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SHORT COMMENT

# Why strategies to control *Leishmania* spp. multiplication based on the use of proteinase inhibitors should consider multiple targets and not only a single enzyme

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**Abstract** The use of proteinases as targets to develop novel chemotherapies against *Leishmania* spp. infections is a very promising strategy. Based on a previous study by Goyal et al. [J Mol Model (2014) 20:2099], we discuss herein the idea that only a combined treatment with distinct proteinase inhibitors would be an effective antileishmanial therapy.

**Keywords** Leishmania · Oligopeptidase B · Proteinase inhibitors · Antileishmanial therapy

## Short comments

Leishmaniases are parasitic tropical diseases that affect humans. Currently, pentavalent antimony compounds (Glucantim and Pentostam) are the drugs of first choice to treat these diseases, while Amphotericin B, Pentamidine, Miltefosine or Aminosidine are used as secondary options [1]. In fact, to date, near 25 compounds or drug formulations have been shown to present antileishmanial effects; however, only a few have been tested properly, and even fewer are in the process to be considered for clinical use [2].

As the current chemotherapy options for these diseases are not entirely efficient and resistant strains are emerging, other components of the parasite have been proposed as potential targets to control the cutaneous or visceral infections; and, one such target are the proteinases [3].

A pivotal advantage of using proteinases as targets for leishmaniasis treatment derives from the fact that proteolysis is a common mechanism of activation or inactivation of enzymes involved in an array of biological processes, such as digestion, blood clotting, cell differentiation and apoptosis [4]. Thus, an increasing number of research articles have become available in the scientific literature that analyze characteristics of *Leishmania* proteinases and their roles during the course of infection, defining those that are more prominently suitable as drug targets.

In this context, serine proteinases are a topic of great interest for developing new chemotherapies, and an interesting study was published recently about the activity of oligopeptidase B (OPB) inhibitors against *Leishmania* parasites [5]. This paper reported relevant information concerning the interactions of naturally occurring compounds with *Leishmania major* OPB. The authors used a structure-based approach to perform a virtual screening of a large library of compounds and two of those, COP and TOA, were selected for further analysis by molecular dynamics simulations. A good binding affinity to OPB was shown for these compounds, as well as a low toxicity to human cells, indicating a promising new path for developing drugs against *Leishmania* spp.

Using OPB as a target allowed the development of a very relevant study, but it is important to bear in mind that other serine proteinases of *Leishmania* could also be targeted in similar approaches. At the present time, the genomes of *Leishmania* (*L.*) *infantum*, *Leishmania* (*V.*) *braziliensis*, *Leishmania* (*L.*) *mexicana* and *Leishmania* (*L.*) *major*, as

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annotated in online databases, harbor 18, 17, 20 and 20 genes for serine proteinases, respectively. In comparison, only 2 genes encoding OPB have been identified in these species: in chromosome 9 (OPB) and chromosome 6 (OPB-like) [6]. Thus, these numbers reveal a great diversity of potential new targets for inhibition studies.

In addition to the importance of considering other serine proteinases as potential targets, studies by Munday et al. [7] and Swenerton et al. [8] showed that using OPB as a single target against *Leishmania* parasites may not be the most effective strategy. It was reported that, although knockout of the OPB gene does cause a defect in the differentiation of *L.* (*L.*) *major* during experimental infection assays in animals, this effect is only transient, suggesting that OPB is important in virulence processes but may play a less relevant role in pathogenesis of the disease.

As indicated in these studies, the inhibited OPB activity may be compensated by a second OPB-like enzyme. This OPB-like enzyme, although exhibiting only low sequence identity to the OPB, may compensate its activity by having a similar substrate specificity. Such specificity may be associated to a conserved amino acid residue, Glu-621, as well as other important residues composing the S1 binding site [7].

So, these observations are in agreement with the idea that targeting a single proteinase, OPB in this case, is not an appropriate strategy to treat leishmaniasis, even more so when one considers the complex profile of proteinases of *Leishmania* spp. [6]. We advocate that, to develop an efficient chemotherapy strategy, it is necessary to consider the use of multiple protease inhibitors and eventually even combine these inhibitors with drugs currently in use. However, to date, no such combination has been tested clinically and the only reported use of a combination of drugs refers to miltefosine associated with amphotericin B [9].

The concept of a chemotherapy strategy based on a combination of proteinase inhibitors has been applied in the treatment of patients with HIV-1 infection [10]. As for infections with protozoan parasites, a combined chemotherapy based on protease inhibitors is still a proposal at the experimental stage with few empirical data available.

An example of a potential chemotherapy against protozoa based on inhibition of multiple proteinase activities has been reported for *Plasmodium falciparum* [11]. It was shown that a combination of inhibitors targeting cysteine proteinases (falcipain) and aspartic proteinases (plasmepsin I and II) had synergistic effects on blocking this parasite's metabolism. Regarding treatment of leishmaniasis, a study reported the control of infection in laboratory mice by *Leishmania* (*Leishmania*) *donovani* through the administration of anticytokine antibody therapy combined with cystatin inhibitor [12]. Such studies shed light on the potential of combined chemotherapies for treating these diseases, but, at the moment, clinical evidence is still lacking.

Nevertheless, classical and novel proteinase inhibitors have been assayed continuously in parasite cultures or in experimental infection models to assess their potential antileishmanial effect [3]. Some of the assayed proteinase inhibitors that could be potentially used to develop therapies are listed in Table 1.

Among these compounds, some specific inhibitors against serine proteinases, such as antipain and leupeptin, have been assayed on *Leishmania* parasites. Antipain is an oligopeptide isolated from actinomycetes and is an inhibitor of trypsin and papain [28] while leupeptin is naturally occurring inhibitor that can affect cysteine-, serine- and threonine-proteinases [29]. Although these inhibitors are known to interfere with *Leishmania* growth in vitro, they still lack clinical validation.

Our group has been continuously publishing new data about proteinases of *Leishmania* spp. [18, 30–34] and *Trypanosoma cruzi* [35–37] and their importance in host–parasite interactions for over a decade. Collectively, these manuscripts make a relevant contribution to the search for proteinases that could serve as targets for developing future leishmaniasis treatments. As consequence, during our ongoing studies, some peculiarities of proteinases of *Leishmania* spp., in the context of potential targets for drugs, have become apparent to us that could be very useful if considered in other studies in this same research field.

One of such point of interest is the fact that *Leishmania* parasites exhibit variations and fluctuations in the levels of proteinase expression during different stages of their life cycle, and, thus, it would be important to target those proteinases expressed at the parasitic stage that inhabits the vertebrate host when considering targets for novel drugs.

Another point we observed is that even minor structural differences in proteinases in the same class can affect their catalytic site microenvironment, and these variations may account for the difficulties in developing proteinase inhibitors that have wide activity against proteinases of the same class in *Leishmania* spp.

Therefore, due to all these observations, as well as the reports that suggest a great variability and importance of proteinases in these parasites, we reinforce our statement that only treatments that combine distinct proteinase inhibitors have the possibility to deliver an effective antileishmanial therapy. To this end, a deep understanding of the expression modulation of different proteinase classes, even of distinct isoforms of the same proteinase, in *Leishmania* parasites infecting vertebrate hosts is required.

**Table 1** Proteinase inhibitors tested in experimental assays against *Leishmania* spp. and against experimental infection with these parasites

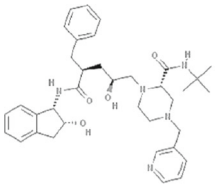
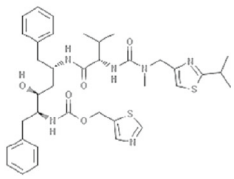
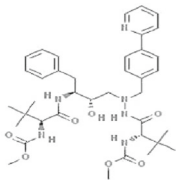
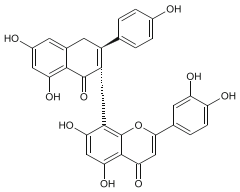
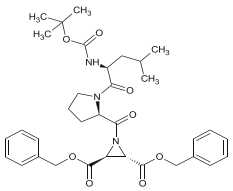
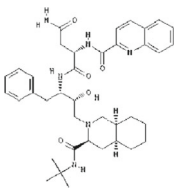
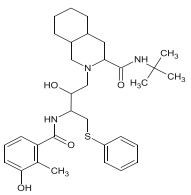
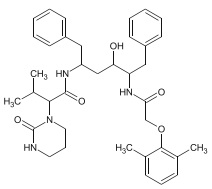
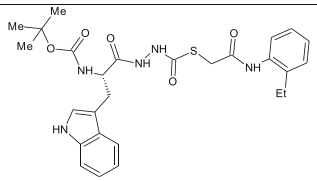
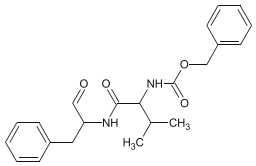
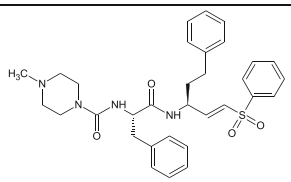
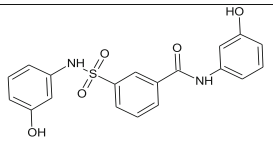
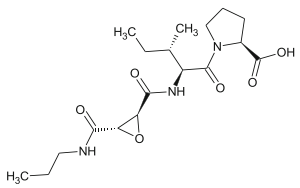
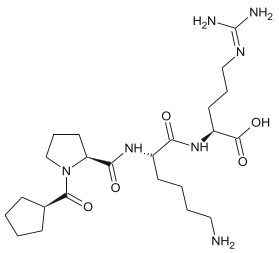
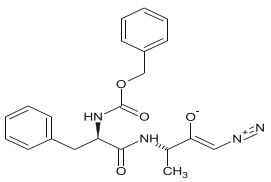
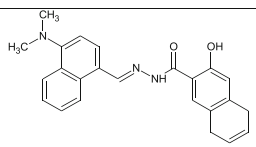
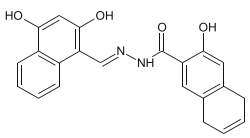
| Drug | 2D structure | Tested parasite | Efficiency | Target enzyme | Reference |
|---|---|---|---|---|---|
| Indinavir (CID 5362440) |  | | $IC_{50}$ 100 and 400 μM (promastigote) | | |
| Ritonavir (CID 392622) |  | *L. (L.) amazonensis and L. (V.) braziliensis* | $IC_{50}$ 40 and 2.3 μM (promastigote) | Aspartic proteinase | [13] |
| Atazanavir (CID 158550) |  | | $IC_{50}$ 266 and 400 μM (promastigote) | | |
| Fukugetin |  | *L. (L.) amazonensis* | $IC_{50}$ 3.2 μM (amastigote) | Cysteine proteinase and Serine proteinase | [14] |
| 13b |  | *L. (L.) major* | $IC_{50}$ 40 μM (promastigote) $IC_{50}$ 3.0 μM (amastigote) | Cysteine proteinase | [15, 16] |
| Saquinavir (CID 441243) |  | *L. (L.) infantum, L. (L.) donovani, L. (L.) mexicana, L. (L.) amazonenses, L. (V.) brazilensis and L. (L.) major* | $IC_{50}$ 48.04/55.21, 51.89, 40.67/24.44, 40, 36 and 46.95 μM (promastigote/amastigote) | Aspartic proteinase | [17] |
| Nelfinavir (CID 64142) |  | *L. (L.) infantum, L. (L.) donovani, L. (L.) mexicana, L. (L.) amazonenses, L. (V.) brazilensis and L. (L.) major* | $IC_{50}$ 18.21/22.86, 14.10, 12.44/13.83, 13.36, 14.60 and 13.37 μM (promastigote/amastigote) | Aspartic proteinase | [17] |
| Lopinavir (CID 92727) |  | *L. (L.) amazonensis* | $IC_{50}$ 15 μM (promastigote) | Aspartic proteinase | [19] |

**Table 1** (continued)

| Drug | 2D structure | Tested parasite | Efficiency | Target enzyme | Reference |
|---|---|---|---|---|---|
| CID 16725315 |  | *L. (L.) major* | $IC_{50}$ 12.5 μM (promastigote) | Cysteine proteinase | [19] |
| MDL 28170 (CID 11199915) |  | *L. (L.) amazonensis* | $LD_{50}$ 23.3 μM (promastigote) | Cysteine proteinase | [20] |
| K11777 (CID 9851116) |  | *L. (L.) mexicana and L. (L.) tropica* | antiparasitic properties with a capacity of reduction in lesion size in treated animals and hinder the survival inside peritoneal macrophages of CD1 mice | Cysteine proteinase (amastigote) | [21, 22] |
| CID 1069242 |  | *L. (L.) donovani* | $IC_{50}$ 0.3 μM (promastigote) | Cysteine proteinase | [23] |
| CA074 (CID 9821383) |  | *L. (L.) donovani* *L. (L.) major* | reduction the parasite survival within the macrophages and capacity induce cure in infected BALB/c mice | Cysteine proteinase | [24, 25] |
| Cystatin (CID 128439) |  | *L. (L.) donovani* | affects amastigotes growth inside macrophages in vitro and had curative effects for infected animals | Cysteine proteinase | [12] |
| Z-FA-DMK (CID 5488522) |  | *L. (L.) mexicana* | prevent infection of peritoneal cells from BALB/c mice (promastigote and amastigote) and also reduced the number of infecting parasites per host cell | Cysteine proteinase | [26] |
| ZLIII115A |  | *L. (L.) major* | prevent parasite replication as well as infection of mouse macrophages | Cysteine proteinase | [27] |
| ZLIII43A |  | | | | |

## References


1. WHO (1998) Technical Report Series. Rapport sur la Santé dans le Monde 1:59
2. Berman J (2005) Recent developments in leishmaniasis: epidemiology, diagnosis and treatment. Curr Infect Dis Repor 7:33–38
3. Pereira BAS, Silva FS, Silva-Almeida M, Santos-de-Souza R, Oliveira LFG, Ribeiro-Guimarães ML, Alves CR (2014) Proteinase inhibitors: a promising drug class for treating leishmaniasis. Curr Drug Target (in press)
4. Beynon RJ, Bond JS (2001) Proteolytical enzymes: a pratical approach, 2nd edn. Oxford University Press, Oxford
5. Goyal S, Grover S, Dhanjal JK, Goyal M, Tyagi C, Chacko S, Grover A (2014) Mechanistic insights into mode of actions of novel oligopeptidase B inhibitors for combating leishmaniasis. J Mol Model 20:2099
6. Silva-Almeida M, Souza-Silva F, Pereira BA, Ribeiro-Guimarães ML, Alves CR (2014) Overview of the organization of protease genes in the genome of *Leishmania* spp. Parasit Vectors 7(1):387
7. Munday JC, McLuskey K, Brown E, Coombs GH, Mottram JC (2011) Oligopeptidase B deficient mutants of *Leishmania major*. Mol Biochem Parasitol 175(1):49–57
8. Swenerton RK, Zhang S, Sajid M, Medzihradszky KF, Craik CS, Kelly BL, McKerrow JH (2011) The oligopeptidase B of Leishmania regulates parasite enolase and immune evasion. J Biol Chem 286(1): 429–40
9. Jha TK (2006) Drug unresponsiveness & combination therapy for kala-azar. Indian J Med Res 123:389–398
10. Hughes PJ, Cretton-Scott E, Teague A, Wensel TM (2011) Protease inhibitors for patients With HIV-1 infection: a comparative overview. P T 36(6):332–336
11. Semenov A, Olson JE, Rosenthal PJ (1998) Antimalarial synergy of cysteine and aspartic protease inhibitors. Antimicrob Agents Chemother 42(9):2254–2258
12. Das L, Datta N, Bandyopadhyay S, Das PK (2001) Successful therapy of lethal murine visceral leishmaniasis with cystatin involves up-regulation of nitric oxide and a favorable T cell response. J Immunol 166(6):4020–4028
13. Demarchi IG, Silveira TG, Ferreira IC, Lonardoni MV (2012) Effect of HIV protease inhibitors on New World *Leishmania*. Parasitol Int 61(4):538–544
14. Pereira IO, Assis DM, Juliano MA, Cunha RL, Barbieri CL, do Sacramento LV, Marques MJ, dos Santos MH (2011) Natural products from *Garcinia brasiliensis* as *Leishmania* protease inhibitors. J Med Food 14(6):557–562
15. Schurigt U, Schad C, Glowa C, Baum U, Thomale K, Schnitzer JK, Schultheis M, Schaschke N, Schirmeister T, Moll H (2010) Aziridine-2,3-dicarboxylate-based cysteine cathepsin inhibitors induce cell death in *Leishmania major* associated with accumulation of debris in autophagy-related lysosome-like vacuoles. Antimicrob Agents Chemother 54(12): 5028–5041
16. Ponte-Sucre A, Vicik R, Schultheis M, Schirmeister T, Moll H (2006) Aziridine-2,3-dicarboxylates, peptidomimetic cysteine protease inhibitors with antileishmanial activity. Antimicrob Agents Chemother 50(7):2439–2447
17. Valdivieso E, Rangel A, Moreno J, Saugar JM, Cañavate C, Alvar J, Dagger F (2010) Effects of HIV aspartyl-proteinase inhibitors on *Leishmania* sp. Exp Parasitol 26(4):557–563
18. Santos LO, Marinho FA, Altoé EF, Vitório BS, Alves CR, Britto C, Motta MC, Branquinha MH, Santos AL, d'Avila-Levy CM (2009) HIV aspartyl peptidase inhibitors interfere with cellular proliferation, ultrastructure and macrophage infection of *Leishmania amazonensis*. PLoS ONE 4(3):e4918
19. Shah PP, Myers MC, Beavers MP, Purvis JE, Jing H, Grieser HJ, Sharlow ER, Napper AD, Huryn DM, Cooperman BS, Smith AB 3rd, Diamond SL (2008) Kinetic characterization and molecular docking of a novel, potent, and selective slow-binding inhibitor of human cathepsin L. Mol Pharmacol 74(1): 34–41
20. d'Avila-Levy CM, Marinho FA, Santos LO, Martins JL, Santos AL, Branquinha MH (2006) Antileishmanial activity of MDL 28170, a potent calpain inhibitor. Int J Antimicrob Agents 28(2):138–142
21. Williams RA, Tetley L, Mottram JC, Coombs GH (2006) Cysteine peptidases CPA and CPB are vital for autophagy and differentiation in *Leishmania mexicana*. Mol Microbiol 61(3):655–674
22. Mahmoudzadeh-Niknam H, McKerrow JH (2004) *Leishmania tropica*: cysteine proteases are essential for growth and pathogenicity. Exp Parasitol 106(3–4):158–163
23. Desai PV, Patny A, Sabnis Y, Tekwani B, Gut J, Rosenthal P, Srivastava A, Avery M (2004) Identification of novel parasitic cysteine protease inhibitors using virtual screening The ChemBridge database. J Med Chem 47(26):660966–660915
24. Somanna A, Mundodi V, Gedamu L (2002) Functional analysis of cathepsin B-like cysteine proteases from *Leishmania donovani* complex. Evidence for the activation of latent transforming growth factor beta. J Biol Chem 277(28):25305–25312
25. Maekawa Y, Himeno K, Ishikawa H, Hisaeda H, Sakai T, Dainichi T, Asao T, Good RA, Katunuma N (1998) Switch of CD4+ T cell differentiation from Th2 to Th1 by treatment with cathepsin B inhibitor in experimental leishmaniasis. J Immunol 161(5):2120–2127
26. Frame MJ, Mottram JC, Coombs GH (2000) Analysis of the roles of cysteine proteinases of *Leishmania mexicana* in the host-parasite interaction. Parasitology 121(4):367–377
27. Selzer PM, Pingel S, Hsieh I, Ugele B, Chan VJ, Engel JC, Bogyo M, Russell DG, Sakanari JA, McKerrow JH (1999) Cysteine protease inhibitors as chemotherapy: lessons from a parasite target. Proc Natl Acad Sci USA 96(20):11015–11022
28. Umezawa S, Tatsuta K, Fujimoto K, Tsuchiya T, Umezawa H (1972) Structure of antipain, a new Sakaguchi-positive product of streptomyces. J Antibiot (Tokyo) 25(4):267–270
29. Maeda K, Kawamura K, Kondo S, Aoyagi T, Takeuchi T (1971) The structure and activity of leupeptins and related analogs. J Antibiot (Tokyo) 24(6):402–404
30. Silva-Almeida M, Pereira BA, Ribeiro-Guimarães ML, Alves CR (2012) Proteinases as virulence factors in *Leishmania* spp. infection in mammals. Parasit Vectors 5:160
31. Pereira BA, Britto C, Alves CR (2012) Expression of infection-related genes in parasites and host during murine experimental infection with *Leishmania* (Leishmania) *amazonensis*. Microb Pathog 52(2):101–108
32. Rebello KM, Britto C, Pereira BA, Pita-Pereira DD, Moraes MO, Ferreira AB, Cysne-Finkelstein L, Otto TD, Côrtes LM, da-Silva GG, Alves CR (2010) *Leishmania (Viannia) braziliensis*: influence of successive in vitro cultivation on the expression of promastigote proteinases. Exp Parasitol 126(4):570–576
33. Rebello KM, Côrtes LM, Pereira BA, Pascarelli BM, Côrte-Real S, Finkelstein LC, Pinho RT, d'Avila-Levy CM, Alves CR (2009) Cysteine proteinases from promastigotes of *Leishmania (Viannia) braziliensis*. Parasitol Res 106(1):95–104
34. Alves CR, Corte-Real S, Bourguignon SC, Chaves CS, Saraiva EM (2005) *Leishmania amazonensis*: early proteinase activities during promastigote-amastigote differentiation in vitro. Exp Parasitol 109(1):38–48
35. Bourguignon SC, Cavalcanti DF, de Souza AM, Castro HC, Rodrigues CR, Albuquerque MG, Santos DO, da Silva GG, da Silva FC, Ferreira VF, de Pinho RT, Alves CR (2011)

*Trypanosoma cruzi*: insights into naphthoquinone effects on growth and proteinase activity. Exp Parasitol 127(1):160–166

36. Bourguignon SC, Castro HC, Santos DO, Alves CR, Ferreira VF, Gama IL, Silva FC, Seguins WS, Pinho RT (2009) *Trypanosoma cruzi*: in vitro activity of Epoxy-alpha-Lap, a derivative of alpha-lapachone, on trypomastigote and amastigote forms. Exp Parasitol 122(2):91–96
37. Pinho RT, Beltramini LM, Alves CR, De-Simone SG (2009) *Trypanosoma cruzi*: isolation and characterization of aspartyl proteases. Exp Parasitol 122(2):128–133