









In vivo study of schistosomicidal action of (Z)-1-(2-chloro-6-fluorobenzyl)-5-thioxo-4-(2,4,6-trimethoxybenzylidene)imidazolidin-2-one

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Schistosoma mansoni is one of the major parasites causing human schistosomiasis, recognized as a major global health problem with over 200 million people afflicted worldwide (Moraes et al., 2012). Schistosomiasis is a chronic and debilitating disease that continues to threaten millions of people, particularly the rural poor in the developing world (WHO, 2011).

The reference drug for treatment of schistosomiasis is praziquantel (PZQ), but this drug is ineffective against immature forms and recent reports of resistance in some strains have worried the world's public health organizations. Therefore many studies have been testing the effectiveness of new drugs against many schistosomiasis strains (Ismail et al., 1999; Pica-Mattocchia and Cioli, 2004).

The imidazolidines are a broad class of bioactive compounds that also have schistosomicidal properties. Niridazole, 1-(5-nitrothiazol-2-yl)imidazolidin-2-one, a drug used during the last century, has been widely applied for clinical purposes and was one of the early treatment options to be administered orally (Caterina et al., 2008).

Imidazolidines and their derivatives comprise a substance class that has shown anti-convulsive and antiarrhythmic pharmacological activities. Furthermore, the imidazolidines have a methylene group very reactive to carbon-5 that allows the synthesis of many derivatives through aromatic aldehyde condensation (Rossi and Zelnik, 2000).

Imidazolidines are a broad class of bioactive pentagonal heterocyclic compounds with diverse biological activity (Rossi and Zelnik, 2000). Imidazolidines have antifungal, antimicrobial, anti-*Trypanosoma cruzi* and schistosomicidal action (Caterina et al., 2008). The schistosomicidal properties of imidazolidine derivatives have been demonstrated by *in vitro* studies with adult *S. mansoni* worms (Rocha-Pitta et al., 2013) and *in vivo* studies (Silva et al., 2016).

Based on the promising results of the imidazolidine-derived (Z)-1-(2-chloro-6-fluorobenzyl)-5-thioxo-

4-(2,4,6-trimethoxybenzylidene)imidazolidin-2-one (LPSF/PTS23) *in vitro*, this study sought to assess the schistosomicidal potential of LPSF/PTS23 in an experimental model of mansonic schistosomiasis (Matos-Rocha et al., 2016).

The compound (LPSF/PTS23)-(Z)-1-(2-chloro-6-fluorobenzyl)-5-thioxo-4-(2,4,6-trimethoxybenzylidene)imidazolidin-2-one were obtained from Laboratory of Planejamento e Síntese de Fármacos at Universidade Federal de Pernambuco (Brazil) and their identities verified by ¹H nuclear magnetic resonance of hydrogen (¹H NMR), infrared (IR) and mass spectroscopy (MS) (Matos-Rocha et al., 2016).

The determination of cytotoxicity Peripheral blood mononuclear cells were obtained from heparinized blood from healthy, nonsmoking donors who had not taken any medication for at least 15 days prior to the sample collection (10 volunteers), and cells were isolated via a standard method of density-gradient centrifugation using a Ficoll Hypaque solution (GE Healthcare). Cells were counted in a Neubauer chamber, and viability was determined by the trypan blue exclusion method. Cells were used only when the viability was at least 98%. All the donors gave informed consent, and the study was approved by the Human Research Ethics Committee of UFPE in the Health Sciences Center (CEP/CCS/UFPE N0 483/10 and 57/10). Cells were plated in 96-well plates (10⁶ cells/well). After 24 h, the test compound was added (1, 10 and 100 μM) in triplicate wells, the cells were incubated for 48 h and then subjected to the MTT assay.

Cytotoxicity was quantified by the ability of living cells to reduce the tetrazolium dye MTT to formazan, a purple compound. Measurements were performed by using enzyme-linked immunosorbent assay (ELISA) kits (eBiosciences, USA, and BD Biosciences, USA) according to the manufacturers' instructions. At the end

of the incubation period, wells were centrifuged, and the medium was replaced by 150 µL of another medium without the compound containing MTT (0.5 mg/mL). Three hours later the MTT formazan was diluted with 100 µL of 20% SDS, and its absorbance was measured at 570 nm in a BioTek EL808 reader. Cytotoxic activity was quantified as the percentage of reduction in absorbance relative to a vehicle treated control. In all the analyzed experiments, the vehicle (DMSO 0.1%) treated group presented > 98% of viability compared to the control cells without vehicle in three independent assays.

Infection was performed percutaneously, using for each mouse 80 *S. mansoni* cercariae. LE (Belo Horizonte) strains of *S. mansoni* adult worms collected from *Biomphalaria glabrata* were obtained by the Departamento de Malacologia do Centro de Pesquisa Aggeu Magalhães (CPqAM). Fifty male albino Swiss mice (*Mus musculus*) were used, aged 25 days. Sixty days after infection, a parasitological examination was done from feces of mice to evaluate the positivity of the infection.

For the assessment of schistosomicidal activity of the imidazolidine derivative, LPSF/PTS23, experimental and control groups consisting of eight mice were used. The allocation of mice to experimental groups was performed randomly. The mice were treated orally 55 days after infection. The recommended doses of the imidazolidine compound and PZQ were 150 mg/kg being administered for five consecutive days. The groups were allocated as follows: Group I (LPSF/PTS23-150 mg/kg); Group II (LPSF/PTS23-75 mg/kg); Group III (praziquantel/150 mg/kg); and the Group IV (vehicle and PEG (polyethylene glycol)).

Fifteen days after treatment with the compounds, the animals were anesthetized with an intraperitoneal injection of ketamine hydrochloride (115 mg/kg) associated with xylazine hydrochloride (10 mg/kg). After anesthesia, the animals underwent perfusion of hepatic portal system for removal of the worms, which were separated in Petri dishes containing 0.85% saline and then the parasites were counted and classified according to sex and vitality (Silva et al., 2016).

The evaluation of the effectiveness of the imidazolidine derivative LPSF/PT-09 and of PZQ was determined by reducing the percentage of parasitic load in each group treated using the following equation: reduction of worms (%) = # of worms in the control group - # of worms in the treatment group x 100/# of worms in the control group (Silva et al., 2016).

A descriptive analysis was performed to display the results obtained. The presentation of the measured variables was done using tables, including also the use of some descriptive measures such as minimum, maximum, average and standard deviation. For comparative analysis of quantitative variables with more than two groups, the following tests were used: Bartlett to test the assumption of homogeneity of variance; Kruskal-Wallis was applied when an assumption of homogeneity was seen. All findings were taken at a significance level of 5%. The software used was GraphPad Prism 5.

Our results showed that LPSF/PTS23 presented nontoxic effects at 1, 10 and 100 µM concentrations. The derivative LPSF/PTS23, at a dose of 150 mg/kg, presented efficacy in the treatment of mansonic schistosomiasis in mice, reducing the number of adult worms after treatment by 50%. The use of the lower dose, 75 mg/kg, also had good efficacy, being able to cut down on the number of worms by 25%. PZQ, in turn, had efficacy of 100% in both the doses evaluated (Table 1).

In the evaluation of liver tissue, no significant differences were observed between the number of eggs found in animals treated with LPSF/PTS23, with PZQ or not treated. While in the evaluation of the intestine, PZQ was effective in reducing the number of eggs at both doses, with the 200 mg/kg dose, interestingly, more effective in this reduction (Table 2).

Chemotherapy is the mainstay of schistosomiasis control and is carried out largely through the use of praziquantel. Many drugs have been compared with praziquantel and their cytotoxicity and ability to kill adult worms of *S. mansoni* have been shown to be equivalent to the reference drug (Sayed et al., 2008).

However, similar to praziquantel, the mechanism by which the imidazolidines exert schistosomicide activity in vitro is still unclear. The efficacy of this compound against adult worms of all schistosome species that infect humans has led to its widespread use (Rocha-Pitta et al., 2013; Silva et al., 2016).

Imidazolidines also show activity in apoptotic cells with melanoma, whose route was identified in *S. mansoni* and has been suggest as the result, possibly, of activity at the cholinergic level receptors, since the nervous system of *S. mansoni*, with its unique pharmacological

Table 1. Effectiveness of treatment of the animals infected and treated with imidazolidine derivative LPSF/PTS23 and PZQ.

Animal groups#	Number of worms	Efficacy of treatment (%)
I	20	50
II	30	25
III	-	100
IV	40	-

#Group I: LPSF/PTS23: 150 mg/Kg; Group II: LPSF/PTS23: 75 mg/Kg; Group III: PZQ: 150 mg/Kg and group IV: (vehicle and PEG [polyethyleneglycol]).

Table 2. Effectiveness of treatment of the animals infected and treated with imidazolidine derivative LPSF/PTS23.

Animal groups#	Tissue egg loads x 10 ³	
	Hepatic	Intestinal
I	4.10±3.33	1.89±1.30
II	2.45±2.31	1.98±1.12
III	2.70±1.23	0.44±0.21***
IV	14.55±3.18	2.35±2.43

*** at P<0.001. #Group I: LPSF/PTS23: 150 mg/Kg; Group II: LPSF/PTS23: 75 mg/Kg; Group III: PZQ: 150 mg/Kg and group IV: (vehicle and PEG [polyethyleneglycol]).

and physiological characteristics, can be used to research compounds for human and animal use (Dubois et al., 2009).

Chemical groups that are essential for the manifestation and intensity of many biological reactions may be considered biofunctional groups. Here, we observed the structural similarity of imidazolidines synthesized with niridazole (Ambilhar®), chemically named 1-(5-nitro-2-fenilo-3-il)-imidazolidin-2-ona. Imidazolidines show the same pharmacophoric group of niridazole and present HC=C-N and C-N=N-C-N substitutions at position 5 of the imidazolidine ring (Korolkovas, 1974).

A study published by our research group demonstrated the activity of 3-benzil-5-(4-cloro-arylazo)-4-tioxi-imidazolidin-2-ona against Schistosomiasis Mansoni in Mice, the use of doses of LPSF/PT-5, significantly reduced the number of recovered worms due to increases in the solubility of the compound in this formulation; the greatest reduction (70.5%) was observed at the dose of 100 mg/kg. The results show the derivative LPSF-PT05 to be a potential candidate in the etiological treatment of schistosomiasis (Silva et al., 2016).

A study published by Silva et al. (2016) demonstrated the schistosomicidal action of 1-benzil-4-[(4-fluorofenil)-hidrazono]-5-tioxi-imidazolidin-2-ona (LPSF/PT-9), during the acute phase of the disease and have demonstrated moderate effectiveness of 30-54,4%, the results obtained in this model showed that the imidazolidine derivative LPSF/PT-09 presented significant antischistosomal activity *in vivo*, posing as a potential candidate for this class of drugs.

More recently, evaluation of the schistosomicidal properties of the derivative (Z)-1-(2-cloro-6-fluorobenzil)-5-tioxi-4-(2,4,6-trimetoxibenzilideno)imidazolidin-2-ona or LPSF/PTS23, showed higher activity *in vitro* against adult male worms, with 100% mortality after 24 hours of contact at all the concentrations tested (Matos-Rocha et al., 2016). This *in vitro* study of LPSF/PTS23 confirmed the promising *in vivo* results.

Based on these results, we conclude that the imidazolidine derivative LPSF/PTS23 showed significant antischistosomal activity *in vivo*, posing as a potential candidate for this class of drugs. However, there is still a need for further *in vivo* and more in-depth studies of their mechanism of action. This project was approved by the Ethics Committee on the Use of Animals of the Centro de Pesquisa Aggeu Magalhães/Fundação Oswaldo Cruz (CPqAM/FIOCRUZ) authorized by license No. 06/2010.

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Erratum

Erratum of the article: “In vivo study of schistosomicidal action of (Z)-1-(2-chloro-6-fluoro-benzyl)-5-thioxo-4-(2,4,6-trimethoxy-benzylidene)-imidazolidin-2-one”, DOI <https://doi.org/10.1590/1519-6984.190062>, published in Brazilian Journal of Biology, ahead of print Epub May 30, 2019.

Which reads:

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Should be read:

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