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# Natural products as inhibitors of recombinant cathepsin L of *Leishmania mexicana*

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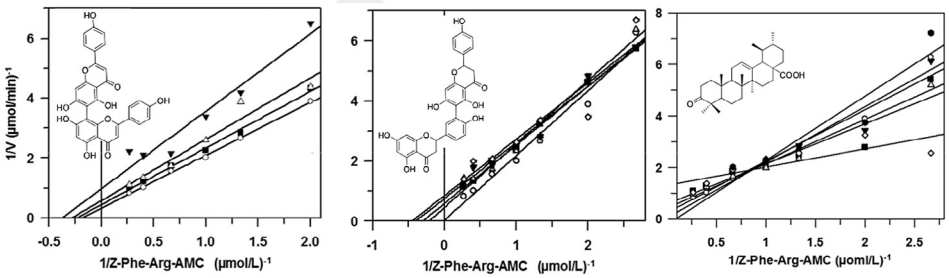
## HIGHLIGHTS

- Natural products were screened against *L. mexicana* cathepsin L (rCPB2.8).
- A triterpene and some flavonoids are inhibitors of rCPB2.8.
- The inhibitors of rCPB2.8 are partially noncompetitive and uncompetitive.
- The mechanism would be an advantage in the search for selective inhibitors.

## GRAPHICAL ABSTRACT




Uncompetitive and partially noncompetitive inhibitors of cathepsin L of *L. mexicana*

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## ABSTRACT

Cysteine proteinases (cathepsins) from *Leishmania* spp. are promising molecular targets against leishmaniasis. *Leishmania mexicana* cathepsin L is essential in the parasite life cycle and a pivotal in virulence factor in mammals. Natural products that have been shown to display antileishmanial activity were screened as part of our ongoing efforts to design inhibitors against the *L. mexicana* cathepsin L-like rCPB2.8. Among them, agathisflavone (**1**), tetrahydrorobustaflavone (**2**), 3-oxo-urs-12-en-28-oic acid (**3**), and quercetin (**4**) showed significant inhibitory activity on rCPB2.8 with $IC_{50}$ values ranging from 0.43 to 18.03 µM. The mechanisms of inhibition for compounds **1**–**3**, which showed $K_i$ values in the low micromolar range ($K_i$ = 0.14–1.26 µM), were determined. The biflavone **1** and the triterpene **3** are partially noncompetitive inhibitors, whereas biflavanone **2** is an uncompetitive inhibitor. The mechanism of action established for these leishmanicidal natural products provides a new outlook in the search for drugs against *Leishmania*.


## 1. Introduction

Leishmaniasis is a tropical disease caused by parasites of *Leishmania* spp. which is transmitted to mammalian host by the bite of sand flies from *Lutzomyia* genus (WHO, 2014). The different species of parasite can lead to cutaneous leishmaniasis (*Leishmania major*, *Leishmania tropica*, *Leishmania mexicana*, *Leishmania braziliensis*, *Leishmania amazonensis*), mucocutaneous leishmaniasis (*Leishmania braziliensis*) or visceral leishmaniasis (*Leishmania donovani*, *Leishmania infantum*/*chagasi*) (Berman, 1997). This disease represents a global problem for which new treatments are needed, since the available chemotherapeutics have many issues related to toxicity, efficacy and administration (Gómez et al., 2014; Santos et al., 2008).

*Leishmania* infection is established when the parasites inside macrophage cells proliferate evading and manipulating the human immune defense mechanism (Sharma and Singh, 2009; Wilson et al., 2005). This suppression of the antileishmanial immune response in mammalian host can be directly related to the activity of

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cathepsin L and B from *Leishmania* spp. parasites (Buxbaum et al., 2003; Onishi et al., 2004; Selzer et al., 1999). Previously, studies demonstrated by genetic approach with deletion of cathepsins (CPs) genes in *L. mexicana*, that these enzymes represent a key determinant for virulence in *Leishmania* infection (Alexander et al., 1998; Denise et al., 2003; Mottram et al., 2004). Among CPs, the cathepsin L has demonstrated to be the most relevant proteinase for infectivity by *Leishmania* parasites when inside host macrophages. Cathepsin L inhibitors have detained parasite infection, such as the compound IV (Calbiochem) that showed effect on *L. mexicana* due its interference of IL-12 expression by macrophages (Pereira et al., 2014).

Although proteinase inhibitors have been an effective approach, it has been suggested that a combination of different proteinase inhibitors would improve efficiency for leishmanicidal therapy (Alves et al., 2014; Pereira et al., 2014). Proteinase genes analysis showed that the different *Leishmania* species presented high synteny. In addition, the cysteine, serine proteinase and metalloproteinase are the majority classes among the proteinase genes in *Leishmania* spp., a useful information for development of new drugs (Silva-Almeida et al., 2014).

The recombinant cathepsin L-like rCPB2.8 from *L. mexicana* is an isoform without the C-terminal extension that has been used as target in the search for new leishmanicidal compounds (Alves et al., 2001; Desai et al., 2006; Gontijo et al., 2012; Judice et al., 2013; Schröder et al., 2013; Steert et al., 2010). The structure of this enzyme has the amino acid residues Asn$^{60}$, Asp$^{61}$ and Asp$^{64}$ in the α-helices (wall of the active site), and previous NMR experiments revealed that rCPB2.8 adopts a type of immunoglobulin-like fold (Juliano et al., 2004; Smith et al., 2006).

Natural products are important sources for antiparasitic drug discovery (Kayser et al., 2003; Ndjonka et al., 2013; Newman and Gragg, 2007). Particularly triterpenes and flavonoids isolated from plants have revealed antiprotozoal potential (Al Musayeib et al., 2013; Gontijo et al., 2012; Mbwambo et al., 2006; Suárez et al., 2003; Tasdemir et al., 2006; Weniger et al., 2004). Tingenin B (22β-hydroxytingenone), a triterpene isolated from *Elaeodendron schlechteranum* (Loes.) Loes. (Celastraceae), presented activity against *Trypanosoma cruzi* ($IC_{50}$ <0.25 μg/mL), *Trypanosoma brucei* (<0.25 μg/mL), *L. infantum* (0.51 μg/mL), and *Plasmodium falciparum* (0.36 μg/mL) (Maregesi et al., 2010). The biflavonoids 2,3-dihydrohinokiflavone and isoginkgetin showed strong leishmanicidal activity, with $IC_{50}$ values of 1.6 and 1.9 μM, respectively (Kunert et al., 2008; Weniger et al., 2006). The biflavonoid fukugetin had activity on amastigote of *L. amazonensis* with $IC_{50}$ of 3.2 μM, and it is a cysteine and serine proteinase inhibitor (Alves et al., 2014; Pereira et al., 2011). Quinone derivatives isolated from *Bignoniaceae* and *Verbenaceae* trees have been described as antileishmanial compounds. Specifically a naphthoquinone derivative epoxy-α-lapachone that showed efficiency on *L. amazonensis* in vitro and in vivo, which mechanism suggested is associated to the inhibition of oligopeptidase B, a serine proteinase from *Leishmania* spp. (Souza-Silva et al., 2014, 2015).

In an effort toward the discovery of new inhibitors of rCPB2.8, this work reports the results of a screening of natural products isolated from plants as well the inhibition type presented by the promising compounds. A triterpene with ursolic skeleton and two biflavonoids (Fig. 1) has showed significant inhibitory activity against rCPB2.8. The inhibition mechanism of these compounds showed that they were not competing directly for the active site.

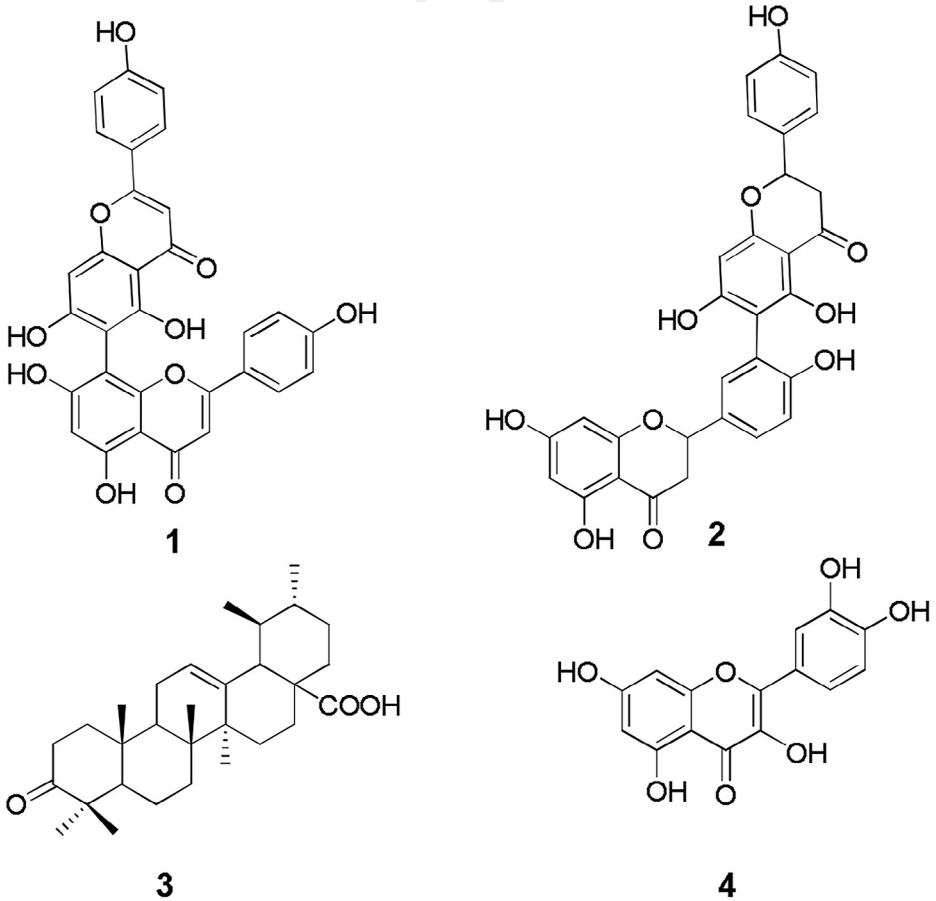


**Fig. 1.** Structures of the natural compound inhibitors of rCPB2.8 activity.

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