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2-Amino-4-arylthiazole Derivatives as Anti-giardial Agents: Synthesis, Biological Evaluation and QSAR Studies

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Abstract: A series of seven 2-amino-4-arylthiazoles were prepared following Hantzsch's modified method under microwave irradiation. A set of 50 derivatives was obtained and the *in vitro* activity against *Giardia intestinalis* was evaluated. The results on the biological activity revealed that, in general, the *N*-(5-bromo-4-aryl-thiazol-2-yl)-acetamide scaffold showed high bioactivity. In particular, compounds **6e** (IC_{50} = 0.39 μM) and **6b** (IC_{50} = 0.87 μM) were found to be more potent than the positive control metronidazole. Citoxicity and acute toxicity tests performed showed low toxicity and high selectivity of the most active compounds (**6e** SI = 139, **6b** SI = 52.3). A QSAR analysis was applied to a data set of 37 obtained 2-amino-4-arylthiazoles derivatives and the best model described a strongly correlation between the anti-giardiasic activity and molecular descriptors as E2M, RDF115m, F10, MATS6v, and Hypnotic-80, with high statistical quality. This finding indicates that *N*-substituted aminothiazole scaffold should be investigated for the development of highly selective anti-giardial agent.

Keywords: 2-amino-4-arylthiazole, Anti-protozoal activity, *Giardia intestinalis*, QSAR

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1 Introduction

Giardia intestinalis (syn. *Giardia duodenalis*, *Giardia lamblia*) is an enteric protozoan pathogen that infects humans, domestic animals and wildlife worldwide. The clinical manifestations of *G. intestinalis* infection are highly variable between individuals and can range from acute to chronic infections, while some hosts may remain asymptomatic. When present, clinical signs of infection may include nausea, weight loss, bloating, abdominal pain, and diarrhea [1]. In Asia, Africa, and Latin America, about 200 million people have symptomatic giardiasis, with more than 500,000 new cases reported every year. In developed countries, where the prevalence of giardiasis varies from approximately 3–7%, it is referred to as a re-emerging infectious disease [2]. Due to these facts, since ten years ago, this parasite was included in the World Health Organization (WHO) Neglected Disease Initiative [3].

Currently, the classes of chemotherapeutic agents available for the treatment of giardiasis are: 5-nitroimidazoles (Metronidazole, Tinidazole, Secnidazole, and Ornidazole), 5-nitrofurans (Furazolidone), 5-nitrothiazoles (Nitazoxanide), benzimidazoles (Albendazole, Mebendazole), quinolines (Chloroquine), acridins (Quinacrine), and aminoglycosides (Paromomycin) [4].

Although 5-nitroimidazoles are the most commonly used anti-giardiasic drugs, recent studies have shown

that Metronidazole (MTZ) has several toxic effects such as headache, vertigo, nausea, and in some cases neurotoxicity [5,6]. Moreover, previous studies have also demonstrated mutagenic, teratogenic, and other cytotoxic effects [7,8]. In addition *Giardia* strains have been developing resistance to MTZ *in vivo* and manifesting cross-resistance to other nitroimidazole analogues, which results in treatment failures in up to 20% of cases [8-10]. Early in the last decade, US Food and Drug Administration has approved the use of nitazoxanide (NIT) for treatment giardiasis. Nitazoxanide, (Alinia®; Romark Laboratories), is a drug introduced as an alternative option that shows excellent *in vitro* activity against a wide variety of protozoa [11]. In a clinical trial involving children with diarrheal illness associated with *G. intestinalis*, this nitrothiazolide was effective at reducing the duration of it [12] and the most common side effect is gastrointestinal upset [13].

In contrast, the other alternative agents for treating giardiasis show variable efficacies besides adverse effects. Then, Furazolidone can cause mild hemolysis in patients deficient in glucose-6-phosphate dehydrogenase. Albendazole can be as effective as Metronidazole, but its efficacy varies markedly (25–90%) depending on the dosing regimen. Quinacrine possess a highly efficacy (90%), however it can produce yellow discoloration of skin sclera, and exfoliative dermatitis. The aminoglycoside Paromomycin is less effective among adults with metronidazole-refractory disease. Chloroquine is an old anti-malarial drug with anti-giardial activity that is no longer available in the US or Canada [13].

The thiazole ring system is a useful structural motif found in numerous biologically active molecules and is an interesting group as anti-infective agents [14]. In the chemotherapy field of protozoan diseases, substituted 2-aminothiazoles have been investigated due to their broad anti-parasitic spectrum. These structures have been tested as pharmacophores in many synthetic compounds and have shown *in vitro* and *in vivo* activities against *Trypanosoma* [15], *Leishmania* [16], *Plasmodium* [17] and *Toxoplasma* [18], among others protozoa [19].

Based on our earlier laboratory experiences where 2-amino-4-arylthiazole derivatives were reported to poses anti-microbial activity [20], it appeared of interest to explore this bioactive system for anti-parasitic activity. Hence, small library around the 2-amino-4-arylthiazole scaffold were developed and evaluated for anti-giardial activity *in vitro*. Further, the 2-D Quantitative Structure Activity Relationship (QSAR) studies were performed using the Hantzsch approach.

2 Experimental procedure

2.1 General

All reagents were obtained from Sigma-Aldrich and used as received. Solvents were dried and distilled before use. Precoated TLC silica gel 60 F254 aluminum sheets from Sigma-Aldrich were used for thin-layer chromatography (0.25 and 0.5 mm layer thickness for analytical and preparative TLC, respectively) and visualized under short (254 nm) and long (366 nm) wavelength UV light or oleum reagent (H_2SO_4:AcOH:H_2O, 1:20:4).

Melting points were measured using an Electrothermal IA 9100 melting point apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded at 400 MHz and 100 MHz, respectively on a Bruker Avance 400 spectrometer at 25°C. The chemical shifts are given in δ (ppm) with the residual solvent peak as an internal. High-resolution mass spectra (HRMS) were measured on a VG Micromass LTD-ZAB-2F Fisons Instrument apparatus operating with electronic impact source at 70 eV. A CEM Discover Focused Microwave Synthesis System operating at 2450 MHz was used at an output of 50 W.

2.2 Synthesis

General procedures

Preparation of compounds 1a-g

A mixture of *p*-substituted acetophenone (8.6 mmol), thiourea 2 (17.2 mmol, 1.3 g) and iodine (8.6 mmol, 2.2 g) was placed in an open vessel containing a Teflon coated stir bar. The vessel was placed in the microwave cavity and subjected to MW irradiation (50 W) at a temperature between 140–150°C for 10 min. After the completion of the reaction, the crude mixture was cooled until 70°C and was triturated, filtered, and washed with Et_2O. The crude product was dissolved in hot water and the pH was adjusted between 11–12 with NH_4OH. The precipitated was filtered and crystallized from EtOH:H_2O (1:4) to obtain the 2-amino-4-aryl-1,3-thiazole (**1a-g**).

Preparation of compounds 2a-f and 9

Sodium acetate (2 mmol) and 2-amino-4-aryl-1,3-thiazole (**1a-g**) (2 mmol) was added to acetic anhydride (10 mL). The resulting solution was stirred at 60°C for 1 h. The reaction was allowed to warm to rt and then were added 25 mL of

cool water. The pH was adjusted between 11–12 with NH_4OH and the formed precipitate was filtered and crystallized from EtOH:H_2O (1:4) to obtain the *N*-(4-aryl-thiazol-2-yl)-acetamide (**2a-f**) and acetic acid 4-(2-acetylamino-thiazol-4-yl)-phenyl ester (**9**).

Preparation of compounds 3a-f and 4a-f

Triethylamine (2 mmol) and 2-amino-4-aryl-1,3-thiazole (**1a-g**) (2 mmol) was added to benzoyl chloride or furan-2-carbonyl chloride (5 mmol). The resulting solution was stirred at 90°C for 1 h. The reaction was allowed to warm to rt and then were added cool water, the formed precipitate was filtered and dissolved in EtOH. To the solution were added cool water and the formed precipitate was filtered and crystallized from EtOH:H_2O (1:4) to obtain the *N*-(4-aryl-thiazol-2-yl)-benzamide (**3a-g**) or the furan-2-carboxylic acid (4-aryl-thiazol-2-yl)-amide (**4a-g**).

Preparation of compounds 5a-e

A solution of Br_2 (2.84 mmol) in glacial acetic acid (2 mL) were added slowly to a solution sulfuric acid (20%, 5 mL) and 2-amino-4-aryl-1,3-thiazole (**1a-h**) (2.84 mmol). The resulting solution was stirred at rt for 18 h. The reaction was then quenched by addition of Et_2O and formed precipitate was filtered. The crude product was dissolved in hot water (25 mL) and the pH was adjusted between 10–11 with NH_4OH. The new precipitated was filtered to obtain the 5-bromo-4-aryl-thiazol-2-ylamine (**5a-e**).

Preparation of compounds 6a-f, 7a-f, 8a-f and 10

To a solution of the N-(4-aryl-thiazol-2-yl)-acetamide (**2a-f**) (1 mmol), N-(4-aryl-thiazol-2-yl)-benzamide (**3a-f**) (1 mmol) or furan-2-carboxylic acid (4-aryl-thiazol-2-yl)-amide (**4a-f**) (1 mmol) in glacial acetic acid (1 mL) at 0°C were added slowly Br_2 (1 mmol) in glacial acetic acid (1 mL). The resulting solution was stirred for 1 h, and then were added cool water, the formed precipitate was filtered and the pH was adjusted between 10–11 with NH_4OH. The precipitated was filtered and crystallized from EtOH:H_2O (1:4) to obtain the *N*-(5-Bromo-4-aryl-thiazol-2-yl)-acetamide (**6a-f**), *N*-(5-Bromo-4- aryl -thiazol-2-yl)-benzamide (**7a-f**), furan-2-carboxylic acid (5-bromo-4- aryl -thiazol-2-yl)-amide (**8a-f**) or acetic acid 4-(2-acetylamino-5-bromo-thiazol-4-yl)-phenyl ester (**10**).

The characterization data of all synthesized compounds are presented as Supporting Information.

2.3 Biological activity

Anti-protozoal assay

Giardia intestinalis IMSS:0696:1 isolate, obtained from an individual with symptomatic giardiasis, was used. Trophozoites were cultured in TYI-S-33 modified medium, supplemented with 10% calf serum, and subcultured twice a week; for the assay, trophozoites were tested in their log phase of growth.

Stock solutions of pure compounds were prepared with dimethyl sulfoxide (DMSO, 20 mg mL^{-1}), from which, by means of two folded serial dilutions with TYI-S-33 modified medium, four final solutions in a range of 1.66–13.33 μg mL^{-1} were obtained. Each solution was inoculated with *G. intestinalis* to achieve an inoculums of 7.5×10^4 trophozoites mL^{-1}. The test included Metronidazole as the drug of reference, a control (culture medium with trophozoites and DMSO), and a blank (culture medium). After 48 h at 37°C, parasites were detached by chilling and 50 μL of each culture tube were subcultured in fresh medium without extracts or drug and incubated for 48 h at 37°C. Cell proliferation was measured with a hemocytometer, and the percentage of trophozoite growth inhibition was calculated by comparison with the controls. The percentage of inhibition calculated for each concentration was transformed into Probit units. The plot of Probit against log concentration was made; the best straight line was determined by regression analysis and the 50% inhibitory concentration (IC_{50}) values were calculated. The experiments were done in duplicate and repeated at least three times.

Acute toxicity assay

The brine shrimp test was performed against freshly hatched nauplii of *Artemia salina*. Briefly, the compounds were dissolved in DMSO and diluted by artificial seawater so that an appropriate range of concentrations was obtained. The final concentration of DMSO was 1% and the number of nauplii was 10. Surviving nauplii were counted after 24 h, and LC_{50} (concentration lethal to 50% of the nauplii) were determined after statistical analysis.

Cytoxicity assay

Vero (African green monkey kidney) cell lines from the American Type Culture Collection (ATCC) were grown as a monolayer in Dulbecco's modified Eagle's medium,

DMEM, supplemented with 5% fetal calf serum and 1% of penicillin-streptomycin mixture (10,000 UI mL^{-1}). The cells were maintained at 37°C in 5% CO_2 and 90% humidity. The cytotoxic activity was assessed using colorimetric MTT 3-(4,5-dimethylthiazol-2-yl)-diphenyltetrazolium bromide) reduction assay. 1.4×10^4 cells/well were incubated in a microtiter 96 well plate with the compounds at 6.25, 12.5, 25 and 50 µg mL^{-1} dissolved in DMSO at a maximum concentration of 0.05%. After 48 h the optical density was measured using a microELISA at 550 nm after dissolving the MTT formazan with DMSO (150 µL). The percentage viability (IC_{50}) was calculated from the curve. All the experiments were repeated three times. The selectivity index (SI) of the compounds is defined as the ratio of cytotoxicity on normal cells to anti-giardial activity (SI = IC_{50} Vero cells/IC_{50} *G. intestinalis*).

2.4 Computational Details

Data set preparation

The QSAR model was derived by multiple linear regressions using the observed biological activity as dependent variables and the chosen descriptors as the independent variables.

The quantum chemical calculations were performed in SPARTAN 06 program [21] and Gaussian 09. [22] The molecular geometries of all compounds were fully optimized using Density Functional Theory (DFT) with a 6–31+ G (d,p) basis set. The water solvent effects are included using the polarizable continuum model (PCM) [23]. The exchange-correlation potential was evaluated using the Beckes´s three parameter hybrid density functional B3LYP [24]. From this calculation the Homo, Lumo, Mulliken, natural, the Hirshfeld, and electrostatic-fit charges; Dipole and higher moments and polarizabilities descriptors were obtained. For each compound, the harmonic-vibrational modes were calculated and no imaginary frequency was observed.

Molecular descriptors selection

The DRAGON Professional 5.5 program was used to calculate 1000 descriptors that encoding the electronic and structural information of the fully optimized structures. All descriptors were auto-scaled because in this way they are less susceptible to the influence of compounds with extreme values [25]. The dataset of 50 compounds was divided into multiple chemically diverse training and test sets with the rational approach hierarchical cluster analyses (HCA) using Ward's algorithm [26]. The splitting procedure led to a training and a validation set consisting of 37 (74%) and 13 (26%) compounds, respectively. The training set was used to generate the QSAR model.

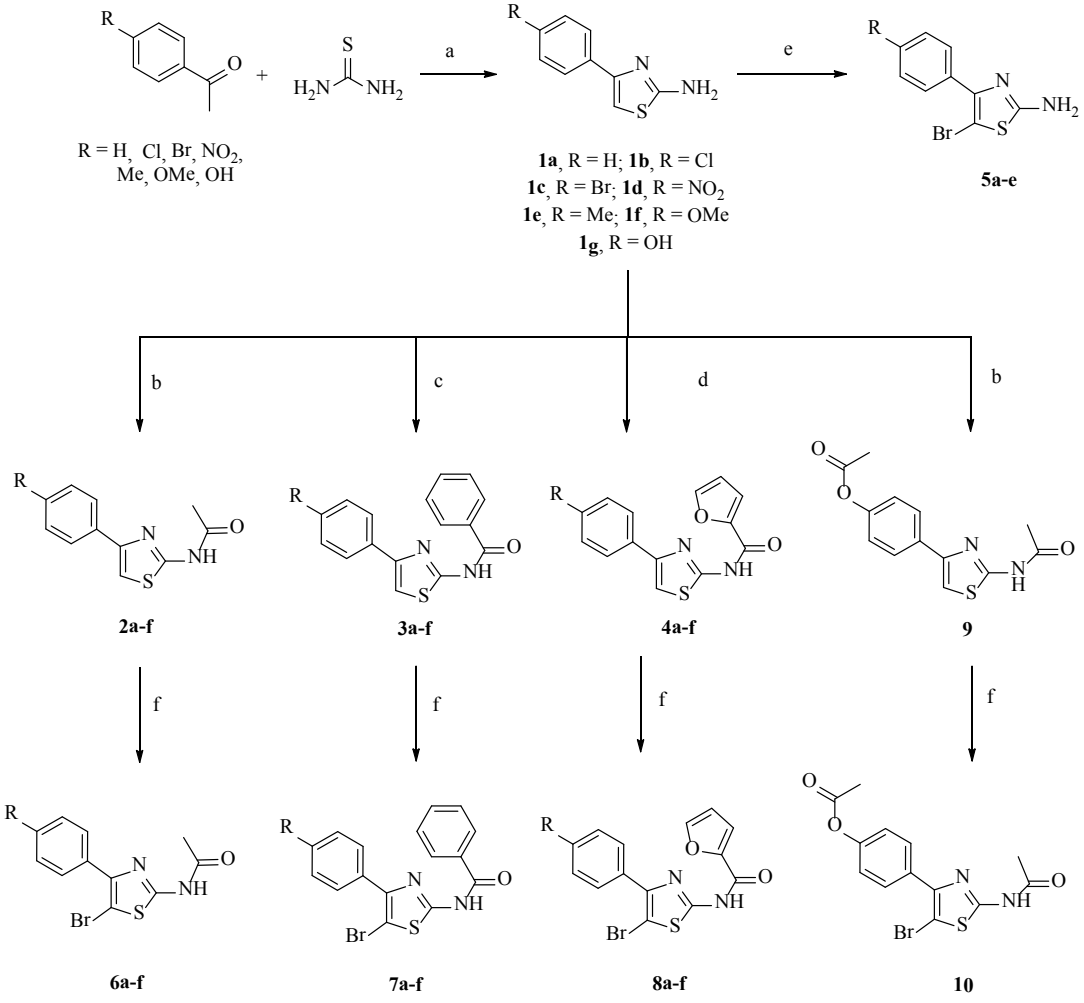
The variable selection for obtain the QSAR were performed using the SYSTAT 12 program with the stepwise linear regression [27]. The stepwise method add to the model only one descriptor at a time, in the order of most significant to lest significant in terms of F-test values with at level of confidence of 0.99. The goodness of the model was tested calculating the regression coefficient (R^2), adjusted square of the correlation coefficient (R^2_{adj}) and the F coefficient. The intercorrelation between descriptors was checked, in order to avoid that the descriptors in the model are highly correlated. The internal predictive ability of the model was assessed by the cross-validate squared correlation coefficient (q^2), which is calculated from the cross-validation method with the leave on out (LOO) scheme [28]. The predictive power of the model was confirmed calculating the correlation coefficient R^2 between the predicted and observed activities for the test set (R^2_{test}). The residuals were calculated in order to confirm the absence of systematic error in the QSAR relationship.

3 Results and discussion

3.1 Synthesis of thiazoles

The thiazole core was synthesized following Hantzsch's modified method reported before and shown in Scheme 1 [29]. The rapid and efficient solvent-free reaction of *p*-substituted acetophenones with thiourea and iodine under microwave irradiation led to 2-amino-4-arylthiazoles (**1a-g**). In order to increase structural diversity a set of 43 derivatives were obtained from seven thiazoles (**1a-g**). Compounds **1a-f** was treated with acetic anhydride, benzoyl chloride and 2-furoyl chloride, respectively, to obtain thiazoles **2a-f**, **3a-f** and **4a-f** (Scheme 1). The reaction of **1g** and acetic anhydride led the diacetyl compound **9**.

The bromine derivatives of **1a-e**, **2a-f**, **3a-f**, **4a-f** and **9** were obtained by the reaction of 2-amino-4-arylthiazoles, *N*-(4-arylthiazol-2-yl)-acetamide, *N*-(4-arylthiazol-2-yl)-benzamide, furan-2-carboxylic acid (4-aryl-thiazol-2-yl)-amide and acetic acid 4-(2-acetylamino-thiazol-4-yl)-phenyl ester, with molecular bromine under acid



Scheme 1: Synthesis of thiazoles derivatives. Reagents and conditions: (a) I_2, neat, MW, 140°C, 10 min.; (b) AcONa, Ac_2O, 60°C, 1 h; (c) Et_3N, BzCl, 90°C, 1 h; (d) Et_3N, FuroylCl, 90°C, 1 h; (e) Br_2, AcOH (glacial), H_2SO_4 (20%), rt. 18 h; (f) Br_2, AcOH (glacial), 0°C, 1 h.

conditions. The synthesis is summarized in Scheme 1. The bromination of **1f** and **1g** did not lead to the brominated compounds.

Compounds **3d**, **4d-e**, **5b-d**, **7a-f** and **9a-f** resulted in new structures and all the spectrometric and spectroscopic data (HRMS, ^1H, and ^{13}C NMR) are in agreement with the expected structures.

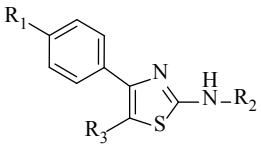
3.2 Biological evaluation of thiazoles

In vitro susceptibility assays were performed using a method previously described [30] and the results are shown in Table 1. The initial structure activity relationship (SAR) studies focused on variation of substituent R_1 (**1a-f**), so the substitution pattern at these positions was carefully selected to confer different electronic environment to the molecules.

As seen in Table 1, compounds **1b**, **1c**, and **1e** having chloro, bromo, and methyl groups at 4' position are relatively active. The unsubstituted phenyl analog (**1a**) was markedly less potent than the remaining compounds in this series.

Knowing that parasiticidal drugs as NIT and other thiazolides[31] possess a benzamide group in the aminothiazole moiety. The amine component of the thiazole core was surveyed for SAR comparison while holding the substitution pattern at R_1 constant. Three classes of *N*-substituted aminothiazoles were obtained through the introduction of acetyl (**2a-f**), benzoyl (**3a-f**), and furoyl (**4a-f**) groups. Introduction of a bigger NH-benzoyl group in compound **1f** resulted in increasing the antiprotozoal activity ten-fold (**3f**); however the presence of this group in compounds **1b** and **1c** led to decrease the bioactivity about 4 and 4.6 times, respectively (compounds **3b** and **3c**). A similar pattern was observed when the acyl or furoyl

Table 1: *In vitro* activity against *Giardia intestinalis* of synthesized 2-amino-4-arylthiazole derivatives.



Comp.	R_1	R_2	R_3	IC_{50} µM	Comp.	R_1	R_2	R_3	IC_{50} µM
1a	H	H	H	108.26	**5a**	H	H	Br	59.97
1b	Cl	H	H	29.29	**5b**	Cl	H	Br	23.21
1c	Br	H	H	16.97	**5c**	Br	H	Br	11.05
1d	NO_2	H	H	35.75	**5d**	NO_2	H	Br	27.82
1e	Me	H	H	20.29	**5e**	Me	H	Br	40.42
1f	OMe	H	H	98.61	**6a**	H	Ac	Br	1.95
1g	OH	H	H	45.88	**6b**	Cl	Ac	Br	0.87
2a	H	Ac	H	50.76	**6c**	Br	Ac	Br	9.31
2b	Cl	Ac	H	142.13	**6d**	NO_2	Ac	Br	9.03
2c	Br	Ac	H	96.21	**6e**	Me	Ac	Br	0.39
2d	NO_2	Ac	H	9.72	**6f**	OMe	Ac	Br	2.51
2e	Me	Ac	H	39.39	**7a**	H	Bz	Br	36.80
2f	OMe	Ac	H	30.77	**7b**	Cl	Bz	Br	11.61
3a	H	Bz	H	44.12	**7c**	Br	Bz	Br	29.05
3b	Cl	Bz	H	119.79	**7d**	NO_2	Bz	Br	16.70
3c	Br	Bz	H	78.42	**7e**	Me	Bz	Br	25.16
3d	NO_2	Bz	H	22.13	**7f**	OMe	Bz	Br	9.97
3e	Me	Bz	H	56.77	**8a**	H	Furoyl	Br	8.68
3f	OMe	Bz	H	8.70	**8b**	Cl	Furoyl	Br	58.83
4a	H	Furoyl	H	16.50	**8c**	Br	Furoyl	Br	42.23
4b	Cl	Furoyl	H	43.64	**8d**	NO_2	Furoyl	Br	7.20
4c	Br	Furoyl	H	38.09	**8e**	Me	Furoyl	Br	31.30
4d	NO_2	Furoyl	H	42.18	**8f**	OMe	Furoyl	Br	1.66
4e	Me	Furoyl	H	17.09	**9**	OAc	Ac	H	56.28
4f	OMe	Furoyl	H	19.41	**10**	OAc	Ac	Br	2.90
MTZ[a]				1.40					

[a] Metronidazol. Positive control

group was introduced in compounds **1b-c**, but the amides of compounds **1d** and **1e** showed a different bioactivity profile. The different patterns in anti-giardial activity of the obtained amides revealed that steric variations are not directly correlated with biological activity.

The nitrothiazole moiety is a structural motif found in antiparasitic drugs such as NIT and Tizoxanide (TIZ). However, it has been shown that nitro group is not crucially instrumental in terms of parasiticidal activity. In addition, the replacement of the potentially harmful nitro group by a bromo moiety does not affect *in vitro* efficacy of these drugs [32]. Considering that bromo thiazolides may represent a valuable alternative to the antiparasitic arsenal [19,33] and the introduction of the heavier halides into aromatic scaffolds produces an affinity gain in protein-ligand complexes [34], we decided to explore the effect of a bromo substitution at position-5 of the thiazole nucleus. The thiazole derivatives with a bromo group attached at carbon-5 of the heterocyclic ring showed an improved anti-giardial activity in contrast with unsubstituted derivatives. Enhanced potency is observed for bromo-analogues bearing an N-acetyl group (**6a-f**). In this series the compounds **6a** (IC_{50} = 1.95 µM) and **6f** (IC_{50} = 2.51 µM) displayed a slightly higher bioactivity against *G. intestinalis* than MTZ (IC_{50} = 1.40 µM). Compounds **6b** (IC_{50} = 0.87 µM) and **6e** (IC_{50} = 0.39 µM) showed the best activity and even the latter was 3.6-times more active than MTZ, the drug of choice for the treatment of giardiasis. The effect of 5-bromo substitution was also observed in compound **10**, derivative of compound **9**, in which the introduction of halogen dramatically improves the anti-giardial activity from 56.28 to 2.90 µM. The bioactivity of these compounds (**6a-b**, **6e-f** and **10**) suggest that the introduction of bromo group into the N-acetamidothiazole core, increase the antiprotozoal activity. Remarkably these compounds, together **8f**, could be considered as hits due their $IC_{50} < 1$ µg mL^{-1}) [35].

According to literature, an anti-parasitic compound must have a selectivity index (SI) ≥ 10 [35], thus the *in vitro* cytotoxicity of the six compounds mentioned above was determined using normal cells (Vero cells line) and preliminarily, their acute toxicity through the brine shrimp bioassay (*Artemia salina*) [36]. Five compounds (**6a-b**, **6e-f**, and **8f**) exhibited selectivity index values higher than **10** (Table 2) when their cytotoxic activity was evaluated; however only three of them showed a similar selectivity when their acute toxicity was measured. It is important emphasize that **6e** was the most potent compound against *G. intestinalis* (0.39 µM), even more than MTZ (Table 1); besides exhibited a selective indexes of 139.0 and 147.6 for cytotoxicity and acute toxicity, respectively.

Table 2: Antigiardial activity, toxicity, and selectivity index of *N*-(5-bromo-4-aryl-thiazol-2-yl)-acetamide derivatives.

Compound	*Giardia intestinalis* IC_{50} µM	Vero Cells IC_{50} µM	Selectivity Index[a]	*Artemia salina* LC_{50} µM	Selectivity Index[b]
6a	1.95	56.7	29.1	31.68	16.2
6b	0.87	45.5	52.3	39.45	45.4
6e	0.39	54.2	139.0	57.55	147.6
6f	2.51	61.3	24.4	9.49	3.8
8f	1.66	52.6	31.7	4.63	2.8
10	2.90	19.2	6.6	0.01	3×10^{-3}

[a]The selectivity index is defined as the ratio of IC_{50} on Vero cells to IC_{50} on *G. intestinalis*.
[b]The selectivity index is defined as the ratio of LC_{50} on *A. salina* to IC_{50} on *G. intestinalis*.

Although, the insertion of halogen atoms on bioactive compounds is predominantly performed to exploit their lipophilic effects, the halogen bonding interactions are responsible for the different conformation of the molecules in the active sites [37]. Thus, in the aim to determine the relationship between the anti-giardial activity and calculated descriptors additional to lipophilicity, a QSAR analysis was performed.

Quantitative Structure–Activity Relationship

The best equation (Eq. 1) for the anti-giardial activity is showed below:

$$\text{Log}(1/C) = 4.7051 + 0.359 \times \text{E2M} - 0.181 \times \text{RDF115m} + 0.162 \times \text{F10} - 0.201 \times \text{MATS6v} + 0.214 \times \text{Hypnotic-80}$$

Where the statistical parameters are:

$$N = 37 \quad R = 0.84 \quad R^2 = 0.70 \quad R^2_{adj} = 0.66 \quad SEE = 0.280 \quad F = 14.81$$
$$p = 0.000 \quad q^2 = 0.50 \quad r^2_{test} = 0.74 \quad R_{max} = 0.37$$

The values of the molecular descriptors for Eq. 1 are shown in Table 3. The value of maximal intercorrelation between the descriptors of the model (R_{max}) showed poor correlation among themselves. The q^2 is not greater than R^2 which means that the model is not over-fitted. External validation was more than 0.7, indicating high predictive ability of the model for external dataset. The p-values of the model indicate a 0.05% probability that the correlations have occurred by chance. Finally, the standard error of the estimate (SEE) was 0.280. It is important to remark that the model contain only five descriptors to describe the activities, following the empirical rule that, in order to minimize the risk of chance correlations, the ratio of number of training set compounds to the number of descriptors in the QSAR/QSPR should be at least 5:1 [38]. The statistical parameters for the coefficients of the descriptors for Eq. 1 are listed in the Table 3. The p-value for all the descriptors shown a probability that the descriptor is there by chance is less than 5%. The standard error of each coefficient is less than the magnitude of the coefficient of the descriptor, and the graph of the residuals (Suppl. Fig. 1) shows the lack of systematic error on the model. The experimental and predicted anti-giardial activity is showed in Figure 1 and Supplementary Table 2.

From Eq. 1, it was found that the most appropriate parameters to describe the antigiardial activity are E2M, RDF115m, F10, MATS6v and Hypnotic-80 [39].

The E2M belongs to Weighted Holistic Invariant Molecular (WHIM) group descriptors that are based on statistical indices calculated on the projections of the atoms along the principal axes of the molecules [40]. The positive sign of the coefficient of this descriptor indicates that a uniform distribution of the atomic mass and polarizability along the second principal axis is significant for the anti-giardial activity. It is important to note that all of the six compounds (**6a-b**, **6e-f**, **8f**, and **10**) considered as hits present a bromine at 5 position of the thiazolic ring and have higher values of E2M descriptor (Table 3). This fact allows relating the assistance of the bromine to get a uniform distribution of the atomic projection along the second principal axis with the observed high anti-giardial activity.

The Hypnotic-80 descriptor, proposed by Ghose–Viswanadhan–Wendoloski, presents a positive coefficient with the second highest value in the equation Eq. 1. This is a descriptor that can take values of 1 or 0. The value 1 means that the bioactive compound presents a calculated log P, calculated molar refractivity, molecular weight, and number of atoms ranged between 0.5–3.9, 43–97, 162–360, and 20–45, respectively [41].

Table 3: Standard error and p values for each descriptor for the best equation.

Descriptor	Coefficient	Standard error	*P* value
E2M	0.359	0.065	0.0000
RDF115m	-0.180	0.045	0.0004
F10[O-O]	0.162	0.050	0.0003
MATS6v	-0.201	0.049	0.0003
Hypnotic-80	0.214	0.053	0.0004

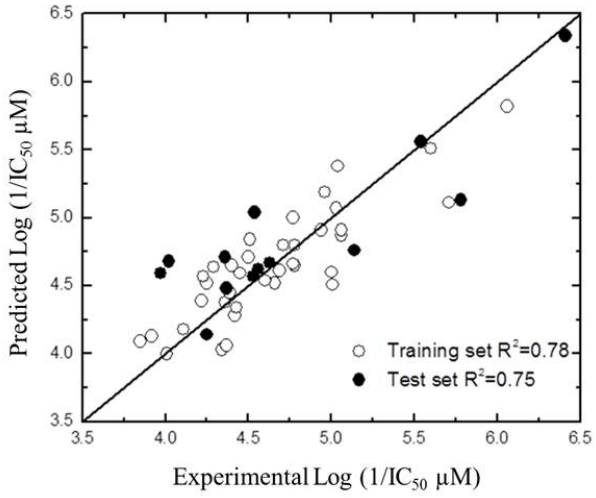


Figure 1: Plot of observed versus predicted Log ($1/IC_{50}$ µM).

The synthetized compounds with anti-giardiasic activity at ≤ 3 µM (**6a-b**, **6e-f**, **8f**, and **10**) have values for the physicochemical properties mentioned above that fall in the ranges stablished by Ghose-Viswanadhan-Wendoloski.

The descriptor F10 is a frequency fingerprint descriptor, and measures the frequency of O-O at topological distance 10 [42]. The positive sign in the coefficient of this descriptor means that the increasing the number of pair of oxygen atoms at this topological distance resulting in an increase of anti-giardial activity. Interesting, only two of the five most active compounds (**8f** and **10**) present this characteristic, unfortunately both compounds showed selective indexes below to 10 (Table 2). Apparently, the presence of the pairs of oxygen atoms at topological distance of 10 improves the anti-giardial activity as well as confers toxicity to compounds.

The last two descriptors present coefficients with negative signs. The RDF is informative descriptors for a molecular structure in a space. The RDF115m encodes a molecule as spheres of radius 11.5 Å and supplies information on the inter-atomic distances in the entire molecule [43]. The presence of the RDF115m descriptor in Eq. 1 suggests the occurrence of some linear dependence between the anti-giardial activity and the 3D molecular distribution of mass, calculated at radius of 11.5 Å from the geometrical centers of each molecule.

Finally, MATS6v is a descriptor belonging to the group 2D autocorrelation between pairs of atoms in the molecule and is also defined to quantify the contribution of the atomic van der Waals volumes of a molecule [44].

The MATS6v descriptor was calculated using the Moran algorithm weighted with the van der Waals volume at topological distance of 6.30. In this case the values for the MATS6v descriptor were mainly negatives, thus the smaller their value the greater activity against *G. intestinalis*. Therefore, the anti-giardial activity is affect by the molecular volume.

4 Conclusions

A set of derivatives of 2-amino-4-arylthiazoles was synthesized. The compounds **6e** (0.39 µM) and **6b** (0.87 µM) displayed the most potent anti-giardial activity. Additionally these thiazoles showed the higher selectivity index (139 and 52 respectively). The developed QSAR model gives us information about the importance of the uniform distribution of the atomic mass and polarizability (E2M), the 3D molecular distribution of mass (RDF115m), the molecular volume (MATS6v), the topological distance of a pairs of oxygen atoms (F10), and the lipophilicity (Hypnotic-80) for the anti-giardial activity of thiazole derivatives. The resulting model displays a good fit with the experimental data. In addition cross-validation coefficients (q^2) and the external validation coefficients (R^2_{test}) are reflecting the predictive power of the regression. Finally, the results may indicate that the anti-giardial activity is enhanced by the bromine at 5 position in thiazole ring but it is necessary to carry out further studies like perform substitutions with other halogens or alkyl groups at that position to prove this hypothesis.

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