containing 1 611 889 entries, was selected. The virtual screening result contained a list of top 400 compounds from ZINC database for each enzyme, which possibly interacts with the target ranked by energy score.

The binding poses of the top 40 ranked compounds were used as starting structures to the refinement step by AutoDock program. To validate the docking protocol at AutoDock program, we used the antipain inhibitor complexed to OPB-binding site of *L. major*. The best parameters set was obtained using Lamarckian Genetic Algorithm, initial population of 100, number of energy assessments of 5 000 000, mutation rate of 0·3, crossover of 0·9, elitism of 10 and 100 runs. The lowest energy conformation (−6·68 kcal mol^{-1}) was superimposed to the crystal structure and showed a RMSD of 3·05 Å, indicating high similarity among conformations. The P3 position participates in just a single interaction through hydrogen bond with Ser253 and it is located in a solvent-filled cavity (McLuskey *et al*. 2010). Consequently, this position showed the most different residue conformation (Fig. S6, Supporting Information).

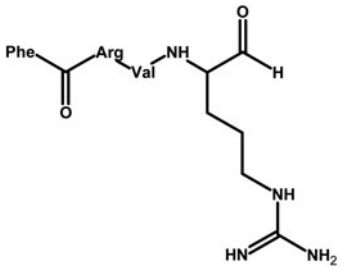
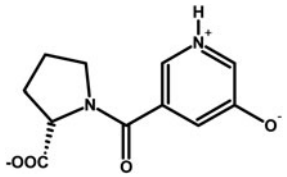
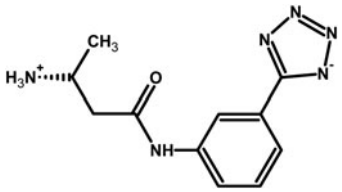
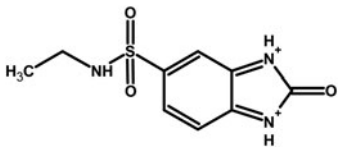
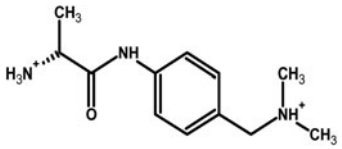
OPB of Leishmania amazonensis

Compound 2, 1-(5-hydroxy-pyridine-3-carbonyl)-pyrrolidine-2-carboxylic acid (ZINC code 19735155), showed the highest score (−71·94 kcal mol^{-1}) when docked into OPB-binding site by DOCK Blaster server. It interacts with the binding site through hydrogen bonds with Ser568 and Glu612, π-stacking with Tyr490, and salt bridges with Glu612 and Arg567 (Fig. 2A).

The binding poses of the top 40 ranked compounds obtained by Virtual Screening to OPB were submitted to refinement by using AutoDock program. The results showed the complex with compound 3 [(3R)-3-amino-N-[3-(1H-tetrazol-5-yl)phenyl]butanamide, ZINC code 37608688] with the lowest estimated binding energy (−10·32 kcal M^{-1}), and the ligand was located close to the catalytic site (Fig. 2A). It interacts through hydrogen bond with Glu612, Pro607, Arg655 and Tyr490, and hydrophobic interactions with His688 and Arg655.

https://doi.org/10.1017/S0031182016002237 Published online by Cambridge University Press

Table 3. Structures and scores of the top hits screened by DOCK Blaster and analysed by AutoDock program

Compound	ZINC code	2D structure	DOCK Blaster Score (kcal mol^{-1})		AutoDock Score (kcal mol^{-1})	
			OPB	OPB2	OPB	OPB2
1 (Antipain)	–		–	–	−5·39	−4·92
2	19735155		−71·94	–	−5·45	−5·95
3	37608688		−66·46	–	−10·32	−9·86
4	63887176		–	−87·6	−6·77	−6·77
5	37042497		–	−60·09	−8·41	−10·14

OPB2 of L. amazonensis

When *VS* was performed against OPB2-binding site, the top-scored compound was the N-ethyl-2-oxo-benzimidazole-5-sulphonamide (compound 4, ZINC code 63887176), interacting through hydrogen bond with Glu659 and π–π stacking interaction with Tyr537 (Fig. 2B).

After docking refinement of results obtained for OPB2, the complex formed with compound 5 [(2R)-2-amino-N-[4-(dimethylaminomethyl)phenyl]propanamide, ZINC code 37042497] showed the lowest estimated binding energy (−10·14 kcal M^{-1}) by AutoDock program. The selected docking pose is also located close to the catalytic site and interacts through hydrogen bonds with Glu539, Ser614, Arg704, Glu659 and Phe641 residues (Fig. 2B). As the compound is protonated, it also makes ionic interactions with Glu539 and Glu659. Besides, it also performs hydrophobic contacts with Tyr537, Phe641, Leu655 and Ala615 (Fig. 2B).

Cross-docking of the top-ranked compounds

In order to find dual inhibitors of OPB and OPB2 from *L. amazonensis*, we performed the cross-docking of the best results obtained by AutoDock program into both binding sites.

Then, compound 5 was also analysed at OPB-binding site (Fig. 3A). The compound showed hydrogen bonds with Arg655 and Glu612 residues. Besides, it also forms an ionic interaction with Glu612 and hydrophobic contacts with Leu608, Tyr490, Ala569 and Val656.

Compound 3 was also docked into OPB2-binding site to observe possible interactions (Fig. 3B). As in OPB, it binds near the catalytic site. This compound performs hydrogen bonds with Tyr537, Pro654, Glu659 and Arg704. Moreover, compound 3 participates on hydrophobic interactions with Ala615, Phe641, Leu653 and Val705.

In order to obtain more information about the inhibition mechanism of OPB and OPB2 from *L.*

https://doi.org/10.1017/S0031182016002237 Published online by Cambridge University Press

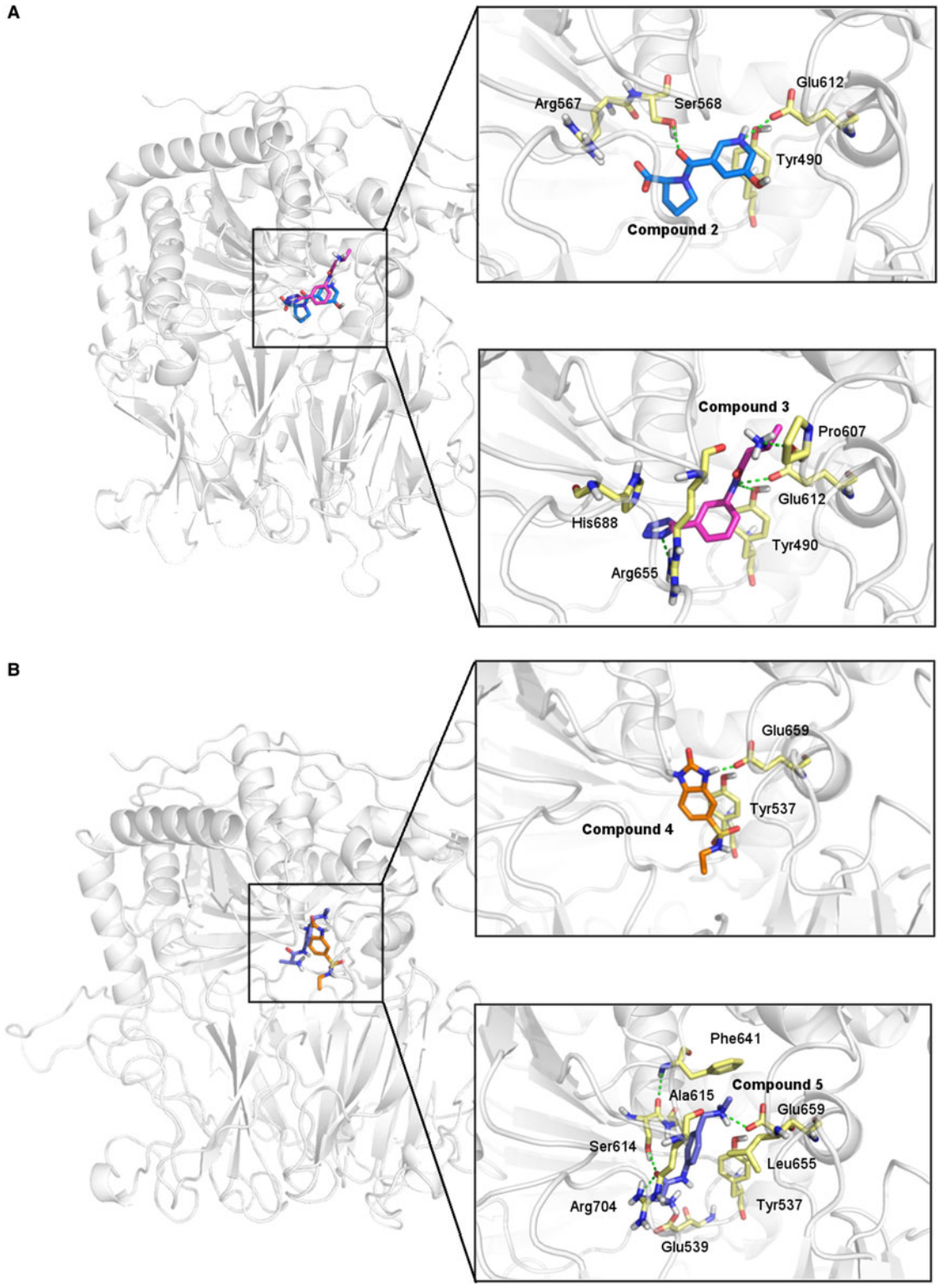


Fig. 2. Binding mode analysis of identified compounds. (A) Compounds 2 (blue) and 3 (pink) at OPB from *L. amazonensis*; (B) Compounds 4 (orange) and 5 (purple) at OPB2 from *L. amazonensis*. Residues involved on the interactions are shown in yellow and hydrogen bonds are coloured in green.

amazonensis, molecular docking of antipain was also performed in both enzymes (Fig. 3).

The result of antipain-OPB complex showed hydrogen bonds with Ser568, Glu612, Glu660 and His688 residues. Besides, it can perform hydrogen bond or ionic interaction with Arg655 residue (Fig. 3A). The antipain also interacts through hydrogen bonds with Tyr537, Glu659 and Arg704 (Fig. 3B).

In silico pharmacokinetic and toxicity analyses

The ADMET evaluations were carried out to the four best compounds and some currently available

https://doi.org/10.1017/S0031182016002237 Published online by Cambridge University Press

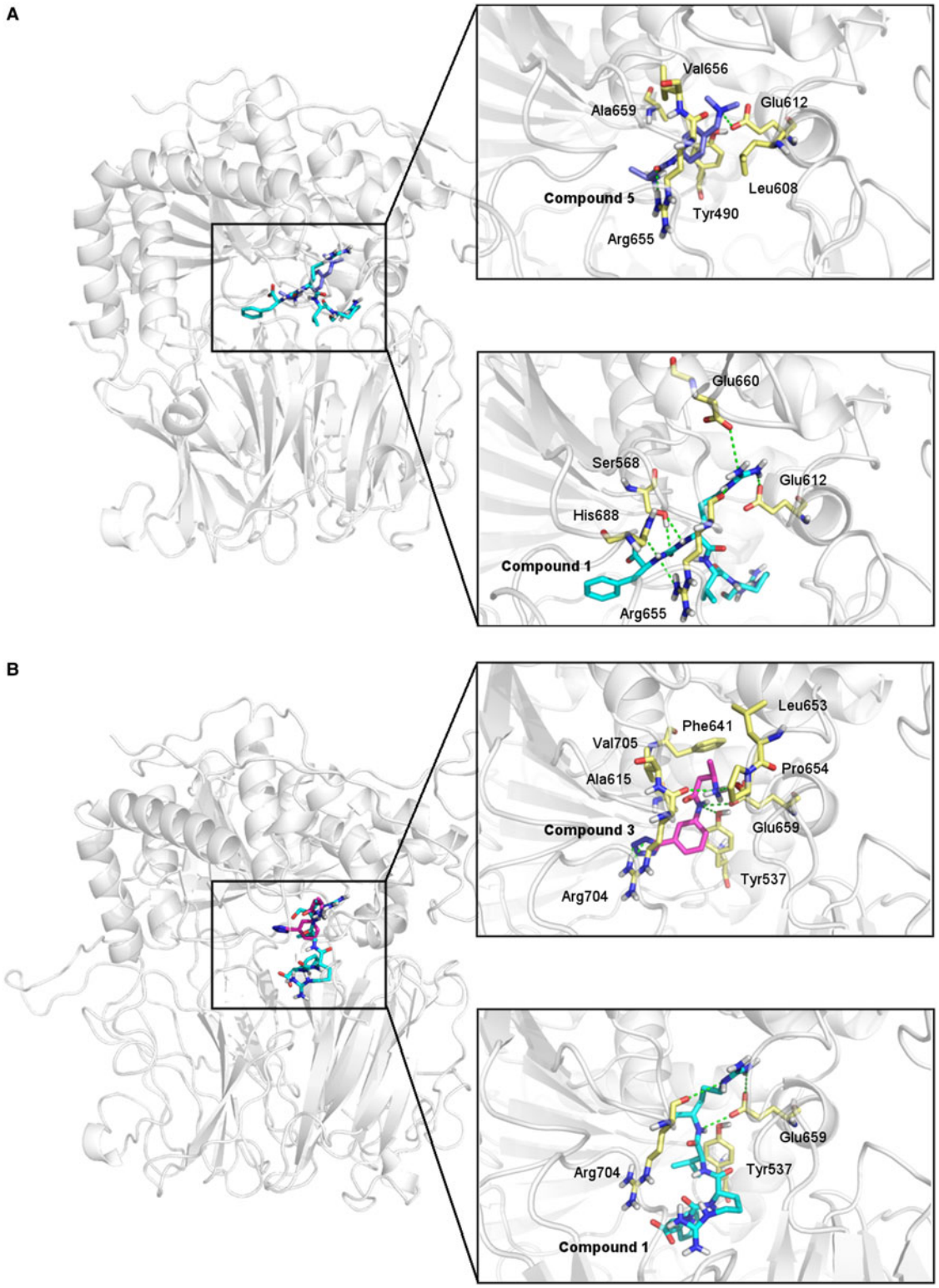


Fig. 3. Binding modes of identified compounds obtained by AutoDock program. (A) Compounds 1 (cyan) and 5 (purple) with OPB from *L. amazonensis*; (B) Compounds 1 (cyan) and 3 (pink) with OPB2 from *L. amazonensis*. Residues involved on the interactions are shown in yellow and hydrogen bonds are coloured in green.

drugs against *Leishmania* sp., as meglumine antimoniate and pentamidine. The selected ZINC compounds were also compared with antipain, a classical serine protease inhibitor (Suda *et al*. 1972). Lipinski rule of 5 were evaluated since is related with compounds bioavailability and all identified compounds respected its criteria, overcoming the currently available drugs and antipain.

Our studies demonstrated that compounds 2, 3 and 5 presented Phase I metabolism mediated by different enzymes: compound 2 may be metabolized by CYP2C9, compounds 3 and 5 by

https://doi.org/10.1017/S0031182016002237 Published online by Cambridge University Press

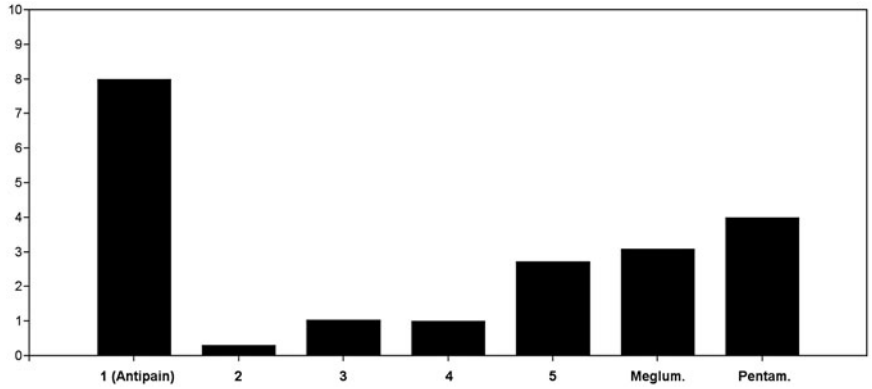


Fig. 4. ADMET Risk evaluation for antipain, selected compounds (2–5) and drugs currently used in leishmaniais therapy, meglumine (meglum.) and pentamidine (Pentam.).

CYP1A2; compound 5 may also be metabolized by CYP2D6.

In the toxicity evaluation, only compound 2 did not showed a risk of carcinogenicity in rats. Furthermore, the analysis indicates low risk of mutagenicity for pentamidine, whereas the new compounds showed no risk of mutagenicity. Previous experimental data showed that pentamidine is not mutagenic but could tight bind to DNA (Stauffert *et al*. 1990). Finally, hepatoxicity was predicted only for pentamidine, based on abnormal blood level elevation of relevant biomarkers as serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase.

After all, pharmacokinetic and toxicological parameters were compiled in ADMET risk. All selected compounds showed a good profile, with values ranging from 0·3 to 3·7 overcoming the meglumine antimoniate, pentamidine and antipain results that showed higher ADMET risk values, ranging from 3·1 to 8·0 (Fig. 4).

DISCUSSION

Leishmaniasis therapy is facing problems due to the lack of effective and safe drugs, besides the emergence of resistance (Vermelho *et al*. 2014). The OPBs are emerging as new targets to leishmaniasis once they are not present in humans (Morty *et al*. 1999; de Matos Guedes *et al*. 2007; de Matos Guedes *et al*. 2008). The knowledge about the 3D structures of proteins is important to provide valuable information about function and for rational drug design (Dill & MacCallum, 2012). Until now, no experimentally determined atomic structures for OPB and OPB2 of *L. amazonensis* were obtained. In this case, comparative modelling is the most commonly used computational structure prediction method (Dill & MacCallum, 2012).

Using comparative modelling was possible to construct molecular models for OPB and for OBP2 (Fig. 1). Based on sequence alignment and phylogenetic analysis, OPB2 is considered a new member of prolyl oligopeptidase family (de Matos Guedes *et al*. 2008). However, it displayed a similar and conserved structure of prolyl oligopeptidase family, as observed in Fig. 1 (Polgar, 2002). Based on the structures, these results support a hypothesis about compensation of OPB2 in absence of OPB (Munday *et al*. 2011). Nevertheless, in the same publication, it was observed that mutant without OPB, that has been reported cleavage Arg substrate (McLuskey *et al*. 2010), lost the capacity to cleavage Bz-R-AMC (Munday *et al*. 2011) indicating a different substrate preference of OPB2 or a different cell localization. Our surface charge analysis demonstrated that OPB2 has a positive charge on surface, different for OPB that has a negative charge. There is an adaptation of protein surface to subcellular localization (Andrade *et al*. 1998), the difference observed for OPB2 indicates a different cell localization and instead a different cell function. OPB was demonstrated to be a cytoplasmatic protein (Munday *et al*. 2011) and we have some evidences that OPB2 is present in vesicles, possible in endosomes (de Matos Guedes *et al*. 2008). Independently of the differences, we observed the similarities of OPB and OPB2, and we studied the possibility of identify new drugs that can inhibit both enzymes at the same time.

The virtual screening approach, by DOCK Blaster server, found compound 2 as the top ranked when docked into OPB-binding site. This compound interacts with Ser568 and Glu612, which are conserved at OPB enzymes and are described as key residues to ligand interaction with OPB from *L. major* (Ser577 and Glu621, respectively) (McLuskey *et al*. 2010; Goyal *et al*. 2014). Besides, Ser568 residue is a member of the catalytic triad, thus it has a special importance to enzymatic activity (McLuskey *et al*. 2010).

After the refinement step, compound 3 was ranked with the lowest estimated binding energy. It

https://doi.org/10.1017/S0031182016002237 Published online by Cambridge University Press

interacts through hydrogen bond with Glu612. There are evidences about the importance of the Glu612 residue, which is conserved throughout the OPB family and is generally involved in substrate specificity (McLuskey *et al*. 2010). Besides, this compound also performs hydrogen bonds with Pro607, Arg655 and Tyr490, and hydrophobic interactions with His688 and Arg655. These residues are conserved at OPB from *L. major* and OPB2 from *L. amazonensis*. Additionally, they were also found as important to the complex stabilization in OPB from *L. major* (Goyal *et al*. 2014).

Compound 4 was the top scored molecule after VS by DOCK Blaster into OPB2-binding site. However, after docking refinement by AutoDock, compound 5 showed the lowest estimated binding energy. When VS was performed against OPB2-binding site, compound 4 was the top scored compound interacting with Tyr537, Glu539, Ser614, Ala615, Phe641, Leu655, Glu659 and Arg704.

As we want to find dual inhibitors of OPBs from *L. amazonensis*, we also performed the cross-docking. Compound 5 was analysed at OPB-binding site and compound 3 was analysed at OPB2-binding site. Both compounds interact with residues conserved at OPBs from *L. amazonensis* and *L. major* and are pointed as important to complex stabilization (McLuskey *et al*. 2010; Goyal *et al*. 2014).

We also studied antipain into binding sites of OPB and OPB2 of *L. amazonensis*. All the residues that interact to antipain were already described as important to interaction between OPB from *L. major* and ligands found by virtual screening (Goyal *et al*. 2014).

Comparing the compounds found by virtual screening and antipain, all presented important interactions, as others studies of OPB from *L. major* (McLuskey *et al*. 2010; Goyal *et al*. 2014). However, the better scores obtained to ZINC molecules suggest that they could have better performance as OPBs inhibitors than antipain.

Compound 4 showed similar and lower estimated binding energy for both OPB and OPB2. It can also be observed a very similar binding position, suggesting that compound 4 can be further modified to better interact with the OPB-binding site, exploring the enzymes specificity.

Nowadays, the leishmaniasis therapy is responsible for several side-effects, mainly associated of high toxicity (Singh & Sundar, 2012). The ADMET evaluations were carried out to compare the four selected ZINC compounds (2, 3, 4 and 5) with some currently available drugs against *Leishmania* sp., amongst them, meglumine antimoniate and pentamidine. Interestingly, the compounds found by VS presented a predicted toxicity risk equal or less to the current drugs used in the treatment, which could represent a good perspective on the search for new compounds against *Leishmania* sp. The next steps would be to evaluate the candidates against both enzymes and against parasites.

Concluding remarks

Herein, *L. amazonensis* OPB and OPB2 3D models were obtained using comparative modelling approach and properly validated. The models were then used for virtual screening, which allowed finding promising compounds against leishmaniasis. The compounds selected in this study showed important interactions with OPB and OPB2, indicating they could act as inhibitors of these proteins. The results indicate that compound 4 could be further explored in order to find dual inhibitors of both enzymes.

The pharmacokinetic and toxicity assessments also showed good results. Furthermore, this study provided possible competitive inhibitors of OPB and OPB2 from *L. amazonensis* and its suggested mechanism of action, which could indicate these molecules as promising compounds for rational development of new antileishmanial drugs.

SUPPLEMENTARY MATERIAL

The supplementary material for this article can be found at https://doi.org/10.1017/S0031182016002237

ACKNOWLEDGEMENTS

Thanks are due to the CNPQ (National Council of Research of Brazil), CAPES and FAPERJ for funding this work.

COMPETING INTERESTS

The authors declare that they have no competing interests.

REFERENCES

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W. and Lipman, D. J. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**, 3389–3402.

Andrade, M. A., O'Donoghue, S. I. and Rost, B. (1998). Adaptation of protein surfaces to subcellular location. *Journal of Molecular Biology* **276**, 517–525.

Berman, H. M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T. N., Weissig, H., Shindyalov, I. N. and Bourne, P. E. (2000). The Protein Data Bank. *Nucleic Acids Research* **28**, 235–242.

Bowie, J. U., Luthy, R. and Eisenberg, D. (1991). A method to identify protein sequences that fold into a known three-dimensional structure. *Science* **253**, 164–170.

Caler, E.V., Avalos, S.V., Haynes, P.A., Andrews, N.W. and Burleigh, B.A. (1998). Oligopeptidase B-dependent signaling mediates host cell invasion by *Trypanosoma cruzi*. *The EMBO Journal* **17**, 4975–4986.

Cerqueira, N. M., Gesto, D., Oliveira, E. F., Santos-Martins, D., Bras, N. F., Sousa, S. F., Fernandes, P. A. and Ramos, M. J. (2015). Receptor-based virtual screening protocol for drug discovery. *Archives of Biochemistry and Biophysics* **582**, 56–67.

de Matos Guedes, H. L., Carneiro, M. P., Gomes, D. C., Rossi-Bergmanmn, B. and Giovanni de Simone, S. (2007). Oligopeptidase B from *L. amazonensis*: molecular cloning, gene expression analysis and molecular model. *Parasitology Research* **101**, 853–863.

de Matos Guedes, H. L. M., Carvalho, R. S. N., Gomes, D. C. O., Rossi-Bergmann, B. and De-Simone, S. G. (2008). Oligopeptidase B-2 from *Leishmania amazonensis* with an unusual C-terminal extension. *Acta Parasitologica* **53**, 197–204.

Dill, K. A. and MacCallum, J. L. (2012). The protein-folding problem, 50 years on. *Science* **338**, 1042–1046.

Genestra, M., Guedes-Silva, D., Souza, W. J. S., Cysne-Finkelstein, L., Soares-Bezerra, R. J., Monteiro, F. P. and Leon, L. L. (2006). Nitric oxide synthase (NOS) characterization in *Leishmania amazonensis* axenic amastigotes. *Archives of Medical Research* **37**, 328–333.

Goyal, S., Grover, S., Dhanjal, J.K., Goyal, M., Tyagi, C., Chacko, S. and Grover, A. (2014). Mechanistic insights into mode of actions of novel oligopeptidase B inhibitors for combating leishmaniasis. *Journal of Molecular Modeling* **20**, 2099.

Grimaldi, G., Jr. and McMahan-Pratt, D. (1991). Leishmaniasis and its etiologic agents in the New World: an overview. *Progress in Clinical Parasitology* **2**, 73–118.

Irwin, J. J. and Shoichet, B. K. (2005). ZINC – a free database of commercially available compounds for virtual screening. *Journal of Chemical Information and Modeling* **45**, 177–182.

Irwin, J. J., Shoichet, B. K., Mysinger, M. M., Huang, N., Colizzi, F., Wassam, P. and Cao, Y. (2009). Automated docking screens: a feasibility study. *Journal of Medicinal Chemistry* **52**, 5712–5720.

Ivens, A. C., Peacock, C. S., Worthey, E. A., Murphy, L., Aggarwal, G., Berriman, M., Sisk, E., Rajandream, M. A., Adlem, E., Aert, R., Anupama, A., Apostolou, Z., Attipoe, P., Bason, N., Bauser, C., Beck, A., Beverley, S. M., Bianchettin, G., Borzym, K., Bothe, G., Bruschi, C. V., Collins, M., Cadag, E., Ciarloni, L., Clayton, C., Coulson, R. M., Cronin, A., Cruz, A. K., Davies, R. M., De Gaudenzi, J. *et al*. (2005). The genome of the kinetoplastid parasite, *Leishmania major*. *Science* **309**, 436–442.

Kaur, P. K., Dinesh, N., Soumya, N., Babu, N. K. and Singh, S. (2012). Identification and characterization of a novel ribose 5-phosphate isomerase B from *Leishmania donovani*. *Biochemical and Biophysical Research Communications* **421**, 51–56.

Killick-Kendrick, R. (1999). The biology and control of phlebotomine sand flies. *Clinics in Dermatology* **17**, 279–289.

Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M. (1993). PROCHECK - a program to check the stereochemical quality of protein structures. *Journal of Applied Crystallography* **26**, 283–291.

Libusova, L., Sulimenko, T., Sulimenko, V., Hozak, P. and Draber, P. (2004). Gamma-tubulin in *Leishmania*: cell cycle-dependent changes in subcellular localization and heterogeneity of its isoforms. *Experimental Cell Research* **295**, 375–386.

Luthy, R., Bowie, J. U. and Eisenberg, D. (1992). Assessment of protein models with three-dimensional profiles. *Nature* **356**, 83–85.

McLuskey, K., Paterson, N. G., Bland, N. D., Isaacs, N. W. and Mottram, J. C. (2010). Crystal structure of *Leishmania major* oligopeptidase B gives insight into the enzymatic properties of a trypanosomatid virulence factor. *Journal of Biological Chemistry* **285**, 39249–39259.

Morris, G. M., Goodsell, D. S., Halliday, R. S., Huey, R., Hart, W. E., Belew, R. K. and Olson, A. J. (1998). Automated docking using a Lamarckian genetic algorithm and an empirical binding free energy function. *Journal of Computational Chemistry* **19**, 1639–1662.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S. and Olson, A. J. (2009). AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *Journal of Computational Chemistry* **30**, 2785–2791.

Morty, R. E., Lonsdale-Eccles, J. D., Morehead, J., Caler, E. V., Mentele, R., Auerswald, E. A., Coetzer, T. H., Andrews, N. W. and Burleigh, B. A. (1999). Oligopeptidase B from *Trypanosoma brucei*, a new member of an emerging subgroup of serine oligopeptidases. *Journal of Biological Chemistry* **274**, 26149–26156.

Mottram, J. C., North, M.J., Barry, J.D. and Coombs, G.H. (1989). A cysteine proteinase cDNA from Trypanosoma brucei predicts an enzyme with an unusual C-terminal extension. *FEBS Letters* **258**, 211–215.

Munday, J. C., McLuskey, K., Brown, E., Coombs, G. H. and Mottram, J. C. (2011). Oligopeptidase B deficient mutants of *Leishmania major*. *Molecular & Biochemical Parasitology* **175**, 49–57.

Notredame, C. (2010). Computing multiple sequence/structure alignments with the T-coffee package. *Current Protocols in Bioinformatics* Chapter 3, Unit 3, **8**, 1–25.

Padmanabhan, P. K., Mukherjee, A., Singh, S., Chattopadhyaya, S., Gowri, V. S., Myler, P. J., Srinivasan, N. and Madhubala, R. (2005). Glyoxalase I from *Leishmania donovani*: a potential target for anti-parasite drug. *Biochemical and Biophysical Research Communications* **337**, 1237–1248.

Polgar, L. (2002). The prolyl oligopeptidase family. *Cellular and Molecular Life Sciences* **59**, 349–362.

Pontius, J., Richelle, J. and Wodak, S. J. (1996). Deviations from standard atomic volumes as a quality measure for protein crystal structures. *Journal of Molecular Biology* **264**, 121–136.

Reguera, R. M., Tekwani, B. L. and Balana-Fouce, R. (2005). Polyamine transport in parasites: a potential target for new antiparasitic drug development. *Comparative Biochemistry and Physiology C – Toxicology & Pharmacology* **140**, 151–164.

Sali, A. and Blundell, T. L. (1993). Comparative protein modeling by satisfaction of spatial restraints. *Journal of Molecular Biology* **234**, 779–815.

Santos, D. O., Coutinho, C. E., Madeira, M. F., Bottino, C. G., Vieira, R. T., Nascimento, S. B., Bernardino, A., Bourguignon, S. C., Corte-Real, S., Pinho, R. T., Rodrigues, C. R. and Castro, H. C. (2008). Leishmaniasis treatment – a challenge that remains: a review. *Parasitology Research* **103**, 1–10.

Singh, B. and Sundar, S. (2012). Leishmaniasis: vaccine candidates and perspectives. *Vaccine* **30**, 3834–3842.

Stauffert, I., Paulini, H., Steinmann, U., Sippel, H. and Estler, C. J. (1990). Investigations on mutagenicity and genotoxicity of pentamidine and some related trypanocidal diamidines. *Mutation Research* **245**, 93–98.

Suda, H., Aoyagi, T., Hamada, M., Takeuchi, T. and Umezawa, H. (1972). Antipain, a new protease inhibitor isolated from actinomycetes. *Journal of Antibiotics (Tokyo)* **25**, 263–266.

UniProt, C. (2012). Reorganizing the protein space at the Universal Protein Resource (UniProt). *Nucleic Acids Research* **40**, D71–D75.

Vermelho, A.B., Supuran, C.T., Cardoso, V., Menezes, D., Silva, J.R. d.A., Ferreira, J.L.P., Amaral, A.C.F. and Rodrigues, I.A. (2014). Leishmaniasis: possible new strategies for treatment. In Leishmaniasis – Trends in Epidemiology, Diagnosis and Treatment (ed. Claborn, D.), pp. 351–376. InTech, DOI: 10.5772/57388. Available from: http://www.intechopen.com/books/leishmaniasis-trends-in-epidemiology-diagnosis-and-treatment/leishmaniasis-possible-new-strategies-for-treatment

Williams, R.A., Kelly, S.M., Mottram, J.C. and Coombs, G.H. (2003). 3-Mercaptopyruvate sulfurtransferase of Leishmania contains an unusual C-terminal extension and is involved in thioredoxin and antioxidant metabolism. *Journal of Biological Chemistry* **278**, 1480–1486.

Wong, J.Y., Harrop, S.A., Day, S.R. and Brindley, P.J. (1997). Schistosomes express two forms of cathepsin D. *Biochimica et Biophysica Acta* **1338**, 156–160.

World Health Organization, WHO (2015). "Leishmaniasis." Retrieved 26.02.2016, 2015, from http://www.who.int/topics/leishmaniasis/en

https://doi.org/10.1017/S0031182016002237 Published online by Cambridge University Press