



The prophylactic and anti-fibrotic activity of phthalimido-thiazole derivatives in schistosomiasis *mansoni*

Carlos André Laranjeira Miranda Filho¹ · Míria de Oliveira Barbosa^{1,2} · Arsênio Rodrigues Oliveira² · Aline Ferreira Pinto² · Daniel Lopes Araújo² · Jéssica Paula Lucena¹ · Roni Evêncio de Araújo¹ · Sheilla Andrade de Oliveira¹ · Ana Cristina Lima Leite²

Received: 9 February 2022 / Accepted: 26 April 2022 / Published online: 23 May 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2022

Abstract

Schistosomiasis *mansoni* is considered a serious public health problem. As praziquantel is the only drug recommended by the World Health Organization for the treatment and control of schistosomiasis, the development of new drugs is of great significance. In this work, we present the antischistosomal activity of a small set of phthalimido-thiazole derivatives against *Schistosoma mansoni*. The effects of those derivatives on the viability of larvae juveniles and adult parasites, production and development of eggs, mortality of schistosomules in vitro by counting worms, and stages of eggs of infected animals in acute and chronic phases were evaluated, resulting in the identification of new multistage antischistosomal compounds. Additionally, a study of liver fibrogenesis was released. The phthalimido-thiazole derivatives, compounds **2b-d**, **2h-j**, had shown activity on schistosomules, achieving 100% mortality even at 5 mg/mL, in the first 24 h. In the chronic phase of schistosomiasis infection, compound **2i** promoted a reduction in the number of immature eggs, an increase in the number of non-viable parasite eggs, a reduction in the average number of eggs in the liver and intestine, decrease in the levels of hydroxyproline in the liver, and a reduction in the areas of hepatic fibrosis. This compound also promoted an increase of IL-10 and a reduction in the level of TNF- α in the liver. Accordingly, the phthalimide-thiazole scaffold is a new starting point for the development of multistage compounds that affect *S. mansoni* viability, egg formation, and production.

Highlights

- Phthalimido-thiazole derivative as multistage compound
- Prophylactic activity of compound **2i**
- 100% on schistosomules mortality
- Reduction of egg number
- Anti-fibrotic activity

Keywords *Schistosoma mansoni* · Schistosomules · Phthalimido-thiazole · Prophylactic activity

Section Editor: Christoph G. Grevelding

✉ Ana Cristina Lima Leite
ana.leite@ufpe.br

¹ Department of Immunology, Laboratory of Immunopathology and Molecular Biology, Aggeu Magalhães Institute, FIOCRUZ, Recife, PE, Brazil

² Department of Pharmaceutical Sciences, Federal University of Pernambuco (UFPE), S/N, Recife, PE CEP 50740-525, Brazil

Introduction

Schistosomiasis is an acute and chronic parasitic disease caused by trematode helminths of the genus *Schistosoma*. Schistosomiasis transmission has been reported in 78 countries, and at least 236.6 million people required preventive treatment in 2019 (WHO 2022). In Brazil, there is only schistosomiasis *mansoni*, caused by the species *Schistosoma mansoni* (*S. mansoni*) and transmitted by snails of the genus *Biomphalaria* (Mesquita et al. 2021).

In its early stages, schistosomiasis is considered an asymptomatic disease that is due to a toxemic and allergic reaction or systemic hypersensitivity to migratory and maturing larvae, while its acuteness is rather a result of the worm burden and the immune response to parasite antigens (Han et al. 2009; Siqueira et al. 2017). The complex migratory lifecycle of the schistosome is initiated by the cutaneous penetration of free-swimming multicellular infective cercariae into the human mammalian host, aided by several environmental signals such as light–dark contrast, motion, chemical, and thermal gradients (Han et al. 2009). The cercariae concurrently lose their bifurcated tails to develop into schistosomule (Masamba et al. 2016).

When *Schistosoma* eggs are trapped in tissues, they trigger an immune reaction causing schistosomiasis. The antigens released from the egg stimulate a reaction that involves helper T cells (Th1), macrophages, eosinophils, and that cause clinical disease. As the disease progresses, the Th2 immune profile becomes predominant, with the occurrence of deposition of collagen fibers and formation of granulomas leading the disease to a more severe condition (Silva et al. 2012; Shaker et al. 2014).

Currently, the treatment of schistosomiasis is mainly based on chemotherapy, using practically a single drug, praziquantel (PZQ) (Ferrari et al. 2003). PZQ is a pyrazinoisoquinoline derivative, which is currently the reference drug in the treatment of schistosomiasis, due to its high efficacy and low cost. However, the drug does not act on the larval forms of the parasite (Lambertucci 2010); therefore, it has no prophylactic effect, and this can cause unsatisfactory results in mass treatment, where there are cases of reinfection. In addition, studies in endemic areas warn of the possibility of developing drug resistance (Liang et al. 2010).

Due to the therapeutic failure of PZQ, in recent years, several studies have stood out in the search for an alternative to the treatment of schistosomiasis. In 2014, Santiago et al. described a promising schistosomicidal activity of phthalimido-thiazole derivatives. Phthalimido-thiazole is a compound composed of two structures known in medicinal chemistry, thiazole, and phthalimide. The structure of thiazole is widely used in medicinal chemistry because it has a broad spectrum of biological action (Giri et al. 2009; Bharti et al. 2010). The phthalimide nucleus is an essential pharmacophoric fragment from thalidomide (Hashimoto 2008), in which some derivatives are known for their immunomodulatory activities, with the inhibition of tumor necrosis factor- α (TNF- α) being the most important (Chelucci et al. 2019; Man et al. 2009). Following this line of research, the phthalimide ring has commonly been employed in the design of potential anti-inflammatory (Assis et al. 2019) and immunomodulatory compounds (Stewart et al. 2007; Zahran et al. 2014).

Regarding the identification of schistosomicidal agents, most of the efforts have been conducted by the Laboratory of Planning and Medicinal Chemistry (LpQM/UFPE), through the investigation of phthalimido-thiazoles derivatives as schistosomicidal agents, as described by Oliveira et al. (2018), Barbosa et al. (2019), and Miranda Filho et al. (2020). Although phthalimido-thiazoles are effective against the adult form, their activity against immature forms of the parasite and their mechanisms of action against *S. mansoni* has not yet been evaluated. It has already been described in the literature that young and adult schistosomes express a number of cysteine proteases, including cathepsins B1, F, and L, which operate synergistically in the parasite's intestine to degrade host proteins as a source of nutrients (Abdulla et al. 2007). Therefore, a drug that acts on these targets could affect not only the adult worm but also the young form of *S. mansoni*.

In the search for more efficient schistosomicidal alternatives, the present study proposes to study six phthalylthiazoles (compounds **2b**, **c**, **d**, **h**, **i**, **j**) with schistosomicidal activity (Miranda Filho et al. 2020), regarding their prophylactic potential in schistosomiasis *mansoni* and try to elucidate the mechanisms of action of the compounds concerning all forms of *S. mansoni*.

Methodology

Compounds

Phthalimido-thiazole derivatives (**2b-d**, **2h-j**) (Table 1) were synthesized as described by Gomes et al. (2016). This compound was planned considering the promising results achieved by compounds bearing a thiazole ring and phthalimides nuclei; they were chosen as common pharmacophores that exist in several drug classes (Ramalho-Pinto et al. 1974). The chemical characterization was done using nuclear magnetic resonance and mass spectroscopy. PZQ (catalog n°. 4668; bench, BCBD3257V) was purchased from Sigma-Aldrich (St. Louis, MO, USA).

Parasites, intermediary, and definitive hosts

The LE strain of *S. mansoni* (Belo Horizonte, Minas Gerais, Brazil) was used throughout this study. Male BALB/c mice were used for the chronic assay. All animals were at the IAM animal research facility. The animal protocol was designed to minimize pain or discomfort to the animals, which were kept in rooms with temperature (22 ± 2 °C) and humidity ($55\% \pm 10\%$) controlled under continuous air renewal conditions. Animals were held in a cycle of 12-h light/12-h dark and had free access to food (Nuvilab,

Table 1 Activity of the phthalimido-thiazoles on schistosomules with 24 h of culture

Compound	Time	Mortality(%) ($\mu\text{g.mL}^{-1}$)					
		100	80	40	20	10	5
2b R= Ph	24 h	0	*	*	*	*	*
	48 h	0	*	*	*	*	*
	72 h	21	*	*	*	*	*
2c R= 4-Me-Ph	24 h	0	*	*	*	*	*
	48 h	15	*	*	*	*	*
	72 h	31	*	*	*	*	*
2d R= 4-MeO-Ph	24 h	0	*	*	*	*	*
	48 h	0	*	*	*	*	*
	72 h	17	*	*	*	*	*
2h R= 4-NO ₂ -Ph	24 h	0	*	*	*	*	*
	48 h	23	*	*	*	*	*
	72 h	37	*	*	*	*	*
2i R= Ph-Ph	24 h	100	100	100	100	100	100
	48 h	100	100	100	100	100	100
	72 h	100	100	100	100	100	100
2j R= 2-Naph	24 h	50	50	50	0	0	0
	48 h	100	100	100	57	41	0
	72 h	100	100	100	100	87	0

Curitiba, Paraná, Brazil) and water. Experimental procedures involving animals complied with the ethical standards of the Oswaldo Cruz Foundation and ethical approval was granted by the Ethics Committee for the Certified Use of Animals (CEUA-IAM 67/2014).

In vitro schistosomula assay

The parasites were obtained by mechanical transformation of cercariae, as reported by Ramalho-Pinto et al. (1974). Schistosomula were cultured in RPMI1640 medium, containing antibiotics and supplemented with 10% fetal bovine serum at 37 °C in an atmosphere of 5% CO₂. This

form of the parasite was cultivated until the 7th day, and schistosomula were obtained corresponding to those observed in the skin, such as the pulmonary phase of infected individuals (Veras et al. 2012; Moraes et al. 2012).

In the in vitro tests, schistosomules (1 and 21 days of age) were incubated in a 24-well plate (approximately 150 parasites per well) containing a complete RPMI 1640 medium. The compounds were added in six concentrations, ranging from 5 to 100 $\mu\text{g/mL}$. As a negative control, DMSO (compounds solvent) at 1.66% was used and as a positive control, PZQ at a concentration of 3 $\mu\text{g/mL}$. The culture plates were monitored daily for 5 days, using an inverted microscope. The death of the parasite was defined in the absence of movement for at

least 1 min of examination. Schistosomule death was also confirmed by an exclusion test using trypan blue dye. The study was done in triplicate and the activity was measured by calculating the percentage of the number of dead schistosomules.

In vivo effect of the phthalimido-thiazol in the chronic phase of schistosomiasis

Thirty male mice were used and submitted to experimental infection by *S. mansoni* (40 cercariae). After 45 days of infection, the positivity of the infection was evaluated using the method of Hoffman et al. (1934) and divided into 3 groups, with 10 animals in each group: group I (control group), treated with 15% Chremophor; group II, treated with PZQ at 400 mg/Kg; group III, treated with compound **2i** at 200 mg/kg for 5 days. After 120 days (chronic phase) of infection, the drug prepared in the 15% Chremophor solution was administered intraperitoneally and oro-gastrically via gavage for 5 days. After the procedure, the animals were returned to their respective boxes and monitored throughout the duration of the experiment. After 15 days of treatment of the compound, the animals were euthanized to collect tissue and blood fragments.

Parasitological evaluation after treatment with the phthalimido-thiazol derivative

The parasitological evaluation was performed by counting the worms, which were obtained after perfusion of the portal venous system, and by the direct collection of the mesentery; the eggs were obtained from the liver and intestine by digestion in 4% potassium hydroxide (KOH) (Cheever 1970) and also from the evaluation of the oogram following the classification of Pellegrino and Faria (1965). Briefly, three fragments of the distal portion of the intestine were compressed between a slide and a cover slip and were analyzed under a microscope for egg classification. For each fragment, 100 eggs were counted and classified according to their developmental stages. The eggs were classified into viable immature eggs (1st and 4th are underwritings), viable mature eggs, and nonviable eggs (calcified, with retracted, semi-transparent miracidium).

The efficacy of the compounds and praziquantel was determined by reducing the percentage of the parasite load in each treated group using the following equation: Reduction of worms (%) = $\frac{(CT)}{C} \times 100$, where *C* is the number of worms that were recovered from the control group and *T* is the number of worms recovered from the treated group.

Hydroxyproline dosage

A liver sample, obtained from the larger lobe, weighing between 100 and 200 mg, was used to determine hydroxyproline, a constituent of collagen. The samples were processed and analyzed according to the methodology of Bergman and Loxley (1963), analyzed in an automatic spectrophotometer (Pharmacia, model Ultrospec 36 3000), at a wavelength of 558 nm, to obtain the values of the molar concentration of each sample (nM).

Histopathological and morphometric analysis

To characterize liver lesions, liver fragments were fixed in Millonig formaldehyde, included in paraffin, and cut (5 µm) in a microtome. The cuts will be stained by hematoxylin–eosin and red picosirius for collagen fibers. Five fields stained with red picosirius will be randomly selected and evaluated by semi-automatic morphometry, using the LEICA Quantimet Q 500 MC image processing and analysis system (Leica Cambridge, Cambridge, England). All periovulares granulomas centralized by eggs or eggshells, present in the selected fields, confluent or not, were evaluated. For stereological calculation, schistosomal granulomas were considered spheroidal structures, with normal size distribution. The data obtained was used to calculate the following morphometric parameters: granuloma volume, an occupied sectional area, volume density, and numerical density of granulomas. The quantification of fibrous hepatic tissue was obtained through the liver sections stained by the red picosirius and was measured and calculated directly, as a percentage of the total area examined.

Immunological assays

Immediately after euthanasia, liver fragments were collected and frozen in liquid nitrogen, homogenized in a lysis buffer containing protease inhibitors. The lysates were centrifuged at 16,000 g at 4 °C for 15 min, and then the supernatants were used to quantify, through sandwich ELISA, the levels of TNF-α and IL-10 (BD OptEIA set to mouse, San Diego, CA, USA). The samples were read with the aid of a microplate reader.

Statistical analysis

Quantitative data were submitted to a normality test (Shapiro–Wilk's). Differences were evaluated using the one-way ANOVA test with post hoc Tukey, two-way ANOVA for parametric analysis, and linear regression for the E_{50} evaluation. Statistical analyses were performed using Prism Software (version 5.0; GraphPad Software, San Diego, CA, USA) and Biostat 5.3 (Mamirauá Institute, Manaus, AM, Brazil). A *P*

value of <0.05 was considered statistically significant. Data were expressed as mean values (mean \pm).

Results and discussion

Previous studies on phthalyl-thiazoles-based synthetic compounds demonstrated that these compounds were active on the adult form of *S. mansoni*. Indeed, Miranda Filho et al. (2020) identified six phthalyl-thiazoles (compounds **2b-d**, **2h-j**) with schistosomicidal activity that motivated us to investigate their activity against immature forms of the parasite and their mechanisms of action against *S. mansoni*.

Importantly, our results show that even small variations in the steric and electronic nature of the R substituent can address compound selectivity toward schistosomules activity. The substitution varied according to the phenyl (compounds **2b-d**, **2h**, **2i**) or naphthyl (compound **2j**) group attached to the 1,3-thiazole ring. Overall, according to our results, the steric nature of the R group affects the schistosomicidal activity.

In vitro susceptibility of *S. mansoni* schistosomules to phthalimido-thiazoles derivatives.

The compounds (**2b-d**, **2h-j**) were first screened for their activity on immature forms of *S. mansoni* with a maximum concentration of 100 $\mu\text{g/mL}$. It was verified that at 100 $\mu\text{g/mL}$, compounds **2i** (251.6 μM) and **2j** (269.2 μM) were considered effective, showing 100% of mortality at all times tested. After that, only compounds **2i** and **2j** were evaluated at 5, 10, 20, 40, and 80 $\mu\text{g/mL}$ to identify the lowest effective concentration of the immature form of *S. mansoni* (Table 1). Compound **2i** promoted 100% of schistosomules mortality even at 5 $\mu\text{g/mL}$ (12.6 μM) at all times tested. Compound **2j** promoted 100% of schistosomules mortality from 20 or 40 $\mu\text{g/mL}$ (53.8 μM and 107.7 μM), after 72 and 48 h, respectively. Besides, with a concentration of 10 $\mu\text{g/mL}$, it exhibited 87% efficiency on schistosomules. Figure 1 demonstrates that the **2i** showed activity on schistosomules at the lowest concentration (5 $\mu\text{g/mL}$, 12.6 μM), causing 100% mortality in the first 24 h. In turn, the PZQ (16.0 μM) and the DMSO-treated group was not able to cause mortality even after 72 h of testing.

After evaluating the action of compounds against schistosomules at 24 h of age, compound **2i** was considered to have the highest activity. Considering that schistosomules undergo several structural and biochemical changes until it reaches the liver, compound **2i** was also evaluated in schistosomules cultured for 21 days, referring to the pulmonary larval phase. It was observed that compound **2i** was effective up to a concentration of 10 $\mu\text{g/mL}$ (25.2 μM) (Table 2), revealing a similar profile that was observed against the larval phase. At 5 $\mu\text{g/mL}$ (12.6 μM), no activity was observed even at 72 h of the experiment.

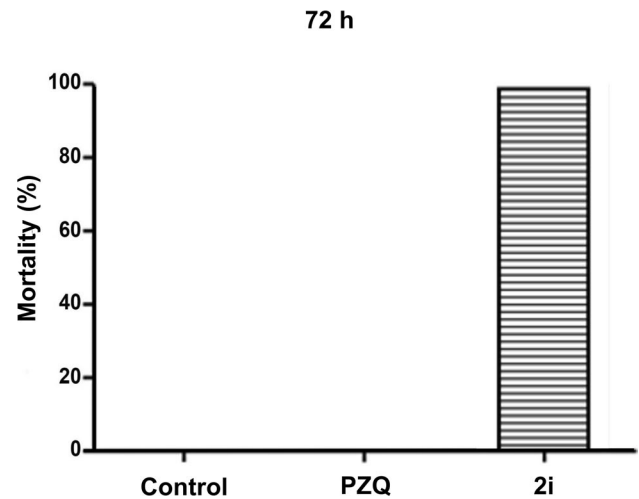


Fig. 1 In vitro effect of compound **2i** on schistosomules. Mortality after 72 h of incubation with DMSO at 1.66%, PZQ at a concentration of 3 $\mu\text{g/mL}$ (9.6 μM), and **2i** at a concentration of 5 $\mu\text{g/mL}$ (12.6 μM)

The activity of compound **2i** was similar when it was screened in larval forms for 24 h and 21 days. The compound was effective up to the concentration of 10 $\mu\text{g/mL}$ (25.2 μM) in the culture for 21 days, while in the initial larval phase, the compound was active even at the lowest concentration tested. Although the compound has been shown to be more effective in the test with schistosomules of 24 h of culture, there is no possibility of verifying that the initial larval form is more sensitive to the compound than the juvenile form.

Effectiveness of treatment with **2i** in the chronic phase of schistosomiasis

Compound **2i** was evaluated after 120 days of schistosomiasis infection (chronic phase), at 200 mg/kg (0.50 M), by using PZQ (400 mg/Kg, 1.28 M) as a positive control, and chremophor, which was used as a vehicle, as a negative control. Compound **2i** promoted a reduction in the number of immature eggs and an increase in the number of non-viable parasite eggs in relation of the negative control (Table 3). This result indicates a decrease in the parasitic load.

In vivo effect of compound **2i** on the number of eggs in the liver and intestine

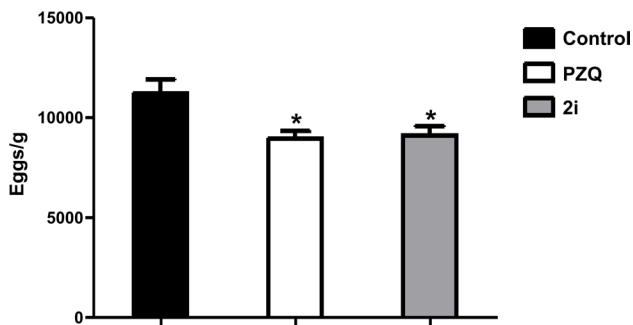
Figure 2 shows a decrease in the average number of eggs in the liver of the groups treated as the compound **2i** and PZQ relative to the control group. Regarding the average amount of eggs found in the intestine, Fig. 3 shows that there was a significant reduction in the groups treated with compound **2i** relative to the control group. The group treated with derivative **2i** demonstrated a similar effect on the number of eggs

Table 2 Activity of the phthalimido-thiazoles on schistosomules with 21 days of culture

Compound	Time	Mortality (%) ($\mu\text{g mL}^{-1}$)					
		100	80	40	20	10	5
2i	24 h	100	100	100	100	100	0
	48 h	*	*	*	*	*	0
	72 h	*	*	*	*	*	0

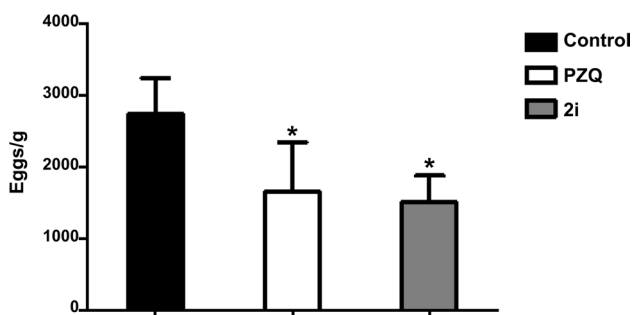
Table 3 Effect of treatment on the staging of *S. mansoni* eggs in the chronic phase of infection

Groups	Reduction in the number of immature eggs	Eggs stages		
		Immatures (%)	Matures (%)	Unviable (%)
Chremophor (OV)		46.25	21.34	46.95
PZQ (OV)	45.41%	0.84	15.14	84.02
2i (OV)	31.77%	14.48	25.48	60.03

**Fig. 2** Average amount of *S. mansoni* eggs per gram of liver of mice treated with the compound **2i** orally. Control, group orally treated with chremophor as the vehicle. PZQ (praziquantel), positive control

found in the intestine to the group treated with PZQ. No significant difference was found between the groups treated with compound **2i** and PZQ.

These results show that treatment with compound **2i** decreased the parasitic load in the chronic phase and decreased the load of eggs found in the tissues. It is worth

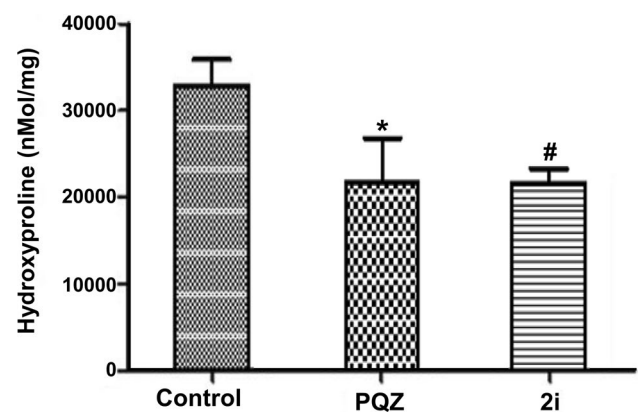
**Fig. 3** Average amount of *S. mansoni* eggs per gram of intestine of mice treated with the compound **2i** orally. Control, treated with the vehicle (chremophor) orally. Praziquantel (PZQ), positive control

mentioning that, in the chronic phase, eggs in the tissues are usually in the form of granulomas. In this way, the elimination of these eggs in the tissues is crucial for a better recovery from the injury caused by schistosomiasis.

Quantification of hydroxyproline in the liver

To quantify collagen deposition in the liver of mice during infection by *S. mansoni*, the hydroxyproline content was quantified as a marker of collagen deposition in the liver of Balb/c animals during *S. mansoni* infection. As shown in Fig. 4, a decrease in the amount of hydroxyproline was detected in the groups treated with PZQ and **2i** in relation to the control group.

As a collagen marker, the hydroxyproline technique serves as an indicator of fibrogenesis. With this in mind, we can observe that both compound **2i** and PZQ significantly

**Fig. 4** Amount of hydroxyproline in 200 mg of liver from mice treated with the compound **2i** orally, control treated with the vehicle orally (control), and positive control treated with praziquantel (PZQ), $p < 0.05$

decrease the levels of hydroxyproline in the liver, indicating a possible therapeutic effect on liver damage resulting from the chronic phase of schistosomiasis *mansoni*.

Hepatic fibrosis

To evaluate liver fibrosis, the quantification of morphometry was used. A significant reduction was observed in the areas of hepatic fibrosis (Fig. 5) in animals treated with PZQ and 2i in relation to the control group. These results corroborate the results verified in the dosage of hydroxyproline.

The compound 2i also demonstrated a possible activity on liver fibrotic lesion by decreasing fibrosis in relation to the control group, as well as the PZQ. Liver fibrosis is a dynamic process, which begins as a response to persistent aggressive stimuli, leading to the exacerbated deposition of extracellular matrix and the consequent replacement of the liver parenchyma by scar tissue (Rockey 2006). In chronic lesions, such as schistosomiasis mansoni, the liver has an increase in extracellular matrix components, including collagens, mainly collagen types I and II, glycoproteins, and proteoglycans that begin to fill the previously injured spaces (Iredale et al. 2013). The chronic deposition of collagen fibers makes fibrous tissue more stable and resistant to degradation (Popov et al. 2011). The possibility of reversing the condition of hepatic fibrosis will depend on the removal of the stimulus of the aggressive etiologic agent (Schuppan and Pinzani 2012).

Immunological analysis

The mice in the chronic phase of schistosomiasis, treated with compound 2i at 200 mg/kg (0.5 μ M) for 5 days, were also investigated concerning the levels of TNF- α and IL-10. Several immunological reactions observed in

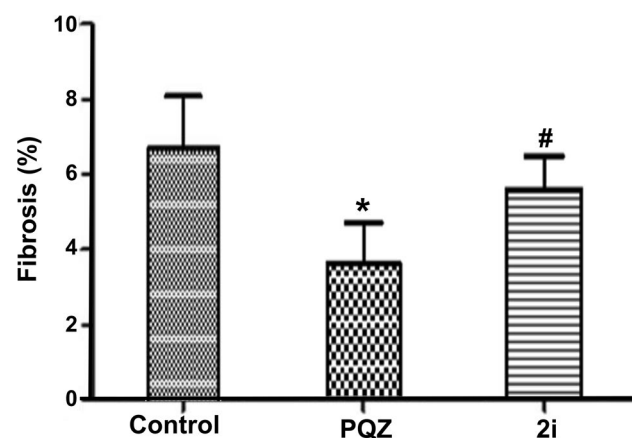


Fig. 5 Morphometric analysis of hepatic collagen in mice treated with the compound 2i orally, control treated with the vehicle orally (control), and positive control treated with praziquantel (PZQ), $p < 0.05$

schistosomiasis are driven by numerous cytokines that interact in a complex network and several studies have shown that the absence or presence of these cytokines could either progress or downregulate schistosomiasis. The understanding of these mechanisms could, consequently, drive treatments for this disease through drug development (Masamba and Kappo 2011).

Once infected with the parasite, the initial response within infected tissues and plasma is characterized by type-1 inflammation, which is driven by tumor necrosis factor- α among others. This response is subsequently stifled during chronic schistosomiasis by the CD4⁺ T helper 2 (Th2) response, which is triggered by antigens secreted by eggs and is driven particularly by IL-10 (Kumar et al. 2015). A reduction in TNF- α concentrations was observed in the group treated with PZQ and compound 2i (Fig. 6B). Although there was an increase in the level of IL-10 in the liver in the PZQ and 2i groups compared to the control group (Fig. 6A).

As can be seen, compound 2i was able to stimulate IL10 production. It is well known that regulatory immune cells not only contribute to disease progression but also play crucial roles in host protection. The IL-10 cytokine represents an important element in disease progression by encouraging inflammation through downregulation of the Th1 response, but also by preventing severe disease during the Th2 response (Masamba and Kappo 2011). Through the expression of IL-10 and stimulation of T (Treg) cells, B cells gain the ability to block pro-inflammatory immune responses (Fairfax et al. 2013).

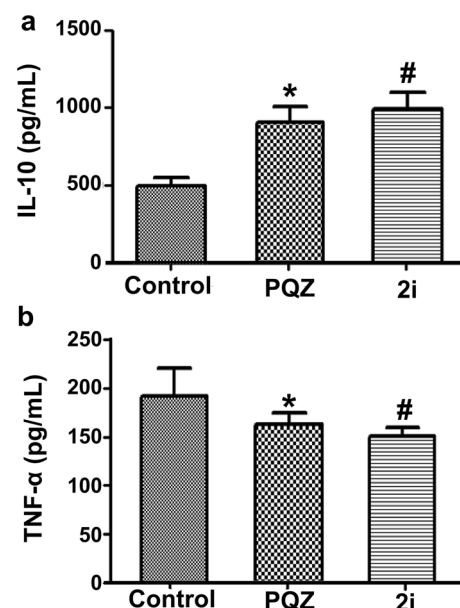


Fig. 6 Cytokine profile after treatment with compound 2i orally. **A** IL 10 level and **B** TNF- α level. Control, treated with the vehicle orally. PZQ, treated with praziquantel

It was also reported that low production of IL-10 by blood mononuclear cells of schistosomiasis-disease patients leads to a higher risk of severe periportal fibrosis (Arnaud et al. 2008; Mutengo et al. 2018) suggesting a protective role of IL-10 against hepatic fibrosis. The anti-inflammatory and immunosuppressive potential of IL-10 that regulates the inflammatory process, central to tissue fibrosis, could explain this protective effect (Bataller et al. 2003).

The compound **2i** was able to decrease the production of TNF- α when compared to the control group. This cytokine was correlated with more severe hepatic fibrosis during *S. mansoni* infections (Jeng et al. 2007). In addition, higher levels of TNF- α in supernatants from whole-blood cultures of patients are strongly associated with an increased risk of periportal fibrosis during hepatosplenic schistosomiasis (Lemos et al. 2008). The effect of compound **2i** on the level of IL-10 and TNF- α corroborates the reduction of hepatic fibrosis observed in morphometric analysis of hepatic collagen in mice treated with compound **2i** orally.

Conclusion

Overall, the results indicate that phthalimido-thiazole derivatives are antiparasitic agents. These compounds showed substantial schistosomicidal properties against schistosomula. Among a small series of different phthalimido-thiazoles derivatives, we identified two compounds as lead candidates, namely compounds **2i** and **2j**, which were active against schistosomules in different stages in vitro in the micromolar range. The compounds **2i** and **2j** were considered effective against 14 h old schistosomules, at 100 $\mu\text{g/mL}$, showing 100% of mortality at all times tested. Compound **2i** showed activity on the schistosomules at the lowest concentration of 5 $\mu\text{g/mL}$ (12.6 μM), causing 100% mortality in the first 24 h. In the schistosomules cultured for 21 days, compound **2i** was effective up to a concentration of 10 $\mu\text{g/mL}$ (25.2 μM). In the in vivo evaluation, in the chronic phase of schistosomiasis infection, compound **2i** promoted a reduction in the number of immature eggs and an increase in the number of non-viable parasite eggs. In addition, similar to PZQ, compound **2i** promoted a reduction in the average number of eggs in the liver and intestine, a decrease in the levels of hydroxyproline in the liver, and a significant reduction in the areas of hepatic fibrosis, indicating a possible therapeutic effect on liver damage resulting from the chronic phase of schistosomiasis mansoni. This compound was also able to promote an increase of IL-10 and a reduction in the TNF- α level in the liver, which could explain the reduction of hepatic fibrosis. Regarding the need to develop a cost-effective and cost-benefit drug therapy, further in vitro and in vivo studies are needed to understand its mechanism/s of

action, and record its activity in other human schistosomes, as well as the efficacy in combination with PZQ.

Acknowledgements The authors would like to thank the animal facilities of the Oswaldo Cruz Foundation (Rio de Janeiro, Brazil) and Aggeu Magalhães Institute, Oswaldo Cruz Foundation, in Recife, Brazil.

Author contribution Carlos André Laranjeira Miranda Filho: investigation, writing—original draft.

Miria de Oliveira Barbosa: investigation

Arsênio Rodrigues Oliveira: compound synthesis

Aline Ferreira Pinto: review and editing

Daniel Lopes Araújo: review and editing

Jéssica Paula Lucena: investigation

Roni Evêncio de Araújo investigation

Sheilla Andrade de Oliveira: supervision, resources, conceptualization

Ana Cristina Lima Leite: supervision, resources, conceptualization, writing—review and editing

Funding This work was funded by the Oswaldo Cruz Foundation (FIOCRUZ), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), and Fundação de Amparo à Ciência e Tecnologia de Pernambuco (FACEPE). Ana Cristina Lima Leite is receiving a CNPq senior fellowship.

Data availability The data supporting the findings of this study are available within the article.

Declarations

Ethics approval Experimentais procedures involving animals were approved by Ethics Committee for the Certified Use of Animals (CEUA-IAM 67/2014) of the Oswaldo Cruz Foundation.

Consent to participate and consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

References

- Abdulla MH, Lim KC, Sajid M, McKerrow JH, Caffrey CR (2007) Schistosomiasis mansoni: novel chemotherapy using a cysteine protease inhibitor. *PLoS Med* 4:20–29. <https://doi.org/10.1371/journal.pmed.0040014>
- Arnaud V, Li J, Wang Y, Fu X, Mengzhi S, Luo X, Hou X, Dessein H, Jie Z, Xin-Ling T, He H, McManus DP, Li Y, Dessein A (2008) Regulatory role of interleukin-10 and interferon-gamma in severe hepatic central and peripheral fibrosis in humans infected with *Schistosoma japonicum*. *J Infect Dis* 198:418–426. <https://doi.org/10.1086/588826>
- Assis SPO, Silva MTD, Silva FTD, Sant’Anna MP, Tenório CMBA, Santos CFBD, Fonseca CSMD, Seabra G, Lima VLM, Oliveira RN (2019) Design and synthesis of triazole-phthalimide hybrids with anti-inflammatory activity. *Chem Pharm Bull* 67(2):96–105. <https://doi.org/10.1248/cpb.c18-00607>
- Barbosa MO, Oliveira SA, Miranda Filho CAL, Oliveira AR, Fernandes CJB, Lucena JP, Sousa FA, Dias MCHB, Brayner FA, Alves LC, Leite ACL (2019) Schistosomicidal and prophylactic

- activities of phthalimido-thiazoles derivatives on schistosomula and adult worms. *Eur J Pharm Sci* 133:15–27. <https://doi.org/10.1016/j.ejps.2019.03.008>
- Bataller R, North KE, Brenner DA (2003) Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. *Hepatology* 37:493–503. <https://doi.org/10.1053/jhep.2003.50127>
- Bergman I, Loxley R (1963) Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Ann Chem* 35:1961–1965. <https://doi.org/10.1021/ac60205a053>
- Bharti SK, Nath G, Tilak R, Singh SK (2010) Synthesis, antibacterial and anti-fungal activities of some novel Schiff bases containing 2,4-disubstituted thiazole ring. *Eur J Med Chem* 45:651–660. <https://doi.org/10.1016/j.ejmech.2009.11.008>
- Cheever AW (1970) Relative resistance of the eggs of human schistosomes to digestion in potassium hydroxide. *Bull World Health Organ* 43:601–603
- Chelucci RC, de Oliveira IJ, Barbieri KP, Lopes-Pires ME, Polesi MC, Chiba DE, Carlos IZ, Marcondes S, dos Santos JL, Chung M (2019) Antiplatelet activity and TNF- α release inhibition of phthalimide derivatives useful to treat sickle cell anemia. *Med Chem Res* 28:1264–1271. <https://doi.org/10.1007/s00044-019-02371-z>
- de Moraes J, Nascimento C, Yamaguchi LF, Kato MJ, Nakano E (2012) *Schistosoma mansoni*: *In vitro* schistosomicidal activity and tegumental alterations induced by pipartine on schistosomula. *Exp Parasitol* 132:222–227. <https://doi.org/10.1016/j.exppara.2012.07.004>
- de Oliveira AS, Barbosa MO, Miranda Filho CAL, Oliveira AR, de Sousa FA, Santiago EF, Filho GBO, Gomes PATM, da Conceição JM, Brayner FA, Alves LC, Leite ACL (2018) Phthalimido-thiazole as privileged scaffold: activity against immature and adult worms of *Schistosoma mansoni*. *Parasitol Res* 117:2105–2115. <https://doi.org/10.1007/s00436-018-5897-4>
- Fairfax KC, Everts B, Smith AM, Pearce EJ (2013) Regulation of the development of the hepatic B cell compartment during *Schistosoma mansoni* infection. *J Immunol* 191:4202–4210. <https://doi.org/10.4049/jimmunol.1301357>
- Ferrari MLA, Coelho PMZ, Antunes CMF, Tavares CAP, da Cunha AS (2003) Efficacy of oxamniquine and praziquantel in the treatment of *Schistosoma mansoni* infection: a controlled trial. *Bull World Health Organ* 81:190–196
- Giri RS, Thaker HM, Giordano T, Williams J, Rogers D, Sudersanam V, Vasu KK (2009) Design, synthesis and characterization of novel 2-(2,4-disubstituted-thiazole-5-yl)-3-aryl-3H-quinazolin-4-one derivatives as inhibitors of NF- κ B and AP-1 mediated transcription activation and as potential anti-inflammatory agents. *Eur J Med Chem* 44:2184–2189. <https://doi.org/10.1016/j.ejmech.2008.10.031>
- Gomes PA, Oliveira AR, Cardoso MV, Santiago EF, Barbosa MO, de Siqueira LR, Moreira DR, Bastos TM, Brayner FA, Soares MB, Mendes AP, de Castro MC, Pereira VR, Leite ACL (2016) Phthalimido-thiazoles as building blocks and their effects on the growth and morphology of *Trypanosoma cruzi*. *Eur J Med Chem* 111:46–57. <https://doi.org/10.1016/j.ejmech.2016.01.010>
- Han ZG, Brindley PJ, Wang SY, Chen Z (2009) *Schistosoma* genomics: new perspectives on schistosome biology and host-parasite interaction. *Annu Rev Genom Hum Genet* 10:211–240. <https://doi.org/10.1146/annurev-genom-082908-150036>
- Hashimoto Y (2008) Thalidomide as a multi-template for development of biologically active compounds. *Arch Pharm Chem Life Sci* 341:536–547
- Hoffman WA, Pons JA, Janer SL (1934) The sedimentation concentration method in Schistosomiasis *mansoni*. *P R J Public Health* 9:283–291
- Iredale JP, Thompson A, Henderson NC (2013) Extracellular matrix degradation in liver fibrosis: biochemistry and regulation. *Biochim Biophys Acta* 1832:876–883. <https://doi.org/10.1016/j.bbdis.2012.11.002>
- Jeng JE, Tsai JF, Chuang LY, Ho MS, Lin ZY, Hsieh MY, Chen SC, Chuang WL, Wang LY, Yu ML, Dai CY, Chang JG (2007) Tumor necrosis factor- α 308.2 polymorphism is associated with advanced hepatic fibrosis and higher risk for hepatocellular carcinoma. *Neoplasia* 9:987–992. <https://doi.org/10.1593/neo.07781>
- Kumar R, Mickael C, Chabon J, Gebreab L, Rutebemberwa A, Garcia AR, Koyanagi DE, Sanders L, Gandjeva A, Kearns MT (2015) The causal role of IL-4 and IL-13 in *Schistosoma mansoni* pulmonary hypertension. *Am J Respir Crit Care Med* 192:998–1008. <https://doi.org/10.1164/rccm.201410-1820OC>
- Lambertucci JR (2010) Acute schistosomiasis: revisited and reconsidered. *Mem Inst Oswaldo Cruz* 105:422–435. <https://doi.org/10.1590/S0074-02762010000400012>
- Lemos DS, Carvalho AT, Filho OAM, Oliveira LFA, Silva MFC, Matoso LF, de Souza LJ, Gazzinelli A, Oliveira RC (2008) Eosinophil activation status, cytokines and liver fibrosis in *Schistosoma mansoni* infected patients. *Acta Trop* 108:150–159. <https://doi.org/10.1016/j.actatropica.2008.04.006>
- Liang YS, Wang W, Dai JR, Li HJ, Tao YH, Zhang JF, Li W, Zhu YC, Coles GC, Doenhoff MJ (2010) Susceptibility to praziquantel of male and female cercariae of praziquantel-resistant and susceptible isolates of *Schistosoma mansoni*. *Helminthol* 84:202–207. <https://doi.org/10.1017/S0022149X0999054X>
- Man HW, Schafer P, Wong LM, Patterson RT, Corral LG, Raymon H, Blease K, Leisten J, Shirley MA, Tang Y, Babusis DM, Chen R, Stirling D, Muller GW (2009) Discovery of (S)-N-(2-[1-(3-ethoxy-4-methoxyphenyl)-2-methanesulfonylethyl]-1,3-dioxo-2,3-dihydro-1H-isoindol-4-yl)acetamide (Apre-milast), a potent and orally active phosphodiesterase 4 and tumor necrosis factor- α inhibitor. *J Med Chem* 52:1522–1524. <https://doi.org/10.1021/jm900210d>
- Masamba P, Adenowo A, Oyinloye B, Kappo A (2016) Universal stress proteins as new targets for environmental and therapeutic interventions of Schistosomiasis. *Int J Environ Res Public Health* 13:972. <https://doi.org/10.3390/ijerph13100972>
- Masamba P, Kappo AP (2011) Immunological and biochemical interplay between cytokines, oxidative stress, and schistosomiasis. *Int J Mol Sci* 22:7216. <https://doi.org/10.3390/ijms22137216>
- Mesquita SG, Neves FGS, Scholte RGC, Carvalho OS, Fonseca CT, Caldeira RL (2021) A loop-mediated isothermal amplification assay for *Schistosoma mansoni* detection in *Biomphalaria* spp. from schistosomiasis-endemic areas in Minas Gerais, Brazil. *Parasitol Vectors* 14:388. <https://doi.org/10.1186/s13071-021-04888-y>
- Miranda Filho CAL, Barbosa MO, Oliveira AR, Santiago EF, Souza VCA, Lucena JP, Fernandes CJB, Santos IR, Leão RLC, Santos FAB, Alves LC, Pereira VRA, Araújo RE, Leite ACL, Oliveira AS (2020) *In vitro* and *in vivo* activities of multi-target phthalimido-thiazoles on Schistosomiasis *mansoni*. *Eur J Pharm Sci* 146:105236. <https://doi.org/10.1016/j.ejps.2020.105236>
- Mutengo MM, Mduluzi T, Kelly P, Mwansa JCL, Kwenda G, Musonda P, Chipeta J (2018) Low IL-6, IL-10, and TNF- α and high IL-13 cytokine levels are associated with severe hepatic fibrosis in *Schistosoma mansoni* chronically exposed individuals. *J Parasitol Res* 2018:1–8. <https://doi.org/10.1155/2018/9754060>
- Pellegrino J, Faria J (1965) The oogram method for the screening of drugs in *S. mansoni*. *The Am J Trop Med Hyg* 14:363–369. <https://doi.org/10.4269/ajtmh.1965.14.363>
- Popov Y, Sverdlov DY, Sharma AK, Bhaskar KR, Li S, Freitag TL, Lee J, Dieterich W, Melino G, Schuppan D (2011) Tissue transglutaminase does not affect fibrotic matrix stability or regression of liver fibrosis in mice. *Gastroenterology* 140:1642–1652. <https://doi.org/10.1053/j.gastro.2011.01.040>
- Ramalho-Pinto FJ, Gazzinelli G, Howells RE, Mota-Santos TA, Figueiredo EA, Pellegrino J (1974) *Schistosoma mansoni*: defined

- system for stepwise transformation of cercaria to schistosomula *in vitro*. *Exp Parasitol* 36:360–372. [https://doi.org/10.1016/0014-4894\(74\)90076-9](https://doi.org/10.1016/0014-4894(74)90076-9)
- Rockey DC (2006) Hepatic fibrosis, stellate cells, and portal hypertension. *Clin Liver Dis* 10:459–479. <https://doi.org/10.1016/j.cld.2006.08.017>
- 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 (2014) Evaluation of the anti-*Schistosoma mansoni* activity of thiosemicarbazones and thiazoles. *Antimicrob Agents Chemother* 54:352–363. <https://doi.org/10.1128/AAC.01900-13>
- Schuppan D, Pinzani M (2012) Anti-fibrotic therapy: lost in translation? *J Hepatol* 56:66–74. [https://doi.org/10.1016/S0168-8278\(12\)60008-7](https://doi.org/10.1016/S0168-8278(12)60008-7)
- Shaker Y, Samy N, Ashour E (2014) Hepatobiliary schistosomiasis. *J Clin Transl Hepatol* 2:212–216. <https://doi.org/10.14218/JCTH.2014.00018>
- Silva ACA, Neves JKAL, Irmão JI, Costa VMA, Souza VMO, de Medeiros PL, da Silva EC, de Lima MCA, Pitta IR, Albuquerque MCPA, Galdino SL (2012) Study of the activity of 3-benzyl-5-(4-chloro-arylazo)-4-thioxo-imidazolidin-2-one against *Schistosoma mansoni* in mice. *Sci World Journal* 2012:1–8. <https://doi.org/10.1100/2012/520524>
- Siqueira LP, Fontes DAF, Aguilera CSB, Timóteo TRR, Ângelos MA, Silva LCPBB, Melo CG, Rolim LA, Silva RMF, Neto PJR (2017) Schistosomiasis: drugs used and treatment strategies. *Acta Trop* 176:179–187. <https://doi.org/10.1016/j.actatropica.2017.08.002>
- Stewart SG, Spagnolo D, Polomska ME, Sin M, Karimi M, Abraham LJ (2007) Synthesis and TNF expression inhibitory properties of new thalidomide analogs derived via Heck cross-coupling. *Bioorg Med Chem Lett* 17:5819–5824. <https://doi.org/10.1016/j.bmcl.2007.08.042>
- Veras LM, Guimaraes MA, Campelo YD, Vieira MM, Nascimento C, Lima DF, Vasconcelos L, Nakano E, Kuckelhaus SS, Batista MC, Leite JR, Moraes J (2012) Activity of epiisopiloturine against *Schistosoma mansoni*. *Curr Med Chem* 19:2051–2058. <https://doi.org/10.2174/092986712800167347>
- World Health Organization (WHO) (2022) Schistosomiasis. <https://www.who.int/news-room/fact-sheets/detail/schistosomiasis>. Accessed: 12/02/2022
- Zahran M, Abdin YG, Osman AMA, Gamal-Eldeen AM, Talaat R, Pedersen EB (2014) Synthesis and evaluation of thalidomide and phthalimide esters as antitumor agents. *Arch Pharm Chem Life Sci* 347:1–8. <https://doi.org/10.1002/ardp.201400073>

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.