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Tegumental changes in adult *Schistosoma mansoni* induced by a new imidazolidinic derivative

ABSTRACT

Aims: verify the potential of the schistosomicidal imidazolidine derivative (5Z)-3-(4-bromo-benzyl)-5-(4-chloro-benzylidene)-4-thioxo-imidazolidin-2-one.
Study design: In this study, we tested the imidazolidinic derivative 3 through *in vitro* evaluations, cytotoxicity assay and analysis of Scanning Electron Microscopy to verify its therapeutic potential in the treatment of schistosomiasis.
Place and Duration of Study: Departamento de Antibióticos, Universidade Federal de Pernambuco (UFPE), Fundação Oswaldo Cruz (FIOCRUZ)/PE and (FIOCRUZ)/BA between January 2013 and march 2014.
Methodology: This study was approved by the Ethics Committee on Animal Use Research Center Aggeu Magalhães/Oswaldo Cruz Fundação (CPqAM/FIOCRUZ) authorized by the license No. 21/2011. Male albino Swiss mice were used *Mus musculus* 25 days old weighing 50 grams. Compound 3 was assayed for its cytotoxicity through cell J774 macrophage lineage. The amount of inhibitory concentration (LC50) was determined by nonlinear regression using the GraphPadPrism version 5.01. Then the compound was evaluated against adult worms of *S. mansoni* by performing the activity *in vitro* at doses 100-20 µg/mL and ultrastructural investigation by Scanning Electron Microscopy (SEM) at doses of 100 and 60 µg/ml. The PZQ was the positive control of the experiment.
Results: The derivative 3 showed LC50 of 29.7 ± 3.9 mM. Compound 3 was able to have decreased motility of *S. mansoni* culminating with a mortality rate of 100% at doses of 60 and 100 µg/mL on the fourth day of observation of the experiment. In the SEM, the compound caused various soft tissue changes of *S. mansoni* parasites such as blistering, destruction of the integument with loss of spines and tubercles, body contraction and windy.
Conclusion: the derivative imidazolidine 3 showed a promising schistosomicidal activity *in vitro*. However, conducting further studies with the completion of work in front of the live schistosomiasis is required.

Keywords: schistosomiasis, imidazolidines, tegument, microscopy.

1. INTRODUCTION

Schistosomiasis is a parasitic disease caused, in Brazil, by the Trematoda *Schistosoma mansoni*. It is still considered a parasitic disease of great importance to public health, as it continues affecting over 230 million people distributed around the world [1, 2, 3]. In Brazil, it is estimated that 2.5 million people are infected at the same time that there are 26 million people living in areas at risk of infection [4].
The outer surface of the adult *Schistosoma mansoni* worms consists of a tegument or syncytial layer that is covered with tiny spines, tubercles and apical membranes. This external layer is the contact interface between the parasite and the host and it is formed by juxtaposed lipid layers, forming the membranocalyx [5, 6, 7].
Knowing that the cutaneous surface is located between the parasite's and the host's environment and that it is responsible for presenting proteins involved in the immune response and in the repair of any damage caused by the definitive host, the tegumental structure becomes a potential biological target for the performance of a antischistosomal drug candidate [8, 9].

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The integrity of the tegument and function of the outer surface are of great significance for the survival and proliferation of *S. mansoni* when it is in contact with the infected host's environment [7]. This is because such structures have a vital role in the invasion of the immune response, nutrient absorption, selective uptake of drugs, metabolism of cholesterol and lipids, and in many other physiological processes [10, 11, 12].

There are various tegumental alterations such as swelling, fusion of the tegumental ridges, formation of vesicles, peeling, erosion and sometimes the collapse of the tegument [13, 14, 15]. Studies indicate that these tegumental changes can lead to the disappearance of the immune response of the worms, leading to increased vulnerability to its host [15]. In addition to this, the ability to absorb nutrients such as glucose is very affected by the destruction of the worm tegument, exerting a huge influence on the metabolism of the worms, resulting in its death [16].

Numerous structural alterations of the tegumental surface of adult *S. mansoni* worms have been observed through studies using antischistosomal compounds such as hicantone [17], niridazol [18], oxaminiquine [19], praziquantel (PZQ) [20, 21, 22], atorvastatin [23], mefloquine [24, 25] and thioxo-imidazolidine derivatives [26].

The Imidazolidines are bioactive heterocyclic compounds that exhibit various biological activities such as antimicrobial activity [27], antihypertensive activity [28], antineoplastic activity [29], anti-*Trypanosoma cruzi* activity [30] and antischistosomal activity [31, 32, 33]. Recent studies about the in vitro antischistosomal activity with adult *S. mansoni* worms have shown promising and similar results to the ones presented by PZQ [34, 26]. However, as PZQ, the mechanism of action of the Imidazolidines has not been fully elucidated yet [26].

Given the results of the imidazolidinic compounds observed so far and due to the great need for a more effective drug, this study aimed to check the antischistosomal potential of the imidazolidinic derivative (5Z)-3-(4-bromo-benzyl)-5-(4-chloro-benzylidene)-4-thioxo-imidazolidin-2-one (3) through an in vitro activity evaluation and an ultrastructural investigation of the parasite, and to analyze the cytotoxicity of the tested compound in a mammalian cell.

2. MATERIAL AND METHODS

2.1 Chemical

The compound (5Z)-3-(4-bromo-benzyl)-5-(4-chloro-benzylidene)-4-thioxo-imidazolidin-2-one (3) was obtained from Laboratório de Planejamento de Síntese de Fármacos at Universidade Federal de Pernambuco (Brazil) and was duly identified by nuclear magnetic resonance of hydrogen as well as infrared (IR) and mass spectroscopy (MS). The figure 1 displays the synthetic route of 3. The starting reagent was imidazolidine-2,4-dione which was reacted with 4-bromo-benzyl chloride under basic conditions to obtain the intermediate 3-(4-bromo-benzyl)-imidazolidine-2,4-dione (1) [35]. After that, the reaction of 3-(4-bromo-benzyl)-imidazolidine-2,4-dione with Lawesson's reagent in anhydrous dioxane gave rise to 3-(4-bromo-benzyl)-4-thioxo-imidazolidin-2-one. The reaction mixture was heated under reflux for 24 hours [36]. Then 2-cyano-3-(4-chlorophenyl)-acrylic acid ethyl ester (2) [37] was synthesised through Knoevenagel condensation between 4-chloro-benzaldehyde and ethyl cyanoacetate. A Michael-type addition was then performed by reacting the ester (2) with the intermediate 3-(4-bromo-benzyl)-4-thioxo-imidazolidin-2-one to form the final compound (3). Reactions were monitored with analytical thin-layer chromatography in silica gel 60 F254 plates and visualized under UV light (254nm). Melting points were determined on a Quimis 340 capillary melting point apparatus and were not corrected. Infrared spectra were recorded as KBr discs using a BRUKER (IFS66) infrared spectrophotometer. Nuclear magnetic resonance ^{1}H NMR and ^{13}C NMR spectra were recorded in a VMMRS 400 MHz VARIAN spectrometer using tetramethylsilane (TMS) as the internal standard and DMSO-d6 as the solvent. Chemical shifts (δ, ppm) were assigned according to the internal standard signal of TMS in DMSO-d6 (δ, ppm). Coupling constants (J) are reported in Hz. ^{1}H NMR spectra are reported in the following order: chemical shift, multiplicity, number and type of proton and coupling constant(s). Mass spectra with MALDI-TOF Autoflex III (Bruker Daltonics, Billerica, MA, USA). Laser Nd:YAG, 355 nm. Freq. laser: 100 Hz. The derivative 3 was isolated as a single isomer. X-ray crystallographic studies and ^{13}C NMR have demonstrated a preferred Z configuration for 5-benzylidene-thiazolidinones [38, 39, 40, 41, 42]. The presence of the arylidene proton peak in ^{1}H NMR for the synthesized derivatives (5Z)-3-(4-bromo-benzyl)-5-(4-chloro-benzylidene)-4-thioxo-imidazolidin-2-one (3) confirmed the completion of the nucleophilic addition reaction. The compound was also confirmed by MS data in negative mode. The IR spectrum of the compound showed characteristic peaks of the carbonile group and

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arilidene. For the preparation of (5Z)-3-(4-bromo-benzyl)-5-(4-chloro-benzylidene)-4-thioxo-imidazolidin-2-one (3), equimolar amounts of -(4-bromo-benzyl)-4-thioxo-imidazolidin-2-one (200mg) and 2-cyano-3-(4-chlorophenyl)-acrylic acid ethyl ester (165 mg) were reacted using absolute ethanol (8 mL) as the solvent and morpholine (1 mL) as the catalyst. The reaction mixture was heated to 50°C for 8 hours and then cooled to room temperature. The solid that precipitated out was filtered under vacuum and washed with water and absolute ethanol. MF: $C_{17}H_{12}BrClN_2OS$; MW: 407.7128; MP: 202-3°C; yield: 44.73%; Rf: 0.56 *n*-hexane/ethylacetate 8:2. IR (υ, cm^{-1}; KBr): 3205; 1732; 1712; 1594; ^{1}H NMR (400 MHZ, DMSO-d6): s (1H,NH) 11,33; d(2H, benzylidene) 7,70; d(2H, benzylic) 7.52; d(2H, benzylidene) 7,48; d(2H, benzylic) 7,29; s(1H=CH) 6,99; s(2HNCH$_2$) 5,03. ^{13}C NMR (δ ppm, DMSO-d6): 44.48 (CH_2); 113,13.(2C); 120,56 (C ring); 128,77(2CH); 129,74 (2CH); 131,25 (2CH); 131,46 (2CH); 131,65(CH); 133,61 (CBr); 134,71 (CCl); 155,56 (C=S), 188,84 (C=O). MS (m/z) relative intensity: expected value $[M]^{+}$ 405.954, found value $(M+H)^{+1}$ 406.936. (Fig. 1).

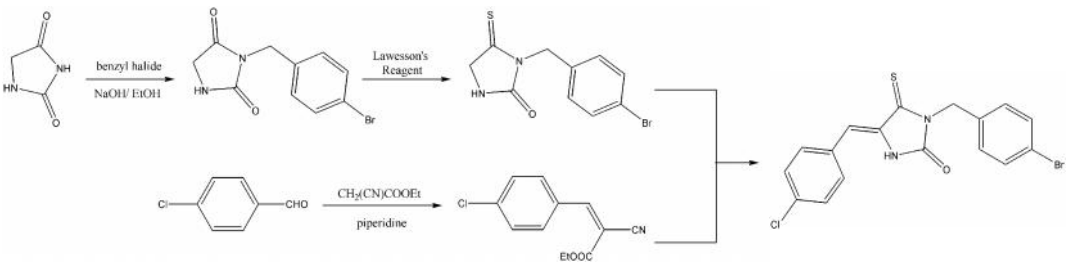

Fig. 1: obtainment of the imidazolidinic derivative.

2.2 Biological Activity

After an initial screening with imidazolidines series compounds through susceptibility testing activity *in vitro* forward to adult worms of *S. mansoni*, the imidazolidinic compound (5Z)-3-(4-bromo-benzyl)-5-(4-chloro-benzylidene)-4-thioxo-imidazolidin-2-one (3) proved to be a potential drug candidate schistosomicidal *in vitro* evaluation well as in testing and scanning electron microscopy (SEM).

2.2.1 Cytotoxicity Assay

Cells of the macrophage cell line J774 ($5x10^4$ cells/mL) were cultured in 96-well flat bottom tissue culture plates (100µL/well) containing RPMI-1640 medium (Sigma-Aldrich, St. Louis, USA) supplemented with 10% Foetal Bovine Serum (FBS) (Gibco Laboratories, Gaithersburg, USA) and 50 µg/mL of gentamicin (Hipolabor, Belo Horizonte, Brazil). The cells were cultured for 24 hours at 37°C in a 5% CO_2 atmosphere. The cells were incubated with the compounds (100µL/well) at concentrations ranging from 100 to 5 µg/mL. Gentian Violet was used as the positive control. The negative control consisted of J774 cells containing only complete RPMI medium. The cells were incubated for 72 hours. Cell viability was measured by Alamar Blue metabolism (Invitrogen, CA, USA). After that, the absorbance was read on a spectrophotometer at 570 nm and 600 nm [43, 44, 45]. Each compound was tested in triplicate in 3 independent experiments. The 50% inhibitory concentration value (IC_{50}) was determined by nonlinear regression using the GraphPad Prism version 5.01 (GraphPad Software).

2.2.2 Parasites and Definitive hosts

Infection for each mouse was performed percutaneously using 100 *S. mansoni* cercariae (Strain LE - Belo Horizonte) that were derived from *Biomphalaria glabrata* freshwater snails maintained at Departamento de Malacologia do Centro de Pesquisa Aggeu Magalhães (CPqAM). Fifty Swiss albino mice (*Mus musculus*) (25 days of age) were used. After 60 days, a parasitological examination of the feces of the mice was conducted to evaluate the positivity of infection [46]. This project was approved by the Animal Ethics Committee from Centro de Pesquisa Aggeu Magalhães/Fundação Oswaldo Cruz (CPqAM/FIOCRUZ) and authorized by the license n°. 21/2011.

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2.2.3 Perfusion by the hepatic portal vein for counting adult *S. mansoni* worms

Adult *S. mansoni* worms were obtained from mice after 60 days of infection. The animals were intraperitoneally anesthetized with ketamine hydrochloride (115 mg/kg) associated with xylazine hydrochloride (10 mg/kg). After anesthesia, the animals were subjected to perfusion by the hepatic portal vein to remove the worms which were separated on Petri dishes with 0.85% saline, and then the parasites were counted and categorized according to the gender and vitality [47].

The parasites removed from the mice infected were washed with a medium (RPMI-1640 containing 20 mM HEPES pH 7.5, 100UI/mL penicillin, 100 µg/mL streptomycin and 10% FBS). After washing, the adult worms were transferred to tissue culture plates containing 2 mL of medium. Each well received two worms, and then they were incubated at 37°C in a 5% CO_2 humidified atmosphere. After a 2-hour period of adaptation to the environment, the imidazolidinic derivative 3 was added at concentrations of 100 µg/mL, 80 µg/mL, 60 µg/mL, 40 µg/mL and 20 µg/mL. The parasites were maintained in culture for 6 days and were monitored every 24 hours for evaluation of their motility, mortality and tegumental changes. PZQ was the standard drug of the experiment (positive control). The worms from the negative control group were treated only with dimethyl sulfoxide (DMSO) in a RPMI medium. The motility of the parasites was analyzed and scored according to the criteria proposed by Horiuchi et al. [48]. The scoring system was as follows: 3 - normal body movement; 1.5 - partial body movement; and 0 - dead.

2.2.4 Scanning electron microscopy

After 24 hours of treatment with the imidazolidinic derivative 3 at concentrations of 60 µg/mL and 100 µg/mL, the worms were fixed with 2.5% glutaraldehyde in a 0.1 M phosphate buffer (pH 7.2) for 2 hours at room temperature. Then, they were washed twice in the same buffer and post-fixed with 1% osmium tetroxide in a phosphate buffer for 1 hour at room temperature. All the worms were dehydrated with 100% ethanol, and then dried with liquid CO_2 in a critical-point dryer machine, mounted on stubs, coated with gold, and examined using an electron microscopy (Field Emission Ambiental FEI Quanta 200 FEG).

3. RESULTS AND DISCUSSION

3.1 Cytotoxicity assay and *in vitro* schistosomicidal activity of the imidazolidinic compound 3

The data relating to the mobility and mortality of the worms are summarized in Tables 01 and 02, respectively. Throughout the 144 hours of observation period, all the adult *S. mansoni* worms incubated in absence of any drug (negative control group) exhibited typical wavy and peristaltic movement along the body axis, with occasional adherence to the bottom of the culture plate through the ventral sucker (score = 3). In the evaluation of the in vitro activity, we observed that the imidazolidinic derivative 3 showed a promising response against adult *S. mansoni* worms. On the fourth day, 100% of the worms treated with 60 and 100 µg/mL of 3 died (score = 0). However, there was only a decrease of movement at other doses (80 µg/mL, 40 µg/mL and 20 µg/mL) (score = 1.5).At the end of the experiment, on the sixth day of observation, 100% of the worms treated with the compound 3 at all doses tested died (score = 0), except for the dose of 20 µg/mL (score = 1.5).

In contrast, the worms exposed to the antischistosomal drug of choice, praziquantel (positive control group), exhibited severe muscle contraction with partial movements or immobile but alive (score = 1.5), which occurred immediately after praziquantel administration. During the first 24 hours of praziquantel treatment at all doses tested, 100% of the worms were dead (score = 0). Additionally, compound 3 interrupted oviposition, the suckers become non adherent and there was clearance of parasites (Not paired).

The cytotoxicity of the compound 3 was determined in cells of the macrophage cell line J774. The derivative 3 showed an IC_{50} of 29.7±3.9 µM. However, reports in the literature indicate that

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PZQ has high toxicity (<1 µg/mL) and is more cytotoxic than the imidazolidinic derivatives [26,34].

Table 1: Motility scores of the worms from the negative control group, and from the groups treated with praziquantel (PZQ) and with the imidazolidinic derivative 3.

Groups	Percent of worms (%) in motility scores after incubation																	
	24 h			48 h			72 h			96 h			120 h			144 h		
	3	1.5	0	3	1.5	0	3	1.5	0	3	1.5	0	3	1.5	0	3	1.5	0
Control	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0
PZQ/40µg/mL	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100	0	0	100
Compound 3																		
100µg/mL	8.3	75	16.7	0	41.7	58.3	0	8.3	91.7	0	0	100	0	0	100	0	0	100
80µg/mL	8.3	58.3	33.4	0	41.7	58.3	0	33.3	66.7	0	33.3	66.7	0	8.3	91.7	0	0	100
60µg/mL	16.7	58.3	25	0	41.7	58.3	0	25	75	0	0	100	0	0	100	0	0	100
40µg/mL	22.2	55.6	22.2	11.1	66.7	22.2	0	66.7	33.3	0	66.7	33.3	0	22.2	77.8	0	0	100
20µg/mL	41.7	58.3	0	41.7	58.3	0	41.7	58.3	0	41.7	58.3	0	33.3	66.7	0	0	66.7	33.3

Score criteria - 3, complete body movement; 1.5, partial body movement or immobile but alive; 0, dead.

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3.2 The imidazolidinic derivative 3 induced ultrastructural alterations in worm tegument

The scanning electron microscopy revealed detailed surface membrane ultrastructural damage caused by the exposure to the imidazolidinic derivative 3 (60 and 100 µg/mL) compared with the negative (untreated) and positive (exposed to praziquatel) controls.

Male worms treated only with DMSO in RPMI-1640 medium were used as a negative control. In the anterior portion of the body, the gynecophoral canal, a longitudinal fold of the middle and posterior body that houses the female for the purpose of mating and reproduction, can be observed (Fig. 2A). Along the body axis, the oral and ventral suckers in normal state can be visualized (Fig. 2B). In the negative control group, the worm tegument was observed with a large number of tubercles and numerous spines (Fig. 2C and D).

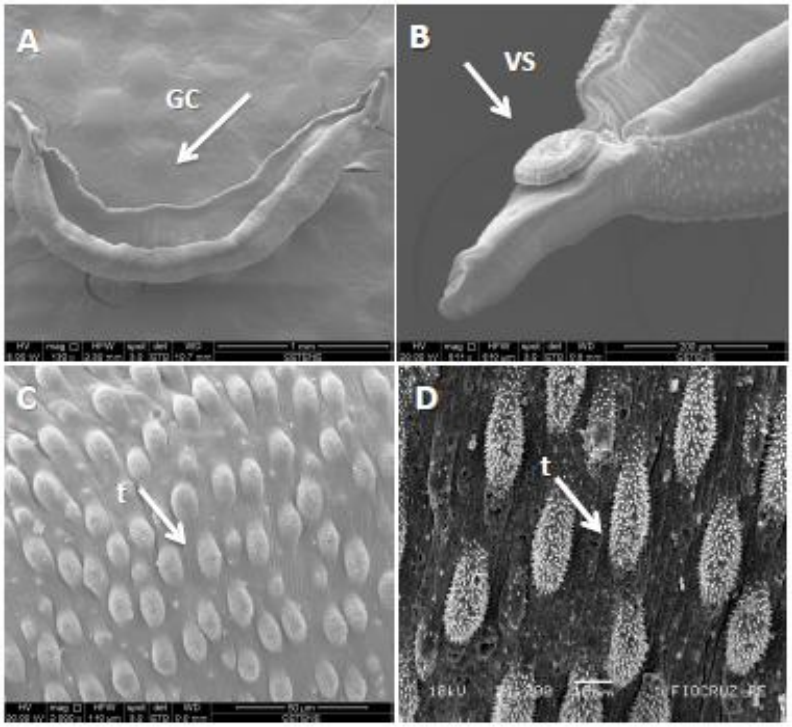

Fig. 2. Images of adult male *S. mansoni* from the negative control group after 24 hours of incubation (A–D): (A, 130x) gynecophoral canal (GC), (B, 544x) ventral sucker (VS), (C, 2000x) worms with normal tegument (t) (arrow) and (D, 1200x) a large number of tubercles (t) with their spines.

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The imidazolidinic derivative 3 and PZQ induced severe damage to the worms. After 24 hours of incubation with the imidazolidinic derivative 3 at the dose of 60 µg/mL, adult *S. mansoni* worms presented severe changes such as the contraction of the body (Fig. 3A), head and suckers (Fig. 3B), loss of spines in the tubercules (Fig. 3C and 2D), tegumental blistering and peeling of the tegument which resulted in the destruction of the tubercules (Fig. 3E and F).

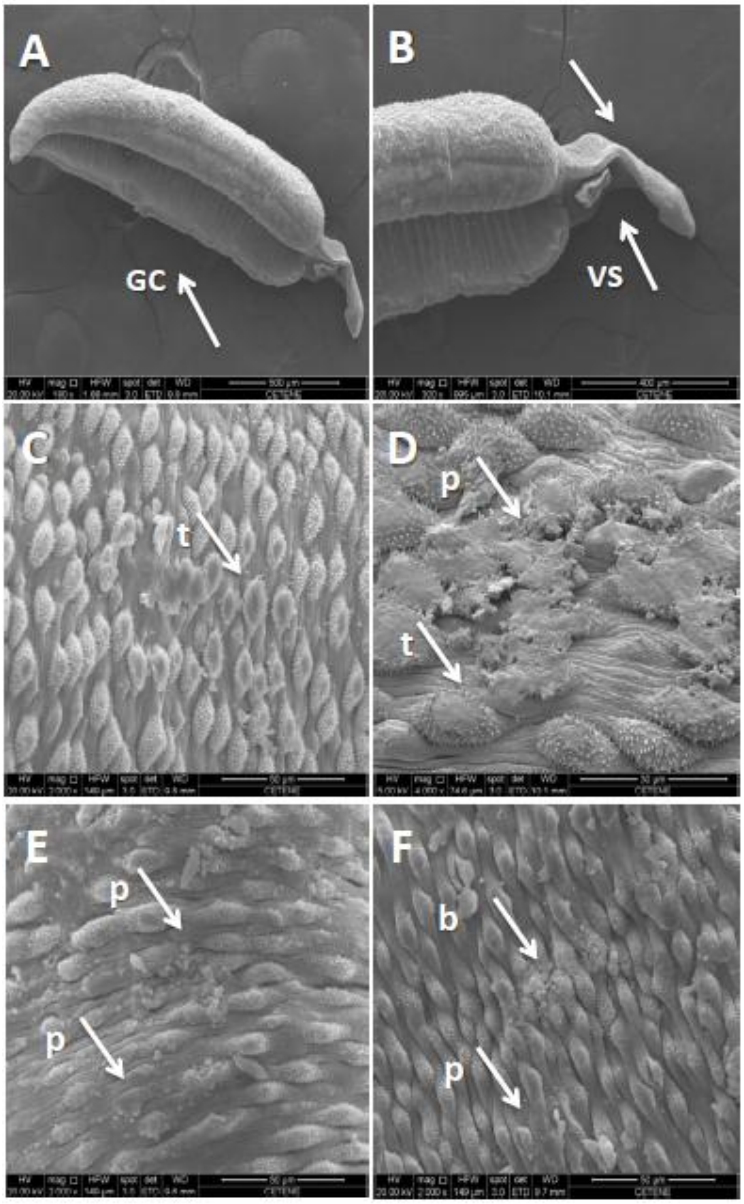

Fig. 3. Scanning electron microscopy of the tegument of an adult *S. mansoni* worm treated with the compound 3 at a dose of 60 µg/mL (A–F), showing the contraction on the body, gynecophoral canal (GC) (A, 180x) and ventral suckers (VS) (B, 300x); loss of spines in the tubercules (t) (C, 2000x); peeling of the tegument (p) (D, 4000x) and (E, 2000x); and blistering (b) (F, 2000x).

After 24 hours of incubation with the imidazolidinic derivative 3 at a dose of 100 µ/mL, adult *S. mansoni* worms had a significant opening of the gynecophoral canal (Fig. 4A), contraction of the head and suckers (Fig. 4B), collapse of the tubercle with erosion of the tegument (E) (Fig. 4C) and a severe lesion revealing the layer of subtegument tissue (ST) (Fig. 4D). In this case, there was a enormous destruction of the subtegument surface (ST) (Fig. 4E and F).

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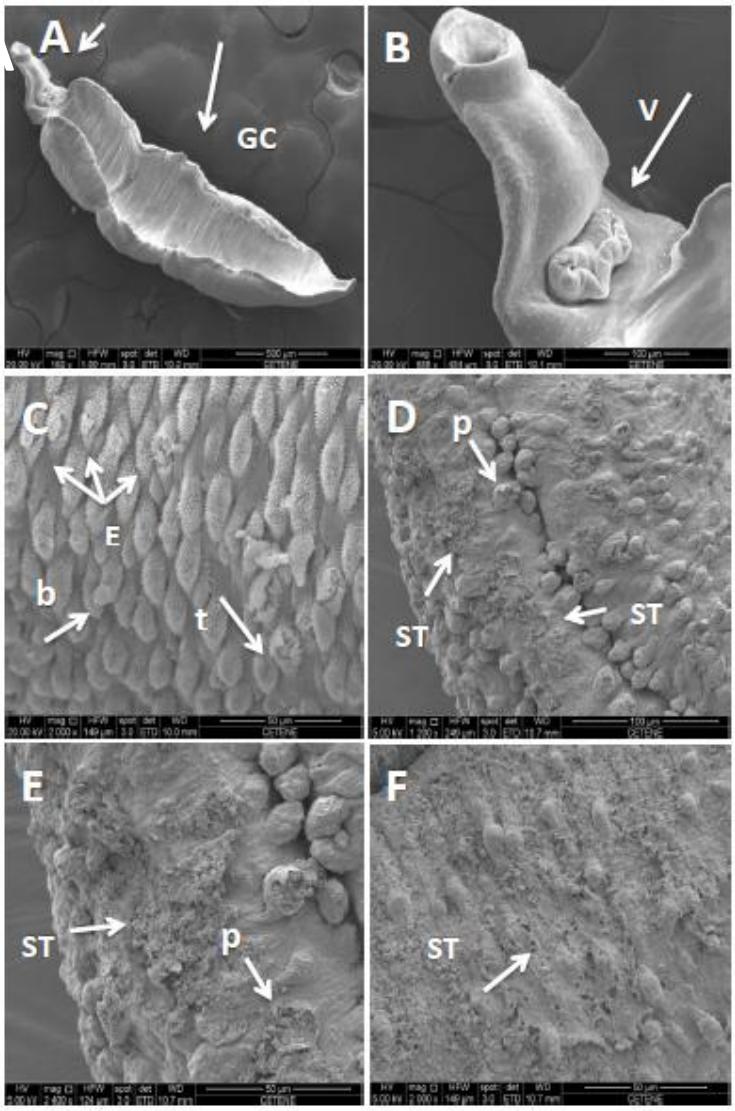

Fig. 4. Scanning electron microscopy of the tegument of an adult *S. mansoni* treated with the compound 3 at a dose of 100 µg/mL (A–F), showing opened gynecophoral canal (GC) (A, 2000x), contraction of the head and suckers (VS) (B, 688x), tegument erosion (E) (C, 2000), and destruction of the subtegument tissue (ST) (D, 1200x), (E, 2400x) and (F, 2000x).

The *in vitro* effects of praziquantel (100 µg/mL) on adult male *S. mansoni* worms promoted an evident contraction of the longitudinal muscles (Fig. 5A and B). The worms were curved and shortened in appearance, and most tubercles were juxtaposed (Fig. 5C and D). Severe lesions became evident including peeling, collapse of the tubercles and appearance of many bubbles (Fig. 5E and F).

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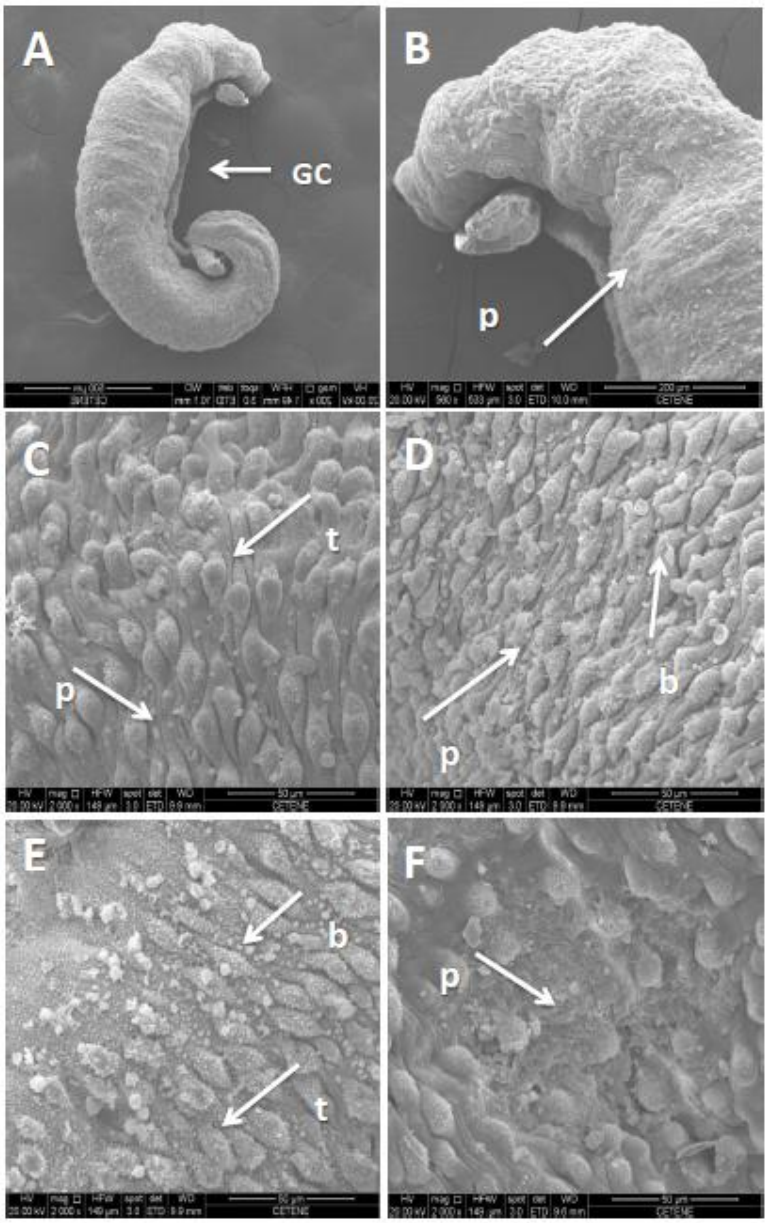

Fig. 5. Scanning electron microscopy of the tegument of the adult *S. mansoni* worms treated with PZQ at a dose of 100 µg/mL (A–F), showing the contraction in the body and the gynecophoral canal (GC) (A, 200x), the peeling of the tegument (p) and the contraction of the suckers (arrow) (B, 560x), loss of spines in the tubercules (t) (arrows) (C, 2000x), peeling (p) and appearance of bubbles (b) (D, 2000x), (E, 2000x) and (F, 2000x).

4. DISCUSSION

The evaluation of antischistosomal drug candidates is of great importance for understanding the biology of the parasite and may be prophylactic causing the death of schistosomula, suppressive for inhibiting oviposition or display a curative activity for being able to cause the death of the adult *S. mansoni* worms [49]. Thus, many parameters need to be analyzed such as motor activity, mortality, oviposition and the structural changes in order to find out what the potential of the compound against the parasite [50].

Among the various antischistosomal compounds already tested, the imidazolidinic derivatives are well known for their activity in several works that are studying their *in vitro* and *in vivo*

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efficacy, showing promising results when compared to praziquantel, a control drug available in the market for the treatment of schistosomiasis [33, 31, 34, 51].

Neves and colleagues have been working with imidazolidinic derivatives conducting *in vitro* activities, scanning electron microscopy analyzes, cytotoxicity and measurement of cytokines during acute and chronic disease. Their studies indicate that these imidazolidinic compounds were able to show similar results to PZQ with 100% mortality of adult *S. mansoni* worms in the first 24 hours of contact with the compound. The compound 3 did not show as fast results as the compounds mentioned above, but it was able to cause the maximum mortality rate after 96 hours of experiment. Furthermore, supporting the work with the Imidazolidines mentioned above, the compound 3, in the cytotoxicity assays, also showed to be less cytotoxic than praziquantel at the cellular level [34, 26].

Adult *S. mansoni* worms have a variety of movements, including rapid shortening and extension of the body, typical wavy and peristaltic movement along the body anterior and posterior axis [52]. The motor activity of the worms could be related to the important neurotransmitters or neuromodulators such as serotonin, norepinephrine, epinephrine, dopamine, acetylcholine, epinephrine, glutamate and neuropeptides [53, 54, 55].

The mechanism of action of the imidazolidinic derivatives is not fully elucidated yet. However, there is evidence that these compounds act at the levels of the cholinergic receptors [56]. Acetylcholine may have an important physiological role as an inhibitory neurotransmitter in *S. mansoni* once its motor activity is reduced by inhibiting acetylcholinesterase, showing a flaccid paralysis with loss of motility followed by the stretching of the worm [53]. These reports corroborate our research because the compound 3 tested in this study, in some parasites, was able to cause muscle relaxation and subsequent elongation of the worms. It can be seen in some images of scanning electron microscopy. On the other hand, some worms showed contraction of the body and suckers, similar to the results seen with PZQ which also does not have a mechanism of action fully discovered, but there is already evidence showing that the contraction is because of the calcium influx responsible for causing the muscle contraction [57].

Acetylcholinesterase is also found on the tegumental surface of the worm and has the function of obtaining glucose. Since this enzyme is inhibited, the absorption of nutrients for the survival of the parasite may be compromised [53]. Thus, inhibition of acetylcholinesterase may be a therapeutic target against the parasite. This has been seen in studies using metrifonate which showed acetylcholinesterase activity [58].

Another very important possible biological target to combat the disease has been the tegumental surface, since this structure is involved in the immune response of the worm against the definitive host. It has sensory activity and the ability to absorb nutrients [8]. Our results, based on the ultrastructural analyzes, demonstrated that the treatment with the imidazolidinic derivative 3, at doses of 60 µg/mL and 100 µg/mL, can be involved in the mortality of the worms, since the compound was able to induce destruction of the tegument, with loss of spines and tubercles, formation of bubbles and destruction of the sub-tegumental surface in adult male *S. mansoni* worms.

Studies indicate that the tegumental changes are more pronounced in male worms than in female ones, since there is not a frequent contact between the female worms and the definitive host environment because they remain in the gynecophoral canal of the male worms [16, 59]. These data corroborate our experiments once it was possible to show that only the male parasites showed greater changes in the tegumental surface in relation to the female parasites. This can also be seen in many previous studies with antischistosomal compounds, such as oxamniquine [60, 19], artemether [15], miltefosine [61], mefloquine [24, 25], praziquantel [62, 63, 21, 64] and thioxo-imidazolidinic compounds [33, 32, 26].

Imidazolidinic compounds such as (Z)-3-(4-chlorobenzyl)-5-(4-nitro-benzylidene)- imidazolidine-2,4-dione, (Z)-3-(4-chloro- benzyl) -5-(4-fluoro-benzylidene)-1-methyl-2- thioxo-imidazolidin-4-one and (Z)-5-(4-fluoro-benzilidene)-1-methyl-3-(4-phenyl-benzyl)-2-thioxo-imidazolidin-4-one induced significant changes in the tegumental surface of the body of adult *S. mansoni* worms, causing damage in the tegument with contraction of the body and of oral and ventral suckers, disorganization and total collapse of the tubercles with loss of spines [32]. The nitro, fluorine and phenyl radicals, present in the imidazolidinic derivative of this work, helped to improve the efficacy of the compound against the worms [65, 66].

Promising results with other imidazolidinic compounds presenting chlorine and fluorine radicals in their structure were also able to cause ultrastructural changes in the tegument of adult worms of *S. mansoni*, such as the derivatives 1-benzyl-4-[(4 -chloro-phenyl)-hydrazono]-5-thioxo-imidazolidin-2-one and 1-(4-chloro-benzyl)-4-[(4-fluoro-phenyl)-hydrazono]-5-thioxo-

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imidazolidin-2-one. According to Thomas [66], the halogens have the ability to enhance the absorption of the derivatives by the cell membranes. Thus, this may have happened in the tegumental surface of the parasite treated with the above compounds as well as with the compound 3 which presents the halogens fluorine and bromine in its chemical structure.

5. CONCLUSION

In conclusion, the imidazolidinic derivative 3 showed a promising in vitro schistosomicidal activity when compared to the reference drug (praziquantel). Thus, it is necessary to investigate the elucidation of the mechanism of action of this compound as well as to invest in further studies to investigate its biological activity such as an in vivo evaluation.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as the ethical principles of the Brazilian Society of Laboratory Animal Science (SBCAL). This project was approved by the Animal Ethics Committee from Centro de Pesquisa Aggeu Magalhães/Fundação Oswaldo Cruz (CPqAM/FIOCRUZ) and authorized by the license no. 21/2011.

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