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PROTOZOOLOGY - ORIGINAL PAPER

Antitrichomonal activity and docking analysis of thiazole derivatives as TvMP50 protease inhibitors

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Abstract

Trichomoniasis, caused by the protozoan *Trichomonas vaginalis*, is the most prevalent non-viral sexually transmitted infection that affects over 170 million people worldwide. The only type of drug recommended for the therapeutic control of trichomoniasis is the 5-nitroimidazoles, although there have been reports of some undesirable side effects and clinical resistance. Hence, the need for the search for new tricomonicidal agents is necessary. In a previous work, we demonstrated that two 2-amino-4-aryl thiazole derivatives (ATZ-1 and ATZ-2) possess a portent antigiardial effect. In the current paper, we investigated the *in vitro* antitrichomonal activity of these thiazole compounds. Both ATZ-1 and ATZ-2 reduced the viability and growth of parasites in a dose-dependent manner, with an IC_{50} value of 0.15 μg/mL and 0.18 μg/mL, respectively. Furthermore, both thiazole compounds were able to decrease the proteolytic activity in *T. vaginalis* trophozoites compared with untreated parasites. Interestingly, a full proteolytic inhibition profile was observed in the 50-kDa region which was associated with the decreased expression of the gene that codes for the trichomonad protease TvMP50. The docking simulations predicted strong interactions of the thiazole compounds in the TvMP50 protease's active site, suggesting a possible role as protease inhibitors. Our results demonstrate the potential of 2-amino-4-aryl thiazole derivatives as trichomonicidal compounds and could be, mechanistically, involved in the inhibition of key trichomonad proteases.

Keywords Trichomoniasis · *T. vaginalis* · Thiazole · Anti-proteolytic · TvMP50

Introduction

Sexually transmitted infections (STIs) are a leading cause of acute illness, sterility, long-term incapacity, and even death, with several and serious consequences among millions of individuals around the world. These infections include those sexually transmitted bacterial, mycological, viral, and parasitic pathogens (Gewirtzman et al. 2011). *Trichomonas vaginalis* is the only known parasite to cause the most frequent non-viral STI worldwide, denominated trichomoniasis (Kissinger 2015). The World Health Organization (WHO) estimates an incidence of 170–190 million new cases of trichomoniasis worldwide every year, affecting both men and women. The only class of antimicrobial drug known to be viable against *T. vaginalis* infections is the nitroimidazoles. Even with adequate available therapy, the trichomoniasis has remained a public health concern on both industrialized and developing countries, partially because of the dependence on a single drug class for its treatment, which could become

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problematic if nitroimidazole resistance between *T. vaginalis* strains increased (Cudmore and Garber 2009; Kirkcaldy et al. 2012). With this situation in mind, research goals for addressing trichomoniasis must include designing and testing new antitrichomonal agents.

T. vaginalis infection is a multifactorial process where some proteinases, such as metalloproteinases, cysteine proteinases, and a rhomboid proteinase (TvROM1), have been implicated as major components on host cell adhesion and cytotoxicity (Mercer and Johnson 2018. The TvMP50 metalloproteinase is an immunogenic protein that is recognized by sera from male patients with trichomoniasis (Quintas-Granados et al. 2013). In previous reports, TvMP50 was identified as a proteolytic band of ~ 50 kDa in gelatin zymogram, either as a native or recombinant protein, indicating that it acts as an active monomer, presumably derived from the ancestral aminopeptidase P-like enzymes (Puente-Rivera et al. 2017; Arreola et al. 2018). Interestingly, the proteolytic activity of TvMP50 is upregulated by zinc and is associated with the cytotoxicity of *T. vaginalis* to the host cell, which was reduced when specific metalloproteinase inhibitors were used (Puente-Rivera et al. 2017). Therefore, TvMP50 is an attractive target for the development of new antitrichomonal drugs.

Thiazole derivatives are an important class of compounds with a wide range of medical applications (Chhabria et al. 2016). Regarding protease inhibition, it is important to mention two drugs: ritonavir and simeprevir, which contain thiazole rings and are potent, are FDA-approved, and are protease inhibitors for the treatment of HIV and HCV infections, respectively (Kempf et al. 1998; Li and De Clercq 2017). In a similar way, thiazole derivatives have been shown to be potent antiparasitic agents through anti-proteolytic activity. For example, trisubstituted thiazole analogs were shown capable of inhibiting the cysteine proteinases falcipain-2 and falcipain-3 from *Plasmodium falciparum* (Goud et al. 2005). In addition, previous reports have shown that proteases from some trypanosomatid species are thiazole derivative targets, which block their action and cause the death of parasites (de Oliveira Cardoso et al. 2014; de Moraes Gomes et al. 2016; Gomes de Oliveira et al. 2020).

Our group conducts research on the identification and development of potent antiparasitic compounds. An example of this was the one performed by Mocelo-Castell et al. in 2015, where a set of 50 thiazole derivatives (2-amino-4-arylthiazoles) were obtained, and their activity against *Giardia intestinalis* was tested. Among them, two bromo-analogues bearing an *N*-acetyl group exhibited the highest *in vitro* activity against *G. intestinalis* trophozoites. Considering these findings, we decided to evaluate the extended antiparasitic capability of these two compounds and further deepen in their mechanism of action. Here, we show that these thiazole derivatives exhibit potent activity against *T. vaginalis* as well as the inhibition of the TvMP50 metalloproteinase.

Materials and methods

Chemistry

Synthesis of the 2-Amino-4-arylthiazole derivatives used herein was described previously (Mocelo-Castell et al. 2015). Production of these derivatives was carried out in the Pharmaceutical Chemistry Laboratory of the Chemistry Faculty of the Autonomous University of Yucatan, in three steps (Fig. 1). During the first step, the thiazole core was obtained by reaction between 1-(4-bromophenyl) ethan-1-one or 1-(p-tolyl)ethan-1-one, with thiourea and iodine under microwave irradiation. In the second step, the compounds obtained, 4-(p-tolyl)thiazol-2-amine and 4-(4-chlorophenyl)thiazol-2-amine, were treated with acetic anhydride to obtain *N*-(4-(4-chlorophenyl)thiazol-2-yl)acetamide and *N*-(4-(p-tolyl)thiazol-2-yl)acetamide, respectively. Finally, these latter compounds were treated with molecular bromine to obtain the desired compounds.

In vitro susceptibility assays against *T. vaginalis* trophozoites

To determine the effect of 2-amino-4-aryl thiazole derivatives on *T. vaginalis* growth, *in vitro* susceptibility assays were performed as previously described (Cáceres-Castillo et al. 2019). Briefly, all compounds were dissolved in DMSO at a stock concentration of 1 mg/mL. For the assay, 12.5×10^6 *T. vaginalis* trophozoites were incubated in the presence of serially diluted thiazole derivatives (0.1 to 1.0 μg/mL) in TYM medium for 24 h at 37 °C. After different time intervals, cells were counted with an automated cell counter (TC10, Bio-Rad). Following incubation, parasite viability was checked by Trypan Blue exclusion assay. Trophozoites treated with either 0.1% DMSO or 6.0 μM metronidazole (MTZ) for 24 h were used as vehicle control and positive control, respectively. All experiments were performed by triplicate. IC_{50} (concentration causing 50% inhibition) values were calculated by probit analysis. The analyses were made by using one-way ANOVA, followed by a post hoc Dunnett's test. Statistical significance was accepted at $p < 0.05$.

Gelatin substrate gel electrophoresis

To evaluate the potential anti-proteolytic activity of the 2-amino-4-aryl thiazole derivatives, a gelatin substrate gel electrophoresis was performed as previously described (Vazquez Carrillo et al. 2011). Briefly, *T. vaginalis* trophozoites were grown in the presence of thiazole derivatives (0.1–0.2 μg/mL), vehicle control (0.1% DMSO), or positive control (6.0 μM MTZ) in TYM medium for 24 h at 37 °C. Following incubation, 2×10^7 cells were harvested by centrifugation at 500×*g* for 10 min at 4 °C and washed twice with

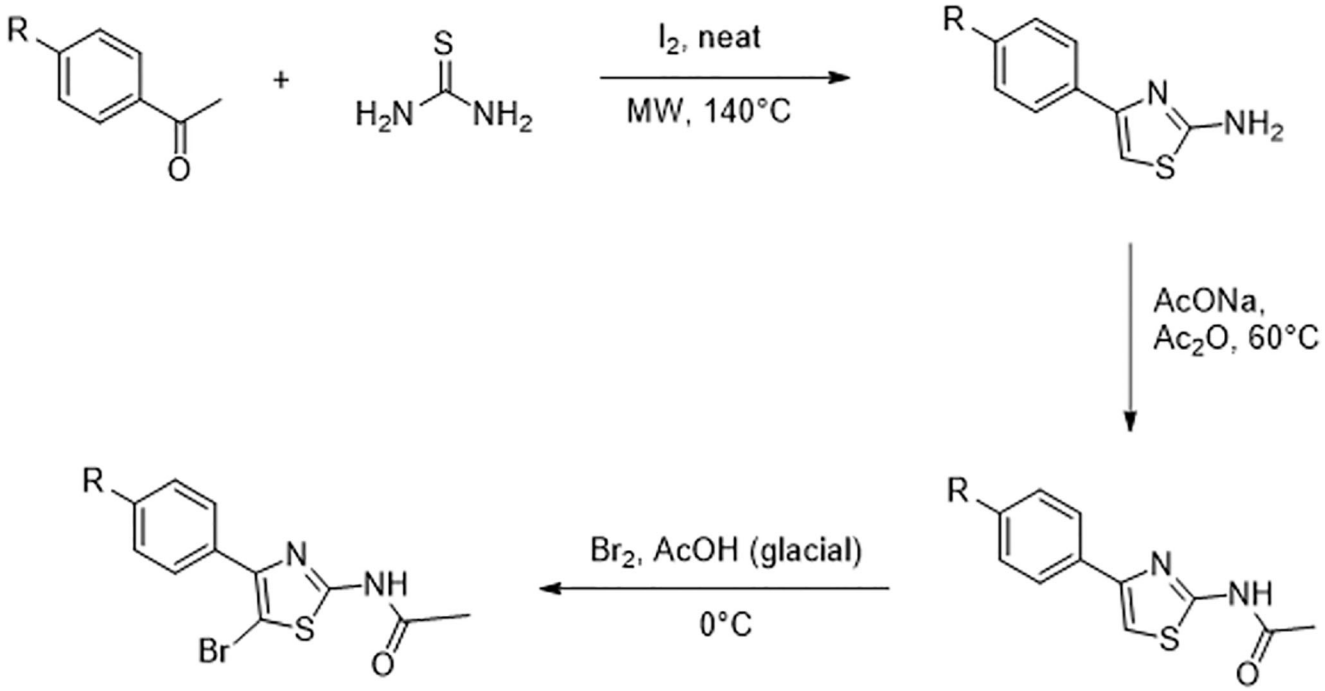


Fig. 1 Reaction scheme for synthesis of the 2-amino-4-arylthiazole derivatives used in this study. In the compounds, R was methyl for *N*-(5-bromo-4-*p*-tolyl-thiazol-2-yl)-acetamide or chloro for *N*-[5-bromo-4-(4-chloro-phenyl)-thiazol-2-yl]-acetamide

cold PBS pH 7.0. The pellet was resuspended in 30 μl of Laemmli buffer without boiling, and the protein concentration was determined by Bradford method, and then 50 μg of the protein was charged for each well onto a 0.2% gelatin copolymerized 10% SDS PAGE and run at 35 mA/gel until the tracking dye reached the bottom of the gel. The gel was soaked in 2.5% Triton-X 100 for 1 h at room temperature to eliminate SDS. The renatured gel was further incubated for 3 h at 37 °C in 0.2 M Tris–HCl buffer pH 8.2 with 0.1% β-mercaptoethanol to activate the proteinases. Finally, the gel was stained with Coomassie blue, and the clear bands were considered as positive proteolytic activity. The degree of proteolytic activity was semi-quantified by densitometric analysis of clear bands using version 4.6.3 of the Quantity One software (BioRad, Hercules, CA). The analyses were made by using one-way ANOVA, followed by post hoc Dunnett's test. Statistical significance was accepted at $p < 0.05$.

Gene expression

A semi-quantitative reverse transcription polymerase chain reaction was performed to evaluate the mRNA levels of *mp50* gene on thiazole derivative-treated *T. vaginalis* trophozoites incubated for 24 h. Trophozoites incubated with vehicle control (0.1% DMSO) or positive control (6.0 μM MTZ) were used for comparison. Briefly, 1×10^6 parasites were harvested by centrifugation at 500×*g* for 10 min at 4 °C and washed twice with cold PBS pH 7.0, and the pellet was resuspended in 1 mL of TRIzol® reagent, and total RNA was extracted according to the manufacturer's instructions.

Following extraction, 1 μg of total RNA was treated with DNAse I to eliminate genomic DNA remnants. Next, 200 ng of DNAse I treated-RNA was retro-transcribed using the SuperScript™ II Reverse Transcriptase Kit. To evaluate the mRNA level of *mp50* gene (TVAG_403460), a reverse transcription polymerase chain reaction was performed as previously described (Puente-Rivera et al. 2017), using 0.2 μL cDNA preparation as a template. The constitutively expressed *β-tubulin* gene in *T. vaginalis* was used as an internal control to normalize the results. PCR amplicons were visualized on 1% agarose gels stained with ethidium bromide, and the semi-quantitative measurement was achieved by digital analysis using version 4.6.3 of the Quantity One software (BioRad, Hercules, CA).

Molecular docking and cavities

Docking calculation was carried out in the Swissdock, an online docking web service accessible via the ExPASy web server from the Swiss Institute of Bioinformatics based on the EADock DSS docking software (Grosdidier et al. 2011a; b). Parameters used in Swissdock were docking type: accurate; passive_flexibility_distance 5.0; wanted_confs 5000; nb_facts_eval 5000; nb_seeds 250; sd_steps 100; abnrsteps 250; clusterin_gradius 2.0 and max_cluster_size 8. The coordinate files (protein and ligands) were prepared with the program Openbabel v. 2.4.0 (O'Boyle et al. 2011), and the visualization of the data result provided by SwissDock was

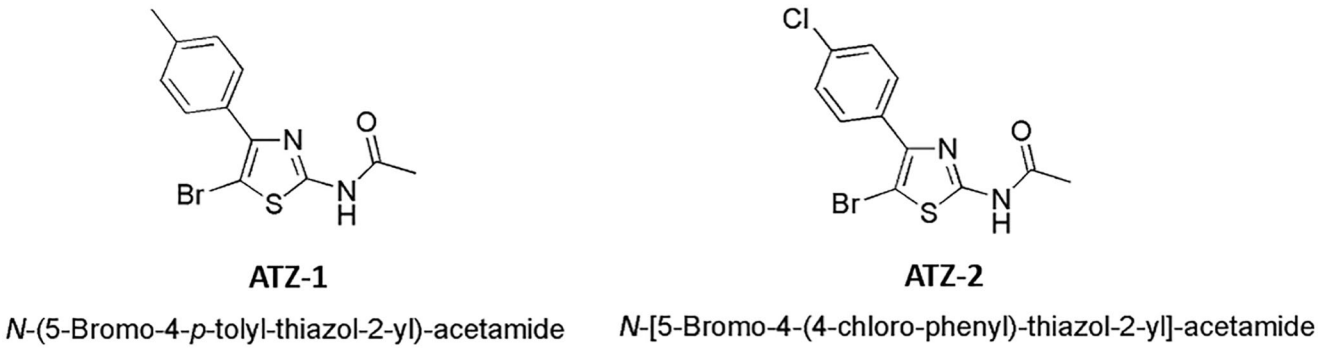


Fig. 2 Chemical structure of thiazole derivatives (ATZ-1 and ATZ-2) evaluated against *T. vaginalis*

analyzed with v. 1.13.1 of the Chimera software (Pettersen et al. 2004) using the Viewdock tool. Protein coordinates of the final published model from TvMP50 were obtained (structure not deposited in the Protein Data Bank. See publication Arreola et al. 2018), and the compounds (thiazole derivatives, MTZ, and fluoxetine) were built in ACD/ChemSketch freeware 5.12 software (www.acdlabs.com) and affined in Chimera with the Molecular Dynamics Simulation Tool. Each compound of interest was tested with three docking conditions: (1) TvMP50 with empty active site (free of metals and inactive), (2) TvMP50 with a Ca^{2+} in the active site (inactive enzyme), and (3) TvMP50 with two Zn^{2+} in the active site (active enzyme). Fluoxetine was used as control molecule not related with the enzyme and metronidazole as active compound against *T. vaginalis*. The crystal structure showed a Ca^{2+} and a Ni^{2+} on the active site; the enzyme was active with Zn^{2+}. We modeled the hypothetical correct functional metals with two Zn^{2+} and a nonfunctional

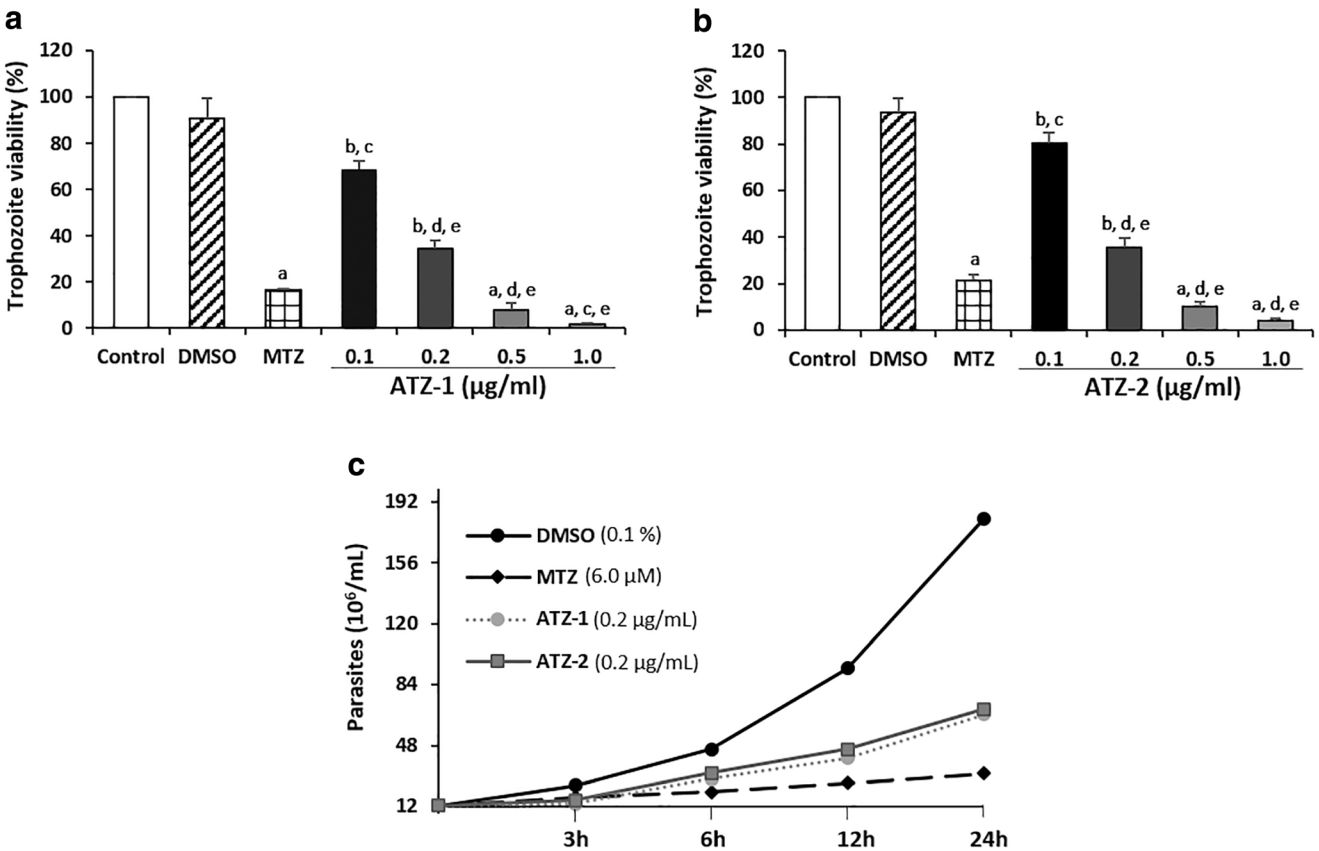


Fig. 3 Trichomonicidal activity of thiazole derivatives. The viability of *T. vaginalis* trophozoites was measured in the presence of increasing concentrations of ATZ-1 compound (**a**) or ATZ-2 compound (**b**) for 24 h. Mean ± standard deviation from three biological replicate experiments are shown. $^{a}p < 0.01$; versus vehicle control (DMSO at 0.1%); $^{b}p < 0.05$; versus vehicle con(DMSO at 0.1%); $^{c}p < 0.01$; versus metronidazole-treated group (MTZ at 6 μM); $^{d}p < 0.05$; versus metronidazole-treated group (MTZ at 6 μM); $^{e}p < 0.05$; versus preceding concentration of thiazol compound. **c** Growth kinetics of *T. vaginalis* trophozoites during 24 h of incubation in the presence of both thiazole compounds at 0.2 μg7mL

enzyme with one Ca^{2+}. The selection criteria for the cluster candidates were using the 40 best predictions considering the Delta G (DG) values and the Fullfitness (FFN) values (20 best predictions for each one). The initial images were built with Chimera and edited with Gimp v 2.10.18.

Results

Previous work in our lab established a high-throughput screening of thiazole derivatives to identify anti-*Giardia* compounds. Through this screening, we identified two potent 2-amino-4-arylthiazoles derivatives, *N*-(5-Bromo-4-*p*-tolyl-thiazol-2-yl)-acetamide and *N*-[5-bromo-4-(4-chloro-phenyl)-thiazol-2-yl]-acetamide, which were named here ATZ-1 and ATZ-2, respectively (Fig. 2).

To confirm the antiparasitic activity of the 2-amino-4-arylthiazoles derivatives mentioned above, we tested their ability to inhibit *T. vaginalis* growth using the *in vitro* susceptibility assay. Results showed that treatment with 0.1 μg/mL of ATZ-1 or ATZ-2 decreased *T. vaginalis* viability by 31% and 20%, respectively, whereas treatment with 0.2 μg/mL of each compound decreased *T. vaginalis* viability by approximately 65% (Fig. 3a, b). Moreover, both compounds reduced the trophozoites viability by > 95% at 1.0 μg/mL after 24-h exposure (Fig. 3a, b), revealing a concentration-dependent inhibition, with an IC_{50} value of 0.15 μg/mL for ATZ-1 and 0.18 μg/mL for ATZ-2. Incubation with vehicle (DMSO, 0.1%) did not exhibit significant differences in parasite viability compared with untreated trophozoites (control).

Considering that the IC50 values for both compounds were close to the tested concentration of 0.2 μg/mL, these were used to evaluate a kinetic growth curve for a period of 24 h by measuring the number of parasites. Both compounds decreased the *T. vaginalis* growth in a similar way, exhibiting a reduction of approximately 35% in comparison with the corresponding vehicle control group after 6-h exposure, and the maximal growth inhibition effect was observed after 24-h exposure, decreasing *T. vaginalis* growth by approximately 63% (Fig. 3c).

Since there is a correlation between the proteolytic activity with the *T. vaginalis* virulence, as well as with the anti-proteolytic capability of the thiazole derivatives, we searched for possible protease inhibitory activity by ATZ-1 and ATZ-2. Figure 4 shows representative photographs of gelatin zymography, which reveal several gelatinolytic proteins in the *T. vaginalis* lysate with different molecular weights (panels A and B, DMSO vehicle control lane 1). ATZ-1 at the highest concentration tested (0.2 μg/mL) was able to

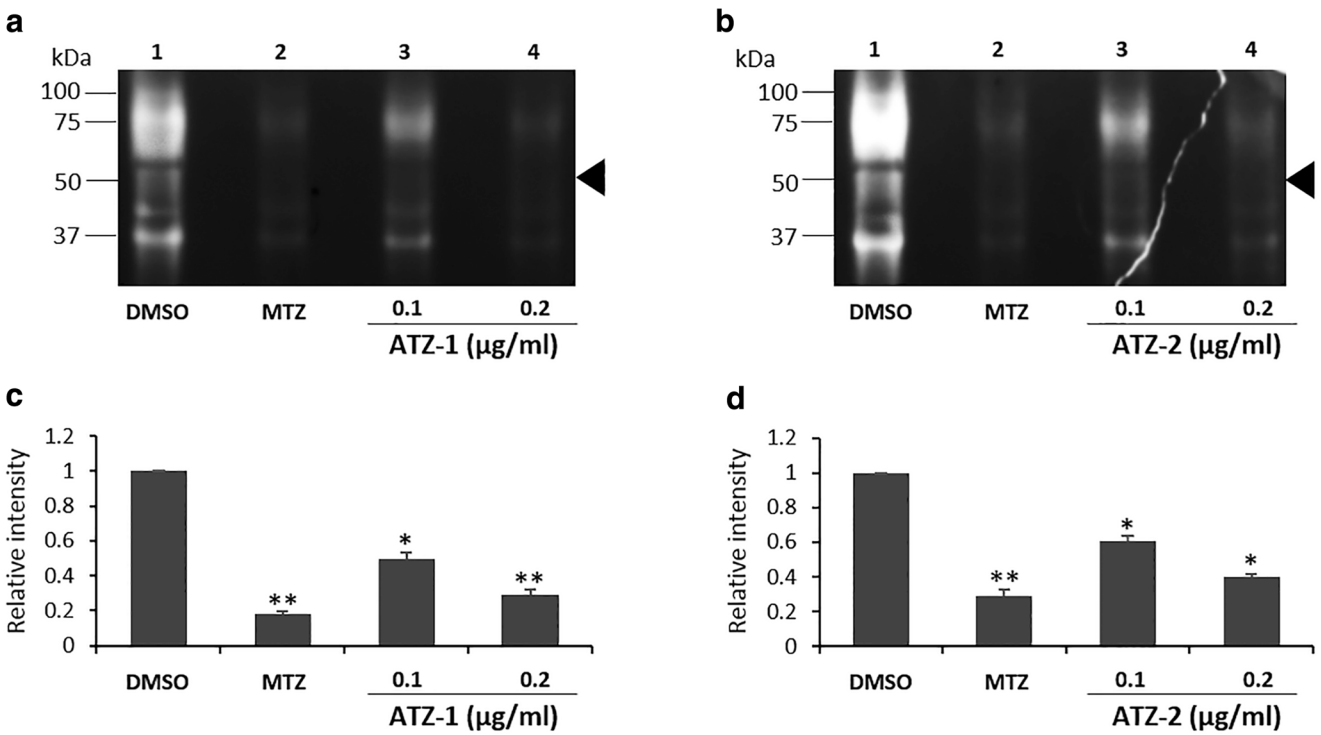


Fig. 4 Effect of ATZ-1 and ATZ-2 compounds on proteolytic activity of total cellular extract of *T. vaginalis*. Gelatin substrate gel electrophoresis showing a decrease of the proteolytic activity of the parasites incubated in the presence of ATZ-1 compound (**a**) or ATZ-2 compound (**b**) for 24 h, in comparison with the corresponding vehicle control group (DMSO at 0.1%). Metronidazole (MTZ) was used as a control to show protease activity inhibition. The complete inhibition of the 50-kDa region is indicated by an arrowhead. **c**, **d** show the densitometric analysis (mean ± standard deviation of at least three independent measurements) to the corresponding inhibition by ATZ-1 and AT-2, respectively. Significances are shown in comparison with the vehicle control. *$p < 0.05$, **$p < 0.01$

inhibit the trichomonal protease activity (Fig. 4a, lane 4) comparable with the classical trichomonicidal drug metronidazole (Fig. 4a, MTZ 6 μM, lane 2). Moreover, the observed protease inhibition activity by ATZ-2 at 0.2 μg/mL concentration was approximately 3-fold lower than the one exhibited with the vehicle (Fig. 4b, lane 4). Also, at a lower concentration of 0.1 μg/mL of the 2-amino-4-arylthiazoles derivatives, a significant reduction in proteolytic activity was observed (Fig. 4a, b, lanes 3). Interestingly, the proteolytic activity in the 50-kDa region was inhibited by both ATZ-1 and ATZ-2 (Fig 4a and b, black arrow head).

These latter results suggest that the mechanism of action of these thiazole derivatives could be related to enzymatic disruption via inhibition of key proteases in *T. vaginalis*, as the metalloproteinase TvMP50, involved in their cytotoxic effect to host cells. To test this hypothesis, we performed gene expression assays to evaluate the transcription levels of the *mp50* gene. The results revealed that the expression levels of the *mp50* transcript were reduced after the treatment with both ATZ-1 and ATZ-2 for 24 h (Fig. 5). Interestingly, the thiazole derivatives exhibited stronger reduction in the transcription of *mp50* rather than the corresponding vehicle control group (Fig. 5a,b). Notably, the expression of *mp50* was almost completely inhibited by ATZ-1, at the highest concentration tested (0.2 μg/mL), which was able to reduce the transcript levels approximately 2-fold lower than the one exhibited by MTZ (Fig. 5a, lane 4).

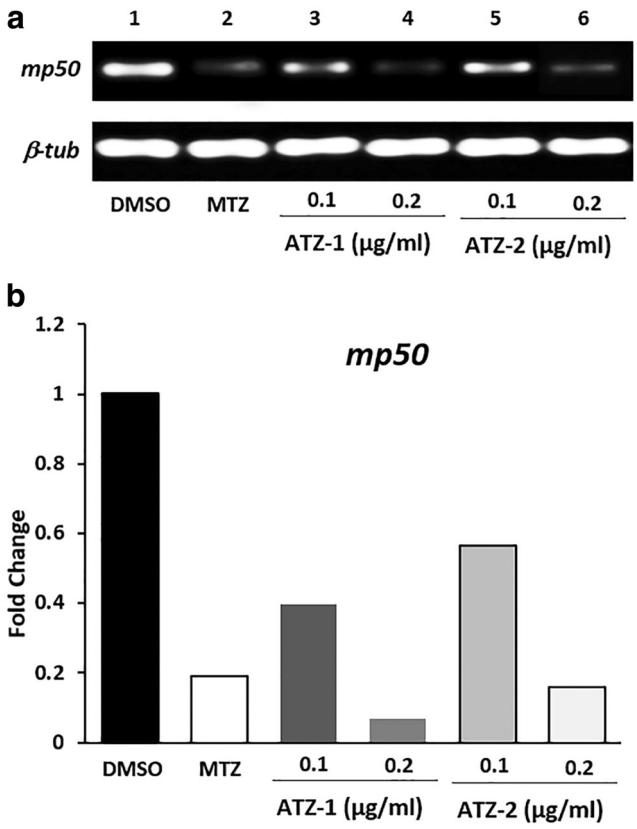


Fig. 5 The expression of TvMP50 is affected by ATZ-1 and ATZ-2 compounds. **a** RT-PCR reaction showing the differential expression of *mp50* after the growth of *T. vaginalis* in presence of thiazole compounds for 24 h. ATZ-1 and ATZ-2 treated-parasites at 0.2 μg/mL showed a significant decrease of *mp50* expression, similar to the one observed in MTZ-treated parasites. **b** Relative expression was analyzed by measuring the RT-PCR band intensity using the Quantity One software version 4.6.3 and represented as relative fold change in expression

To determine the possible mechanism for TvMP50 inhibition through their possible interaction with the ATZ-1 and ATZ-2 derivatives, a molecular docking was carried out. The selected prediction results suggest that ATZ-1 and ATZ-2 compounds can bind to the active site of the TvMP50 metalloproteinase (Fig. 6). The presence of ATZ-1 and ATZ-2 in the active site of TvMP50 can be observed only in the complex with Zn^{2+} and, accordingly, the DG and FFN values increase (Table 1). The TvMP50 empty and the complex with Ca^{2+} do not show a binding mode, and the DG and FFN values are lower than in the TvMP50-Zn^{2+} complex. The control molecule fluoxetine, as expected for a non-related compound, is not observed in the active site in presences of metals. However, we observed that it could bind to the active site when it is empty, probably due to the presence of the acidic cluster residues with negative charges. Regarding MTZ, both TvMP50 metal complexes showed the presence of the molecule with elevated DG and FFN values unlike with the empty enzyme, although TvMP50-Zn^{2+} complex exhibited the higher DG values. Docking suggests that MTZ is capable of binding different metal clusters on proteins, probably a secondary drug activity, not described before.

Discussion

Two indisputable trichomoniasis facts are that it is the most common non-viral STI and that it is the only known STI caused by a protozoan parasite. Nowadays, chemotherapy against trichomoniasis involves mainly the 5-nitroimidazole members, either metronidazole or tinidazole. However, all of these have been reported having undesirable side effects, and antibiotic resistance has already increased considerably because of treatment failure (Alessio and Nyirjesy 2019). Therefore, the identification of new molecules with trichomonicidal activity is still necessary to develop new chemotherapeutic alternatives against this parasite.

Thiazole and its derivatives have established as sources of lid compounds for new promises of medicinal agents. In a previous work, we showed a significant antigiardial effect of two 2-amino-4-arylthiazoles derivatives, *N*-(5-bromo-4-*p*-tolyl-thiazol-2-yl)-acetamide (ATZ-1) and *N*-[5-Bromo-4-(4-chloro-phenyl)-thiazol-2-yl]-acetamide (ATZ-2) (Mocelo-Castell et al. 2015). Considering the close relationship between the metabolisms and treatment for *Giardia* and

Table 1 Docking predictions. Delta G (DG) and Fullfitness (FFN) values of the 40 best predictions

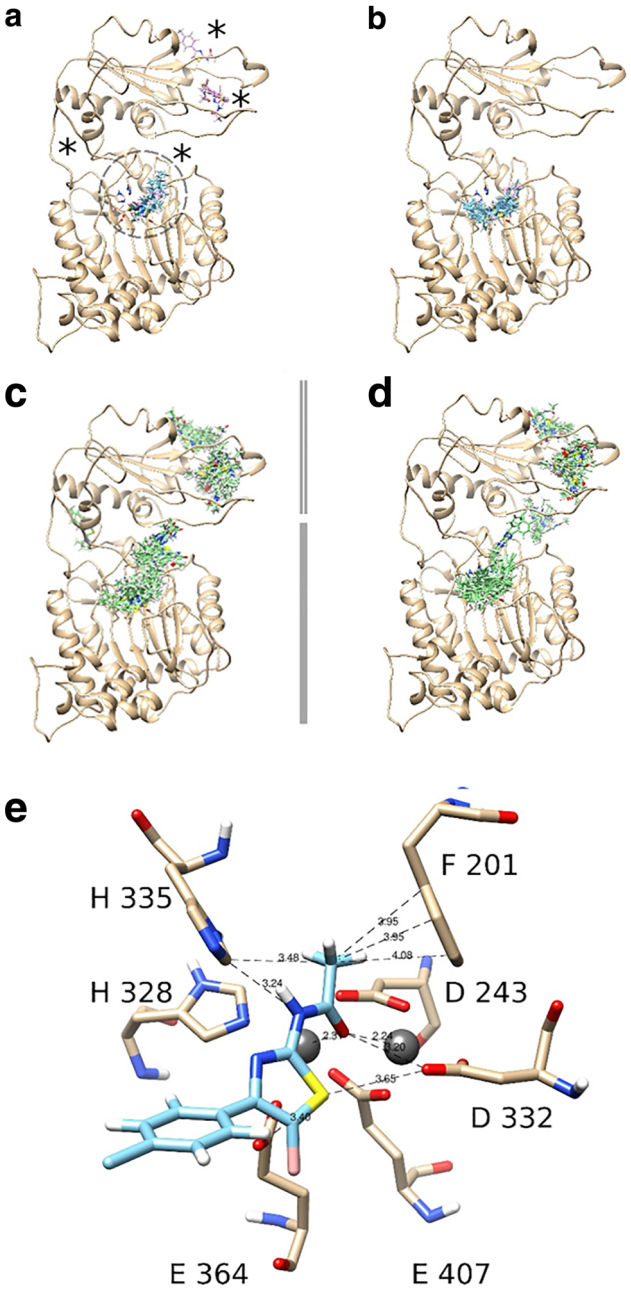
	Active site empty	TvMP50-Ca^{2+}	TvMP50-Zn^{2+}
ATZ-1	256 predictions	256 predictions	255 predictions
Delta G (kcal/mol)	− 7.5165 to − 7.1492	− 7.5134 to − 7.1686	− 8.1060 to − 7.8420
Fullfitness	− 2738.79 to − 2734.98	− 3141.96 to − 3138.20	− 3121.26 to − 3115.34
ATZ-1 presence on the active site, in the 40 best predictions	Neither	Neither	Yes DG 20 FFN 15
ATZ-2	254 predictions	256 predictions	256 predictions
Delta G (Kcal/mol)	− 7.2714 to − 7.1418	− 7.3707 to − 7.2086	− 8.8475 to − 8.0580
Fullfitness	− 2737.57 to − 2735.26	− 3140.17 to − 3137.73	− 3127.58 to − 3115.31
ATZ-2 presence on the active site, in the 40 best predictions	Neither	Neither	Yes DG 20 FFN 20
Metronidazole	256 predictions	255 predictions	256 predictions
Delta G (Kcal/mol)	− 7.1060 to − 6.2916	− 11.7528 to − 6.7329	− 15.2291 to − 14.0447
Fullfitness	− 2674.40 to − 2672.43	− 3086.21 to − 3076.66	− 3152.62 to − 3135.38
Metronidazole presence on the active site, in the 40 best predictions	neither	Yes DG:2 FFN:11	Yes DG:20 FFN:20
Fluoxetine	256 predictions	256 predictions	250 predictions
Delta G (Kcal/mol)	− 11.453 to − 9.9405	− 9.2410 to − 8.6421	− 9.2339 to − 8.8467
Fullfitness	− 2726.42 to − 2721.21	− 3129.83 to − 3124.12	− 3105.93 to − 3099.9
Fluoxetine presence on the active site, in the 40 best predictions	Yes DG 20 FFN 5	Neither	Neither

T. vaginalis, the present study was performed to test the trichomonicidal effect of these thiazole derivatives.

Our results show that ATZ-1 and ATZ-2 were able to exhibit an anti-*T. vaginalis* activity in a dose-dependent manner (Fig. 3, panels A and B), presenting an IC_{50} value of 0.15 μg/mL and 0.18 μg/mL, respectively. In addition, both compounds were able to reduce the *T. vaginalis* trophozoites growth in a time course of 24 h, which was not significantly different from that of MTZ (Fig. 3, panels C). These results highlight the antiparasitic activity of the arylthiazoles derivatives and agree with previously mentioned reports that have shown that these compounds are effective against diverse parasites (Brito et al. 2019; Pereira et al. 2019; Georgiadis et al. 2020).

Considering that the thiazole derivatives have been reported as anti-proteolytic agents, we speculated that the ATZ-1 and ATZ-2 compounds might play a role in altering the proteolytic activity leading to the anti-*T. vaginalis* activity. In the present study, the anti-proteolytic activity of both the ATZ-1 and ATZ-2 compounds was demonstrated, by using gelatin substrate gel electrophoresis. ATZ-1 was able to reduce the *T. vaginalis* proteolytic activity to a level that was not significantly different to the one exhibited by MTZ (Fig. 4, panel A). A similar proteolytic inhibition profile was observed with ATZ-2 (Fig. 4, panel B). It has been previously reported that protozoan parasite proteases are considered good drug targets to develop a therapeutic strategy against diverse parasitic diseases (McKerrow et al. 2008; Das et al. 2013; McKerrow 2018).

Interestingly, both thiazole derivatives were able to fully reduce the proteolytic activity in a range of 46 to 50 kDa, considering the possibility that these compounds could, in part, affect the activity of a key *T. vaginalis* protease located at that region, TvMP50. The transcription levels of the *mp50* gene exhibit downregulation associated with ATZ-1 and ATZ-2 treatment, mainly at the 0.2 μg/mL concentration (Fig. 5a). Based on the biological assays, docking studies were conducted to determine the plausible binding mode of ATZ-1 and ATZ-2 with the TvMP50 protease. Notably, the docking simulations for both thiazole derivatives showed their interaction principally with the dinuclear Zn^{2+} cluster and coordinated residues in the active site of TvMP50, mainly in the presence of zinc, in a stronger way (Fig. 6, panel E). Respect the MTZ-docked binding mode results in comparison with ATZ1 and ATZ2 an interesting chemical property cloud be of interest, the atoms in contact with the Zn^{2+} cluster share a trigonal planar geometry (acetamide oxygen of thiazole derivatives and imidazole nitrogen of MTZ) with a predicted hybridized sp2 orbital and unhybridized p2 orbital. Although, the electronic



◀ **Fig. 6** Predictions of the docking TVMP50-Zn^{2+} conditions for ATZ-1 and ATZ-2 compounds. **a** ATZ-1 docking. The 40 predictions selected by DG and FFN better values depicted on the TvMP50 structure. Most of the predictions appear on the active site cavity in similar binding modes and few predictions on the other two cavities generated by three loops in the N-terminal domain. The dashed circle depicts the active site cavity. The residues of the metal cluster and two Zn^{2+} are represented but are hidden by the predictions. The asterisk (*) represents the sites where CASTp program detects the most relevant cavities on the N-terminal. **b** ATZ-2 docking. The 40 predictions selected by Delta G and FFN better values depicted on the TvMP50 structure. All predictions appear on the active site cavity in a similar binding mode. The variation on the binding modes is related to the position of the two rings of ATZ-2 and ATZ-1. **c** ATZ-1 docking. All ATZ-1 docking predictions depicted on the TvMP50 structure. Many predictions are in the active site cavity and the close cavity between both domains, although a considerable number of predictions appear on the two cavities of the N-terminal domain. An additional small cavity on the back of the structure appears with few predictions. **d** ATZ-2 docking. All ATZ-2 docking predictions depicted on the TvMP50 structure. Many predictions are in the active site cavity and few predictions on the close cavity between both domains; considerable predictions are located on the two cavities of the N-terminal domain. The double bar line indicates the N-terminal domain, and the C-terminal domain is represented by the solid bar. Colors key: in cyan DG predictions, in pink FFN predictions, all predictions on green. **e** The binding mode of ATZ-2 top prediction (higher DG, sFFN, and energy values), as a representative, to show the hypothetical acetamide interactions with Zn^{2+} with 2.2–2.3 Å. The other atoms interactions of ATZ-2 are maintained between 3.2 and 4 Å

distribution of the sp2 orbitals is different and hence the interaction with Zn^{2+} cluster. However, future studies should include these observations to re-design ATZ1 and ATZ2 compounds to search for better properties to find alternative new drugs based on these compounds. The TvMP50 protease plays a major role during the *T. vaginalis* pathogenesis process; therefore, it is worth mentioning that the results suggest that the mechanism of action of thiazole derivatives tested here may be associated, in part, with the inhibition of this key protease and lead to damage to parasites. Although, it was reported that the addition of iron to *in vitro* culture of *T. vaginalis* decreases MTZ minimal lethal concentration (Elwakil et al. 2017), probably contributing to the reductive activation of metronidazole (cytotoxic form produces in the hydrogenosome), which suggest a secondary mode activation mediated by an enzyme as TvMP50, reduction of MTZ, or an enzyme inhibition. However, it is less well known the mechanism that leads to the treatment of ATZ-1 and ATZ-2 to downregulation of the *mp50* gene transcription. The identification of the mechanism could be an interesting additional research way to find other targets of thiazole derivatives with antitrichomonal activity.

However, further studies are necessary to evaluate structural modifications that could improve trichomonicidal activity, as well as studies to elucidate the mechanisms associated and the possible anti-proteolytic effects. In conclusion, our results demonstrate that the 2-amino-4-arylthiazoles derivatives possess potent antiparasitic activity against *T. vaginalis* trophozoites as plausible trichomonad TvMP50 protease inhibitors, revealing the potential use of these thiazoles as novel antitrichomonal agents.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

References

Alessio C, Nyirjesy P (2019) Management of resistant trichomoniasis. Curr Infect Dis Rep 21:31. https://doi.org/10.1007/s11908-019-0687-4

Arreola R, Villalpando JL, Puente-Rivera J, Morales-Montor J, Rudiño-Piñera E, Alvarez-Sánchez ME (2018) *Trichomonas vaginalis* metalloproteinase TvMP50 is a monomeric aminopeptidase P-like enzyme. Mol Biotechnol 60:563–575

Brito CCB, Silva HVCD, Brondani DJ, Faria ARD, Ximenes RM, Silva IMD, Albuquerque JFCD, Castilho MS (2019) Synthesis and biological evaluation of thiazole derivatives as Lb SOD inhibitors. J Enzyme Inhib Med Chem 34:333–342

Cáceres-Castillo D, Pérez-Navarro Y, Torres-Romero JC, Mirón-López G, Ceballos-Cruz J, Arana-Argáez V, Vázquez-Carrillo L, Fernández-Sánchez JM, Alvarez-Sánchez ME (2019) Trichomonicidal activity of a new anthraquinone isolated from the roots of *Morinda panamensis* Seem. Drug Dev Res 80:155–161

Chhabria M, Patel S, Modi P, Brahmkshatriya P (2016) Thiazole: a review on chemistry, synthesis and therapeutic importance of its derivatives. Curr Top Med Chem 16:2841–2862

Cudmore SL, Garber GE (2009) Diagnosis and treatment of metronidazole-resistant *Trichomonas vaginalis* infection. In: Mayers DL (ed) Antimicrobial drug resistance, 1st edn. Humana Press, Totowa, pp 1091–1100

Das P, Alam MN, Paik D, Karmakar K, De T, Chakraborti T (2013) Protease inhibitors in potential drug development for Leishmaniasis. Indian J Biochem Biophys 50:363–376

De Moraes Gomes PAT, de Oliveira BM, Santiago EF, de Oliveira Cardoso MV, Costa NTC, Hernandes MZ et al (2016) New 1, 3-thiazole derivatives and their biological and ultrastructural effects on *Trypanosoma cruzi*. Eur J Med Chem 121:387–398

De Oliveira Cardoso MV, de Siqueira LRP, da Silva EB, Costa LB, Hernandes MZ, Rabello MM et al (2014) 2-Pyridyl thiazoles as novel anti-*Trypanosoma cruzi* agents: structural design, synthesis and pharmacological evaluation. Eur J Med Chem 86:48–59

Elwakil HS, Tawfik RA, Alam-Eldin HY, Nassar DA (2017) The effect of iron on metronidazole activity against *Trichomonas vaginalis* in Vitro. Exp Parasitol 182:34–36. https://doi.org/10.1016/j.exppara.2017.09.021

Georgiadis MO, Kourbeli V, Papanastasiou IP, Tsotinis A, Taylor MC, Kelly JM (2020) Synthesis and evaluation of novel 2, 4-disubstituted arylthiazoles against *T. brucei*. RSC Med Chem 11:72–84

Gewirtzman A, Bobrick L, Conner K, Tyring SK (2011) Epidemiology of sexually transmitted infections. In: Gross G, Tyring SK (eds) Sexually transmitted infections and sexually transmitted diseases, 1st edn. Springer, Berlin, pp 13–34

Gomes de Oliveira VV, Aranda de Souza MA, Mororó Cavalcanti RR, de Oliveira Cardoso MV, Lima Leite AC, da Silva Junior VA, Bressan Queiroz de Figueiredo RC (2020) Study of *in vitro* biological activity of thiazoles on *Leishmania* (Leishmania) *infantum*. J Glob Antimicrob Resist 22:414–421. https://doi.org/10.1016/j.jgar.2020.02.028

Goud PM, Sheri A, Desai PV, Watkins EB, Tekwani B, Sabnis Y, Gut J, Rosenthal PJ, Avery MA (2005) Design, synthesis and evaluation of trisubstituted thiazoles targeting *Plasmodium falciparum* cysteine proteases. Med Chem Res 14:74–105

Grosdidier A, Zoete V, Michielin O (2011a) Fast docking using the CHARMM force field with EADock DSS. J Comput Chem 32:2149–2159

Grosdidier A, Zoete V, Michielin O (2011b) SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic Acids Res 39(suppl_2):W270–W277

Kempf DJ, Sham HL, Marsh KC, Flentge CA, Betebenner D, Green BE, McDonald E, Vasavanonda S, Saldivar A, Wideburg NE, Kati WM, Ruiz L, Zhao C, Fino LM, Patterson J, Molla A, Plattner JJ, Norbeck DW (1998) Discovery of ritonavir, a potent inhibitor of HIV protease with high oral bioavailability and clinical efficacy. J Med Chem 41:602–617

Kirkcaldy RD, Augostini P, Asbel LE, Bernstein KT, Kerani RP, Mettenbrink CJ, Pathela P, Schwebke JR, Secor WE, Workowski KA, Davis D, Braxton J, Weinstock HS (2012) *Trichomonas vaginalis* antimicrobial drug resistance in 6 US cities, STD Surveillance Network, 2009–2010. Emerg Infect Dis 18:939–943

Kissinger P (2015) *Trichomonas vaginalis*: a review of epidemiologic, clinical and treatment issues. BMC Infect Dis 15:307. https://doi.org/10.1186/s12879-015-1055-0

Li G, De Clercq E (2017) Current therapy for chronic hepatitis C: the role of direct-acting antivirals. Antivir Res 142:83–122

McKerrow JH (2018) Update on drug development targeting parasite cysteine proteases. PLoS Negl Trop Dis 12:e0005850. https://doi.org/10.1371/journal.pntd.0005850

McKerrow JH, Rosenthal PJ, Swenerton R, Doyle P (2008) Development of protease inhibitors for protozoan infections. Curr Opin Infect Dis 21:668–672

Mercer F, Johnson PJ (2018) *Trichomonas vaginalis*: Pathogenesis, symbiont interactions, and host cell immune responses. Trends Parasitol 34:683–693

Mocelo-Castell R, Villanueva-Novelo C, Cáceres-Castillo D, Carballo RM, Quijano-Quiñones RF, Quesadas-Rojas M, Cantillo-Ciau Z, Cedillo-Rivera R, Moo-Puc RE, Moujir LM, Mena-Rejón GJ (2015) 2-amino-4-arylthiazole derivatives as anti-giardial agents: synthesis, biological evaluation and QSAR studies. Open Chem 13:1127–1136. https://doi.org/10.1515/chem-2015-0127

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011) Open Babel: an open chemical toolbox. Aust J Chem 3:33. https://doi.org/10.1186/1758-2946-3-33

Pereira AS, Silveira GO, Amaral MS, Almeida SM, Oliveira JF, Lima MC, Verjovski-Almeida S (2019) *In vitro* activity of aryl-thiazole derivatives against *Schistosoma mansoni* schistosomula and adult worms. PLoS One 14:e0225425. https://doi.org/10.1371/journal.pone.0225425

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera–a visualization system for exploratory research and analysis. J Comput Chem 25:1605–1612

Puente-Rivera J, Villalpando JL, Villalobos-Osnaya A, Vázquez-Carrillo LI, León-Ávila G, Ponce-Regalado MD, López-Camarillo C, Elizalde-Contreras JM, Ruiz-May E, Arroyo R, Alvarez-Sánchez ME (2017) The 50 kDa metalloproteinase TvMP50 is a zinc-mediated *Trichomonas vaginalis* virulence factor. Mol Biochem Parasitol 217:32–41

Quintas-Granados LI, Villalpando JL, Vázquez-Carrillo LI, Arroyo R, Mendoza-Hernández G, Álvarez-Sánchez ME (2013) TvMP50 is an immunogenic metalloproteinase during male trichomoniasis. Mol Cell Proteomics 12:1953–1964

Vazquez Carrillo LI, Quintas Granados LI, Arroyo R, Mendoza Hernández G, González Robles A, Carvajal Gamez BI, Alvarez Sánchez ME (2011) The effect of Zn2+ on prostatic cell cytotoxicity caused by *Trichomonas vaginalis*. J Integr OMICS 1:198–210