British Journal of Pharmaceutical Research
4(16): 1988-2005, 2014

SCIENCEDOMAIN *international*
www.sciencedomain.org

Tegumental Changes in Adult *Schistosoma mansoni* Induced by a New Imidazolidinic Derivative

Anekécia Lauro da Silva[1*], Sheilla Andrade de Oliveira[2], Jamerson Ferreira de Oliveira[1], Edna de Farias Santiago[2], Antônio Sérgio Alves de Almeida Júnior[1], Íris Trindade Tenório Jacobi[1], Christina Alves Peixoto[3], Vinícius Pinto Costa Rocha[4], Milena Botelho Pereira Soares[4], Ivan da Rocha Pitta[1] and Maria do Carmo Alves de Lima[1]

[1]Department of Antibiotics, Institute of Biology, Federal University of Pernambuco (UFPE). Av. Moraes Rego s/n, Ciudad Universitaria, 50670-901 Recife, PE, Brazil.
[2]Laboratório of Immunology and Molecular Biology (libm), Aggeu Magalhães Research Center, Oswaldo Cruz Foundation (FIOCRUZ), 50670-420 Recife, PE, Brazil.
[3]Laboratório of Ultrastructure, Department of Entomology, Center Aggeu Magalhães, Fundação Oswaldo Cruz (FIOCRUZ), 50670-420 Recife, PE, Brazil.
[4]Laboratório of Tissue Engineering and Immunopharmacology (LETI), Gonçalo Moniz Research Center, Oswaldo Cruz Foundation (FIOCRUZ), 40296-710 Salvador, BA, Brazil.

Authors' contributions

This work was carried out in collaboration between all authors. Author ALS participated in all experiments of biological activity as well as the manufacture of writing and discussion of all results of the manuscript. Authors JFDO and ALS assisted in the preparation and correction of written of the manuscript. Author JFO participated in all experiments of biological activity. Author EFS participated in all experiments of biological activity. Author ASAAJ participated in all experiments of biological activity. Author ITTJ participated in all the syntheses for obtaining imidazolidinic derivative. Author CAP participated of experiments of biological activity. Author VPCR participated in experiments of biological activity. Author MBPS participated in experiments of biological activity. Author IRP assisted in the preparation and correction of written. Author MCAL participated in all the syntheses for obtaining imidazolidinic derivative, assisted in the preparation and correction of written and discussion of all results of the manuscript.

Original Research Article

Received 2nd July 2014
Accepted 29th July 2014
Published 16th August 2014

**Corresponding author: Email: nenalima.mariadocarmo@gmail.com;*

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 (LC_{50}) was determined by nonlinear regression using the Graph Pad Prism version 5.01. Then the compound was evaluated against adult worms of *S. mansoni* by performing the activity *in vitro* at doses 100-20µg/mL and ultrastructural investigation by Scanning Electron Microscopy (SEM) at doses of 100 and 60µg/ml. The PZQ was the positive control of the experiment.
Results: The derivative 3 showed LC_{50} of 29.7±3.9mM. Compound 3 was able to have decreased motility of *S. mansoni* culminating with a mortality rate of 100% at doses of 60 and 100µg/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 by the trematoda *Schistosoma mansoni*. It is still considered a parasitic disease of great importance to public health, being responsible for affecting 391-597 million people worldwide [1-3], especially in continents such as Latin American, Caribbean, African and Middle America [4]. 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 [5].

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 [6-8].

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 [9,10].

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 [8]. 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 [11-13].

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 [14-16]. 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 [16]. 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 [17].

Numerous structural alterations of the tegumental surface of adult *S. mansoni* worms have been observed through studies using antischistosomal compounds such as hicantone [18], niridazol [19], oxaminiquine [20], praziquantel (PZQ) [21-23], atorvastatin [24], mefloquine [25,26] and thioxo-imidazolidine derivatives [27].

The Imidazolidines are bioactive heterocyclic compounds that exhibit various biological activities such as antimicrobial activity [28], antihypertensive activity [29], antineoplastic activity [30], anti-*Trypanosoma cruzi* activity [31] and antischistosomal activity [32-34]. 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 [35, 27]. However, as PZQ, the mechanism of action of the Imidazolidines has not been fully elucidated yet [27].

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. MATERIALS 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 (Fig. 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) [36]. 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 [37]. Then 2-cyano-3-(4-chlorophenyl)-acrylic acid ethyl ester (2) [38] 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 [39-43]. 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 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 (165mg) were reacted using absolute ethanol (8mL) as the solvent and morpholine (1mL) as the catalyst. The reaction mixture was heated to 50ºC 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: 202-3ºC; 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).

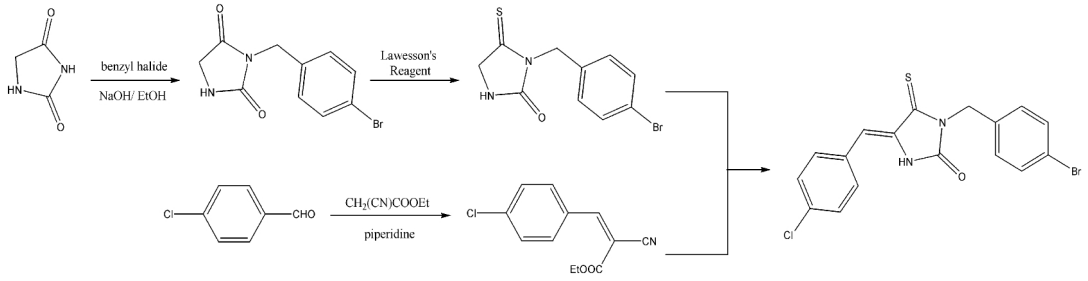

Fig. 1. Obtainment of the imidazolidinic derivative

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 50µg/mL of gentamicin (Hipolabor, Belo Horizonte, Brazil). The cells were cultured for 24 hours at 37ºC in a 5% CO_2 atmosphere. The cells were incubated with the compounds (100µL/well) at concentrations ranging from 100 to 5µg/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 570nm and 600nm [44-46]. Each compound was tested in triplicate in 3 independent experiments. The 50% inhibitory concentration value (IC_{50}) was determined by nonlinear regression using the Graph Pad 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 [47]. 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.

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 (115mg/kg) associated with xylazine hydrochloride (10mg/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 [48].

The parasites removed from the mice infected were washed with a medium (RPMI-1640 containing 20mM HEPES pH 7.5, 100UI/mL penicillin, 100µg/mL streptomycin and 10% FBS). After washing, the adult worms were transferred to tissue culture plates containing 2mL of medium. Each well received two worms, and then they were incubated at 37ºC 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 100µg/mL, 80µg/mL, 60µg/mL, 40µg/mL and 20µg/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 praziquantel was purchase from Sigma–Aldrich®). The imidazolidinic derivative 3 and PZQ were dissolved in 1.6% dimethyl sulphoxide (DMSO) [49]. The worms from the negative control group were

treated only with dimethyl sulfoxide (DMSO) in a RPMI medium. The scoring system was as follows: 3-normal body movement; 1.5-partial body movement; and 0-dead. The motility of the parasites was analyzed and scored according to the criteria proposed by Horiuchi et al. [50].

2.2.4 Scanning electron microscopy

After 24 hours of treatment with the imidazolidinic derivative 3 at concentrations of 60µg/mL and 100µg/mL, the worms were fixed with 2.5% glutaraldehyde in a 0.1M 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 100µg/mL of 3 died (score=0). However, there was only a decrease of movement at other doses (80µg/mL, 40µg/mL and 20µg/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 20µg/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 was the decoupling of parasites.

The cytotoxicity of the compound 3 was determined in cells of the macrophage cell line J774. The derivative 3 showed an IC_{50} of 13,8µg/mL. However, reports in the literature indicate that PZQ has high toxicity (<1µg/mL) and is more cytotoxic than the imidazolidinic derivatives [27,35].

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 100µg/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 (Figs. 2C and D).

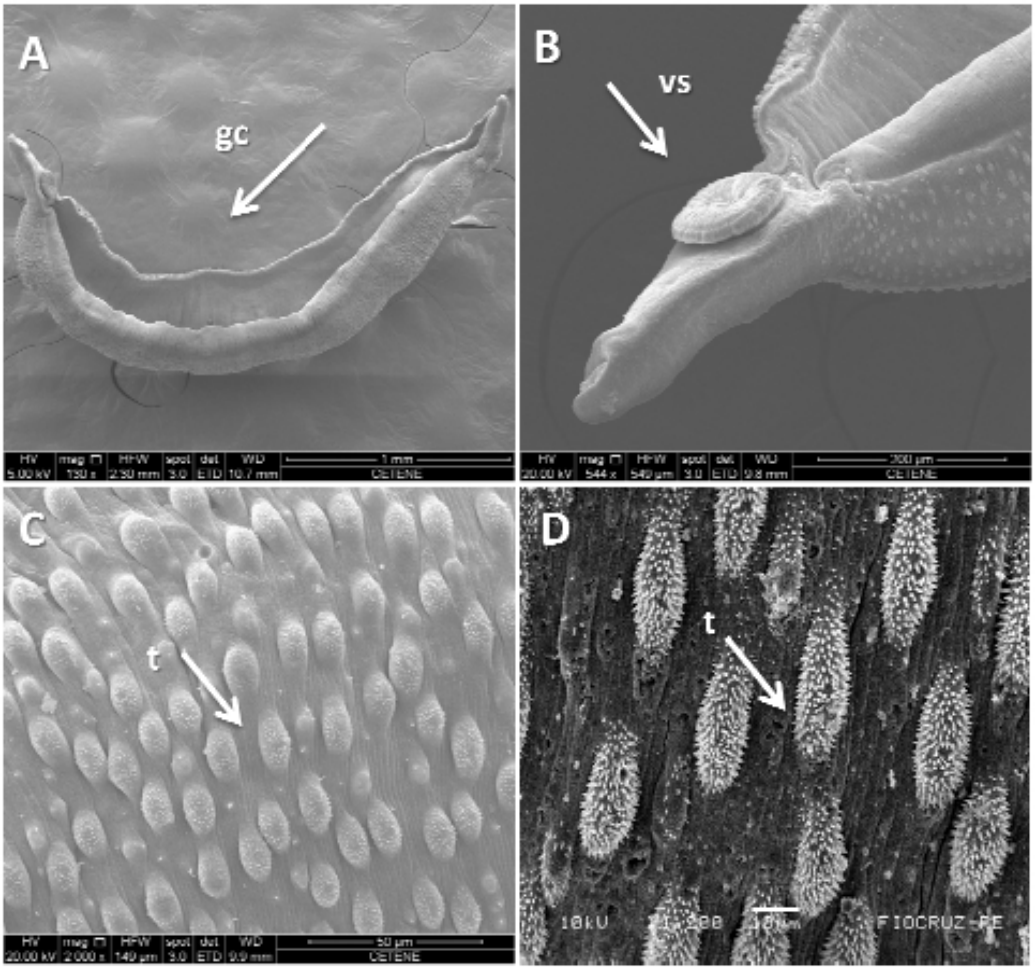

Fig. 2. Images of adult male *S. mansoni* from the negative control group after 24 hours of incubation (A–D): (A, 130x) gynecophoral canal (gc), (B, 544x) ventral sucker (vs), (C, 2000x) worms with normal tegument (t) (arrow) and (D, 1200x) a large number of tubercles (t) with their spines

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																	
	24h			48h			72h			96h			120h			144h		
	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

The imidazolidinic derivative 3 (Figs. 3 and 4) and PZQ (Fig. 5) induced severe damage to the worms. After 24 hours of incubation with the imidazolidinic derivative 3 at the dose of 60µg/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 (Figs. 3C and 2D), tegumental blistering and peeling of the tegument which resulted in the destruction of the tubercules (Figs. 3E and F).

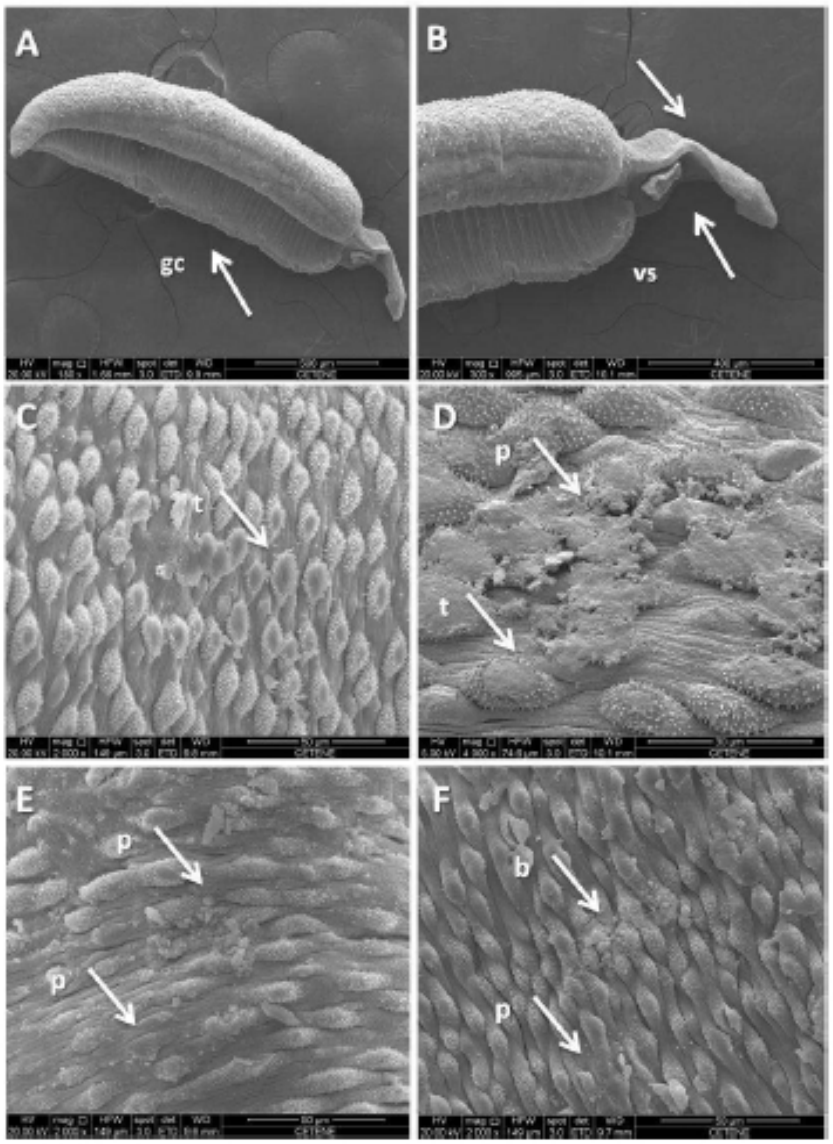

Fig. 3. Scanning electron microscopy of the tegument of an adult *S. mansoni* worm treated with the compound 3 at a dose of 60µg/mL (A–F), showing the contraction on the body, gynecophoral canal (gc) (A, 180x) and ventral suckers (vs) (B, 300x); loss of spines in the tubercules (t) (C, 2000x); peeling 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 100µ/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) (Figs. 4E and F).

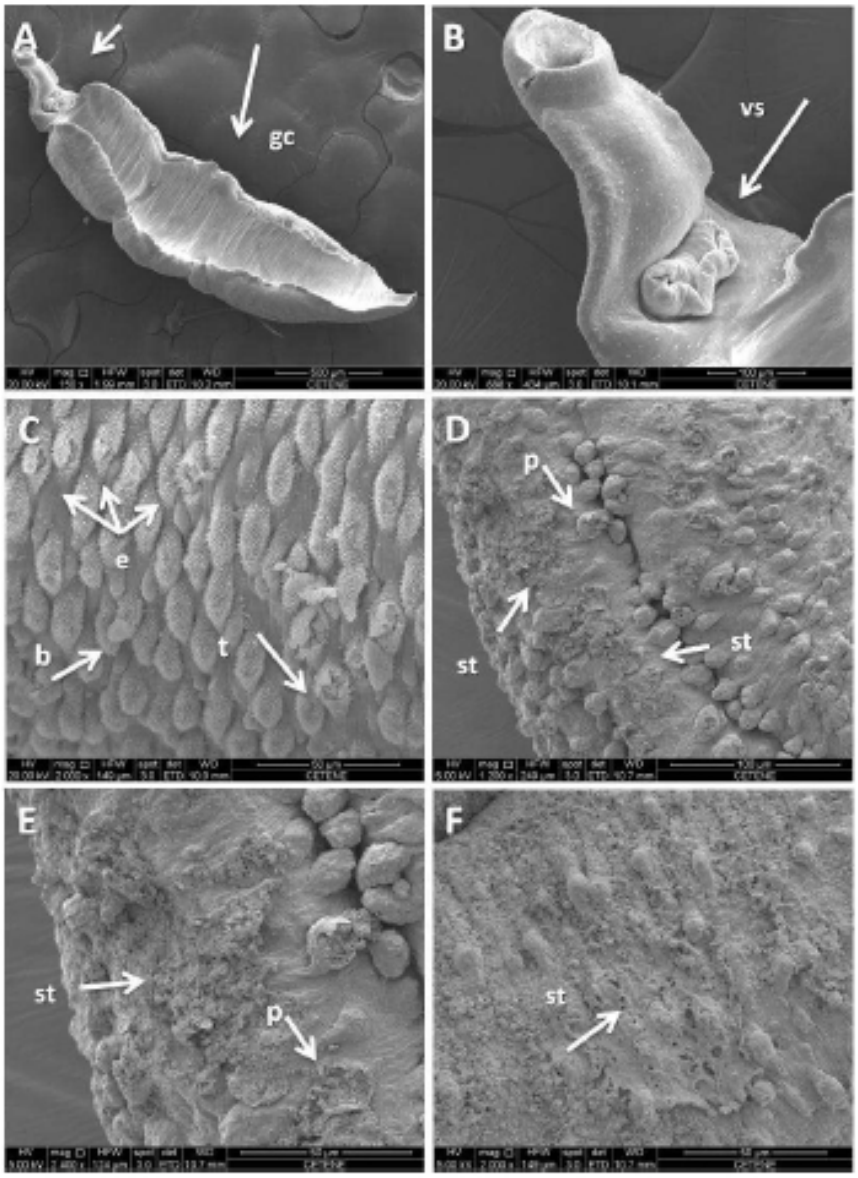

Fig. 4. Scanning electron microscopy of the tegument of an adult *S. mansoni* treated with the compound 3 at a dose of 100µg/mL (A–F), showing opened gynecophoral canal (gc) (A, 2000x), contraction of the head and suckers (vs) (B, 688x), tegument erosion (e) (C, 2000), and destruction of the subtegument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

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

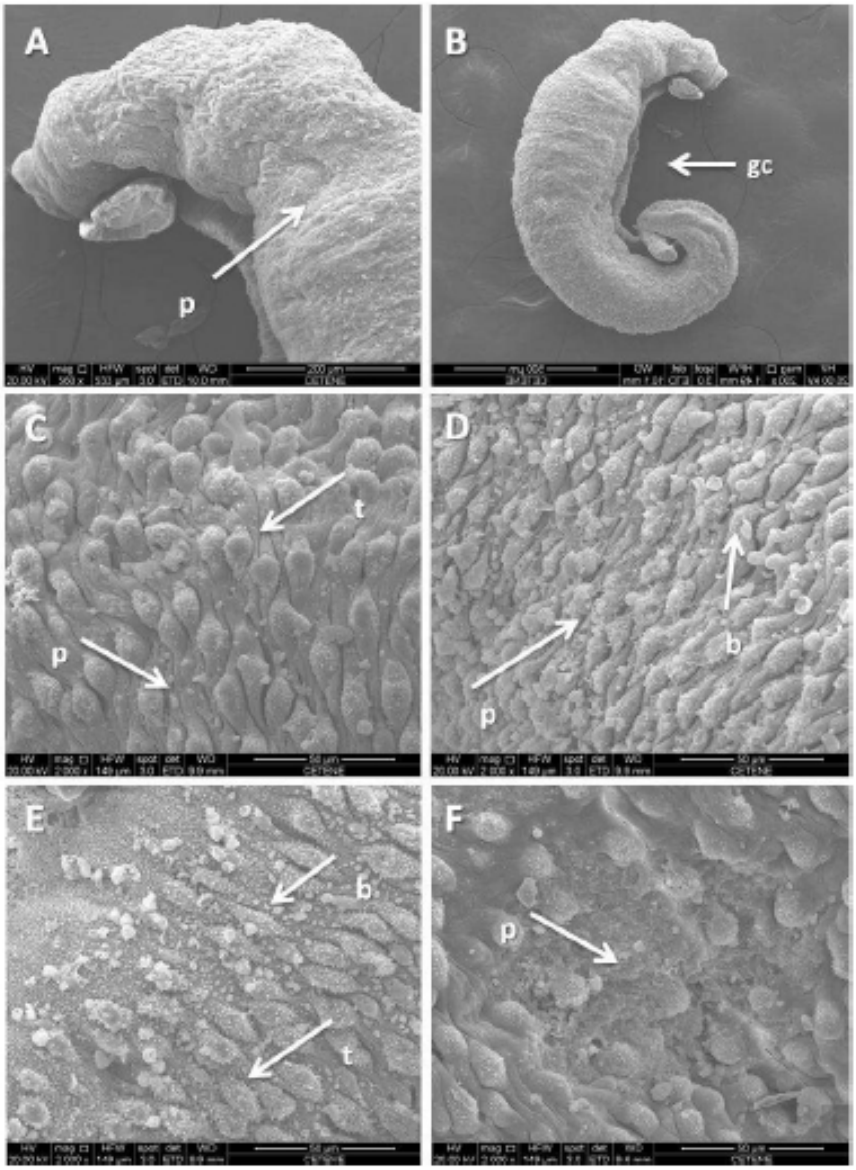

Fig. 5. Scanning electron microscopy of the tegument of the adult *S. mansoni* worms treated with PZQ at a dose of 100µg/mL (A–F), showing the contraction in the body and the gynecophoral canal (gc) (A, 200x), the peeling of the tegument (p) and the contraction of the suckers (arrow) (B, 560x), loss 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 [51]. Thus, many parameters need to be analyzed such as motor activity, mortality, oviposition and the structural changes in order to find out that the potential of the compound against the parasite [52].

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* efficacy, showing promising results when compared to praziquantel, a control drug available in the market for the treatment of schistosomiasis [34,32,35,53].

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 [35,27]. Compound 3 showed no significant results as mentioned above, but was able to cause a maximum rate of mortality after 96 hours of exposure.

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 [54]. 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 [55-57].

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 [58]. 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 [55]. 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 [59].

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 [55]. Thus, inhibition of acetylcholinesterase may be a therapeutic target against the parasite as noted in studies using metrifonate [60].

Another possible biological target for 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 [9]. Our results, based on the ultrastructural analyzes, demonstrated that the treatment with the

imidazolidinic derivative 3, at doses of 60µg/mL and 100µg/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 no frequent contact between the female worms and the definitive host environment because they remain in the gynecophoral canal of the male worms [17, 61]. 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 [62,20], artemether [16], miltefosine [63], mefloquine [25,26], praziquantel [64,65,22,66] and thioxo-imidazolidinic compounds [34,33,27].

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 [33]. Thus, the nitro, fluorine and phenyl radicals can justify the good activity of the imidazolidinics derivatives mentioned above [67,68].

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-imidazolidin-2-one. According to Thomas [68], 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.

CONSENT

Not applicable.

ETHICAL APPROVAL

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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. King CH. Parasites and poverty: the case of schistosomiasis. Acta Trop. 2010;113:95-104.
2. Gray DJ, McManus DP, Li Y, Williams GM, Bergquist R, Ross AG. Schistosomiasis elimination: Lessons from the past guide the future. Lancet Infect Dis. 2010;10:733-6.
3. Siddiqui AA, Siddiqui BA, Ganley-Leal L. Schistosomiasis vaccines. Hum Vaccin. 2011;7:1192-7.
4. World Health Organization (WHO). Initiative for Vaccine Research (IVR). Parasitic Diseases.
   Available at: www.who.int/vaccine_research/diseases/soa_parasitic/en/index5.html. Acessado no dia 14/01/2013.
5. Vitorino RR, Souza FPC, Costa AP, Faria-junior FC, Santana LA, Gomes AP. Schistosomiasis: Diagnosis, treatment, epidemiology, prevention and control. Rev Bras Clin Med. 2012;10(1):39-45.
6. Morris GP, Threadgold LT. Ultrastructure of the tegument of adult *Schistosoma mansoni*. J Parasitol. 1968;54:15-27.
7. Smith JH, Reynolds ES, Von Lichtenberg F. The integument of *Schistosoma mansoni*. Am J Trop Med Hyg. 1969;18:28-49.
8. Hockley DJ. Ultrastructure of the tegument of *Schistosoma mansoni*. Advances in Parasitology. 1973;11:233-305.
9. Hoffmann KF, Strand M. Molecular identification of a *Schistosoma mansoni* tegumental protein with similarity to cytoplasmic dynein light chains. The Journal of Biological Chemistry. 1996;271:26117-26123.
10. Xiao SH, Shen BG, Utzinger J, Chollet J, Tanner M. Ultrastructural alterations in adult *Schistosoma mansoni* caused by artemether. Memories of the Oswaldo Cruz Institute. 2002;97: 717-724.
11. Ruppel A, Mclaren DJ. *Schistosoma mansoni*: Surface membrane stability in vitro and in vivo. Exp Parasitol. 1986;62(2):223-236.
12. Faghiri Z, Skelly PJ. The role of tegumental aquaporin from the human parasitic worm, *Schistosoma mansoni*, in osmoregulation and drug uptake. Faseb J. 2009;23:2780-2789.
13. Pereira AS, Padilha RJ, Lima-filho JL, Chaves ME. Scanning electron microscopy of the human low-density lipoprotein interaction with the tegument of *Schistosoma mansoni*. Parasitol Res. 2011;109:1395-1402.
14. Xiao SH, Shen BG, Catto BA. Effect of artemether on ultrastructure os *Schistosoma japonicum*. Chin J Parasitol Parasit Dis. 1996;14:181-187.
15. Xiao S, Binggui S, Chollet J, Tanner M. Tegumental changes in 21-day-old *Schistosoma mansoni* harboured in mice treated with artemether. Acta Trop. 2000;75:341-348.
16. Xiao SH, Shen BG, Chollet J, Utzinger J, Tanner M. Tegumental alterations in juvenile *Schistosoma haematobium* harboured in hamsters following artemether treatment. Parasitol Int. 2001;50:175-183.

17. Nahed HA, Riad HAT, Yomna IM. Effects of garlic on albino mice experimentally infected with *Schistosoma mansoni*: A parasitological and ultrastructural study. Trop Biomed. 2009;26:40-50.
18. Hillman GR, Gibler WB, Anderson JB. Comparative effects of hycanthone in *Schistosoma mansoni* and *Schistosoma japonicum*. Am J Trop Med Hyg. 1977;26:238-242.
19. Popiel I. & Erasmus DA. *Schistosoma mansoni*: Ultrastructure of adults from mice treated with oxamniquine. Exp Parasitol. 1984;58:254-262.
20. Fallon PG, Fookes RE, Wharton G.A. Temporal differences in praziquantel–and oxamniquine-induced tegumental damage to adult *Schistosoma mansoni*: Implications for drug-antibody synergy. Parasitology. 1996;112:47-58.
21. Becker B, Mehlhorn H, Andrews P, Thomas H, Eckert J. Light and electron microscopic studies on the effect of praziquantel on *Shistosoma mansoni, Dicrocoelium dendriticum* and *Fasciola hepatica* (trematoda) in vitro. Z Parasitenkd. 1980;63:113-128.
22. Liang YS, Coles GC, Dai JR, Zhu YC, Doenhoff MJ. Adult worm tegumental damage and egg-granulomas in praziquantel-resistant and–susceptible *Schistosoma mansoni* treated in vivo. J Helminthol. 2002;76:327-333.
23. Mohamed AH, Ezz El-din N, Fahmy ZH, El-Shennawy AM, Hassan E. Parasitological, hematological and ultrastructural study of the effect of COX-2 inhibitor, pyocyanin pigment and praziquantel, on *Schistosoma mansoni* infected mice. J Egypt Soc Parasitol. 2006;36:197-220.
24. Soliman MFM, Ibrahim MM. Antischistosomal action of artovastatin alone and concurrently with medroxyprogesterone acetate on *Schistosoma haematobium* harboured in hamster: Surface ultrastructure and parasitological study. Acta Trop. 2005;93:1-9.
25. Manneck T, Haggenmüller Y, Keiser J. Morphological effects and tegumental alterations induced by mefloquine on schistosomula and adult flukes of *Schistosoma mansoni*. Parasitology. 2010;137:85–98.
26. Xiao SH, Xue J, Shen B. Transmission electron microscopic observation on ultrastructural alterations in *Schistosoma japonicum* caused by mefloquine. Parasitol Res. 2010;106(5):1179-1187.
27. Neves JK, De Lima MC, Pereira VR, De Melo CM, Peixoto CA, Pitta IR, Albuquerque MC, Galdino SL. Antischistosomal action of thioxo-imidazolidine compounds: an ultrastructural and cytotoxicity study. Exp Parasitol. 2011;128(1):82–90.
28. Oliveira SM, Silva JBP, Hernandes MZ, Lima MCA, Galdino SL, Pitta IR. Structure, reactivity and biological properties of hydantoins. New Quim. 2008;31:614-622.
29. Amemiya Y, Hong SS, Venkataraman BV, Patil PN, Shams G, Romstedt K, Feller DR, Hsu FL, Miller DD. Synthesis and alpha-adrenergic activities of 2- and 4-substituted imidazole analogues. J Med Chem. 1992;35:750-755.
30. Peng GW, Marquez VE, Driscoll JS. Potential central nervous system antitumor agents. Hydantoin derivatives. J Med Chem. 1975;18:846-849.
31. Caterina MC, Perillo IA, Boiani L, Pezaroglo H, Cerecetto H, Gonzalez M, Salerno A. Imidazolidines as new anti-*Trypanosoma cruzi* agents: Biological evaluation and structure–activity relationships. Bioorg Med Chem. 2008;16:2226-2234.
32. Oliveira SM, Albuquerque MCPA, Pitta MGR, Malagueño E, Santana JV, Lima MCA, Pitta IR, Galdino SL. A Resposta do *Schistosoma mansoni* Mantido *In Vitro* frente a Derivados Imidazolidinônicos. Acta Farm Bonaerense. 2004;23(3):343-8.

33. Albuquerque MCA, Silva TG, Pitta MGR, Silva ACA, Silva PG, Malagueño, E, Santana JV, Wanderley AG, Lima MCA, Galdino SL, Barbe J, Pitta IR. Synthesis and schistosomicidal activity of new substituted thioxoimidazolidine compounds. Pharmazie. 2005;60:13-17.
34. Pitta MGR, Silva ACA, Neves JKAL, Silva PG, Irmão JI, Malagueño E, Santana JV, Lima MCA, Galdino SL, Pitta IR, Albuquerque MCPA. New imidazolidinic bioisosters: potential candidates for antischistosomal drugs. Mem Inst Oswaldo Cruz. 2006;101:313-316.
35. Neves JKAL, Botelho SPS, Melo CML, Pereira VRA, Lima MCA, Pitta IR, Albuquerque MCPA, Galdino SL. Biological and immunological activity of new imidazolidines against adult worms of *Schistosoma mansoni*. Parasitol Res. DOI 10.1007/s00436-010-1886-y; 2010.
36. Brandao SSF, Andrade AMC, Pereira DTM, Barbosa Filho JM, Lima MCA, Galdino SL, Pitta IR, Barbe J. A novel way of synthesis of 1,3,5-trisubstituted-2-thioxoimidazolidinones. Heterocycl Comm. 2004;10:9-14.
37. Gouveia FL, Oliveira RMB, Oliveira TB, Silva IM, Nascimento SC, Sena KXFR, Albuquerque JFC. Synthesis, antimicrobial and cytotoxic activities of some 5-arylidene-4-thioxo-thiazolidine-2-ones. Eur J Med Chem. 2009;44:2038-2043.
38. Mourão RH, Silva TG, Soares ALM, Vieira ES, Santos JN, Lima MCA, Lima VLM, Galdino SL, Barbe J, Pitta IR. Synthesis and Biological Activity of Novel Acridinylidene and Benzylidene thiazolidinediones. Eur J Med Chem. 2005;40:1129-1133.
39. Tan et al. Tan F, Ang KP, Fong YF. (Z)- and (E)-5-arylmethylenehydantoins: Spectroscopic properties and configuration assignment. Journal of the Chemical Society, Perkin Transactions II. 1986;12:1941-1944.
40. De Simone, De Simone CA, Zukerman-Schpector J, Pereira MA, Luu-Duc C, Pitta IR, Galdino SL, Amorim ELC, et al. 3-(4-Bromobenzyl)-5-(4-fluorobenzylidene)-imidazolidine-2,4-dione. Acta Crystallogr. 1995;2620–2621.
41. Guarda VLM, Pereira MA, De Simone CA, Albuquerque JC, Galdino SL, Chantegrel J, Perrissin M, Beney C, Thomasson F, Pitta IR, Luu-Duc C. Synthesis and structural study of arylidene thiazolidine and benzothiazine compounds. Sulfur Letters. 2003;26:17-27.
42. Karolak-Wojciechowska J, Szymanska E, Mrozek A, Kiec-Kononowicz K. Crystallographic and spectroscopic studies of 5-arylidene-2-amino-imidazol-4-ones. J Mol Struct. 2009;930:126-134.
43. Handzlik J, Szymanska E, Wojcik R, Dela A, Jastrzebska-Wiesek M, Karolak-Wojciechowska J, Fruzinski A, Siwek A, Filipek B, Kiec-Kononowicz K. Synthesis and SAR-study for novel arylpiperazine derivatives of 5-arylidenehydantoin with a1-adrenoceptor antagonistic properties. Bioorg Med Chem. 2012;20:4245–4247.
44. Keenan M, Abbott MJ, Alexander PW, Armstrong T, Best WM, Berven B, Botero A, Chaplin JH, Charman SA, Chatelain E, Von Geldern TW, Kerfoot M, Khong A, Nguyen T, Mcmanus JD, Morizzi J, Ryan E, Scandale I, Thompson RA, Wang SZ, White KL. Analogues of fenarimol are potent inhibitors of *Trypanosoma cruzi* and are efficacious in a murine model of Chagas disease. J Med Chem. 2012;55:4189-4204.
45. Romanha AJ, Castro SL, Soeiro MN, Lannes-Vieira J, Ribeiro I, Talvani A, Bourdin B, Blum B, Olivieri B, Zani C, Spadafora C, Chiari E, Chatelain E, Chaves G, Calzada JE, Bustamante JM, Freitas-Junior LH, Romero LI, Bahia MT, Lotrowska M, Soares M, Andrade SG, Armstrong T, Degrave W, Andrade ZA. In vitro and in vivo experimental models for drug screening and development for Chagas disease. Memories of the Oswaldo Cruz Institute. 2010;105:233-238.

46. Becerra MC, Guiñazú N, Hergert LY, Pellegrini A, Mazzieri MR, Gea S, Albesa I. In vitro activity of N-benzenesulfonylbenzotriazole on *Trypanosoma cruzi* epimastigote and trypomastigote forms Exp Parasitol. 2012;131:57-62.
47. Hoffman WA, Pons JA, Janer SL. The sedimentation concentration method in Schistosomiasis mansoni. PRJ Public Health Trop Med. 1934;9:283-291.
48. Duvall RH, Dewitt WB. An improved perfusion technique for recovering adult schistosomes from laboratory animals. Am J Trop Med Hyg. 1967;16:483-486.
49. Santiago EF, Oliveira SA, Filho, GBO, Moreira DRM, Gomes PAT, Silva, AL, Barros AF, Silva AC, Santos TAR, Pereira VRA, Gonçalves GGA, Brayner FA, Alves LC, Wanderley AG, Leite ACL. Evaluation of the Anti-*Schistosoma mansoni* activity of Thiosemicarbazones and Thiazoles. Antimicrob Agents Chemother. 2013;352-363.
50. Horiuchi A, Satou T, Akao N, Koike K, Fujita K, Nikaido T. The effect of free and polyethylene glycol-liposome-entrapped albendazole on larval mobility and number in *Toxocara canis* infected mice. Vet Parasitol. 2005;129(1-2):83- 87.
51. Moraes J, Nascimento C, Lopes PO, Nakano E, Yamaguchi LF, Kato MJ, Kawano T. *Schistosoma mansoni*: In vitro schistosomicidal activity of piplartine. Exp Parasitol. 2011;127:357-364.
52. Katz N. The discovery of *Schistosomiasis mansoni* in Brazil. Acta Trop. 2008;69-71.
53. Silva ACA, Neves JKAL, Irmão JI, Costa VMA, Souza VMO, Medeiros PL, Silva EC, Lima MCA, Pitta IR, Albuquerque MCPA, Galdino SL. Study of the activity of 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one against *Schistosomiasis mansoni* in Mice. Scientific World Journal. 2012;520-524.
54. Mendonça-Silva DL, Pessôa RF, Noel F. Evidence for the presence of glutamatergic receptors in adult *Schistosoma mansoni*. Biochem Pharmacol. 2002;64:1337-1344.
55. Noel F. Sistema neuromuscular e controle da motilidade do verme adulto. In: Carvalho OS, Coelho PMZ, Lenzi HL. *Schistosoma mansoni* & Esquistossomose: Uma interdisciplinary vision. Rio de Janeiro: Fiocruz. 2008;207-244.
56. Marks NJ, Maule AG. Neuropeptides in helminths: Occurrence and distribution. Adv Exp Med Biol. 2010;692:49-77.
57. Taman A, Ribeiro P. Glutamate-mediated signaling in *Schistosoma mansoni*: A novel glutamate receptor is expressed in neurons and the female reproductive tract. Mol. Biochem. Parasitol. 2011;176:42-50.
58. Thibaut JPB, Monteiro LM, Leite LCC, Menezes CMS, Lima, LM, Noel F. The effects of 3-methylclonazepam *on Schistosoma mansoni* musculature are not mediated by benzodiazepine receptors. Eur J Pharmacol. 2009;606:9-16.
59. Tallima H, Ridi R. Praziquantel binds *Schistosoma mansoni* adult worm actin. Int J Antimicrob Agents. 2007;29:570–575.
60. Camacho M, et al. The amount of acetylcholinesterase on the parasite surfasse reflects the differential sensitivity of *Schistosome* species to metrifonate. Parasitology. 1994;108:153-160.
61. Mostafa OM, Soliman MI. Experimental use of black-seed oil against *Schistosoma mansoni* in albino mice: II. Surface topography of adults worms. Egyptian Journal of Medical Laboratory Sciences. 2002;11:79-85.
62. Kohn A, Lopez-Alvarez ML, Katz N. Transmission and scanning eléctron microscopical studies in the tegumento f male *Schistosoma mansoni* after oxamniquine treatment. Ann Parasitol Hum Comp. 1982;57:285-291.
63. Bertão HG, Silva RAR, Padilha RJR, Albuquerque MCPA, Rádis-Baptista G. Ultrastructural analysis of miltefosine-induced surface membrane damage in adult *Schistosoma mansoni* BH strain worms. Parasitol Res. 2012;110:2465-2473.

64. Mehlhorn H, Becker B, Andrews P, Thomas H, Frenkel JK. In vivo and in vitro experiments on the effects of praziquantel on *Schistosoma mansoni*, a light and electron microscopic study. Arzneimittelforschung/Drug Research. 1981;31:544-547.
65. Cioli D, Pica-Mattoccia L. Praziquantel. Parasitol Res. 2003;90:3-9.
66. Xiao SH, Keiser J, Xue J, Tanner M, Morson G, Utzinger J. Effect of single-dose oral artemether and tribendimidine on the tegument of adult *Clonorchis sinensis* in rats. Parasitol Res. 2009;104(3):533-541.
67. Paula FR, Serrano SHP, Tavares LC. Mecanísticos aspects of bioatividade and toxicity of nitrocompostos. New Quim. 2009;32:1013-1020.
68. Thomas G. Medicinal chemistry: an introduction. Editora Guanabara Koogan; 2003.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=626&id=14&aid=5759