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Synthesis, *in vitro* Toxicity, and Antitrypanosomal Activity of Arylated and Diarylated Thiazoles

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Chagas disease is a relevant public health threat that affects over 6 million people worldwide, resulting in devastating social and economic consequences. Moreover, the therapeutic options are limited, highlighting the urgency in searching for novel active antitrypanosomal molecules. Compounds with either thiazole or biaryl units have been described as possessing anti-*Trypanosoma cruzi* activities. Therefore, here, we describe the synthesis of nine arylated and diarylated thiazole derivatives and the evaluation of their *in vitro* toxicity on mammalian cells as well as their anti-*T. cruzi* activity. The compounds were prepared in straightforward synthetic routes, using Hantzsch thiazole synthesis and cross-coupling reactions as key steps. A pyridyl-phenyl-thiazole (PPT) derivative (**4c**) presented 76% of *T. cruzi* growth inhibition in preliminary tests using a fixed concentration of 20 μM. This compound was used as a scaffold for the synthesis of two novel PPT analogs (**4g** and **4h**). Dose-response assays on intracellular forms of *T. cruzi* demonstrated that these three compounds presented high antiparasitic potency (half maximal effective concentration (EC_{50}) values ranging from 1.15 to 2.38 μM) and low toxic profile against L929 cell lines. Hence, these findings highlight the pyridyl-phenyl-thiazole backbone as a novel privileged scaffold in the search for active molecules against *T. cruzi*.

Keywords: thiazole, Suzuki cross-coupling, pyridine, *Trypanosoma cruzi*, antitrypanosomal activity

Introduction

Chagas disease (CD) is a neglected illness that affects more than 6 million people around the world, mainly in underdeveloped areas of Latin America, although also represents a relevant public health problem in non-endemic areas due to population migration.[1-3] CD is the deadliest parasitic disease in Latin America and it has two clinical forms: the acute and the chronic stages. The acute stage starts with the parasite infection, displays patent parasitemia, lasting up to eight weeks, and is usually asymptomatic.[4,5] Due to host immune action, there is a control on the parasite proliferation but no cure, and the infected people move to the chronic phase in which, after years or even decades, about 30-40% of the individuals develop progressive cardiomyopathy and/or digestive abnormalities that may result in death.[4,5] In addition, only the nitroderivatives nifurtimox (NF) and benznidazole (BZ) are currently available for CD treatment,[6,7] and both display important limitations, including (*i*) considerable toxicity; (*ii*) lack of efficacy, especially in the chronic stage of CD; (*iii*) severe side effects, which lead to high drop-out rates (up to 20%); and (*iv*) lack of activity against strains resistant to nitroderivatives.[8,9] In this scenario, urges the development of new chemical entities which could represent alternatives to the treatment of CD.

Several classes of drug candidates have been evaluated against *Trypanosoma cruzi*, including triazoles, quinazolines, arylaminoketones, izoxazoles, and phosphorous compounds.[10,11] Also, thiazole derivatives possessing different substitution patterns have been described as active chemical entities against *T. cruzi*.[10,12,13] In this context, thiazolylhydrazones derived from 1-indanones displayed activity against epimastigote, trypomastigote and amastigote

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forms of the parasite, with a chloroaryl-substituted thiazole presenting the best results.[14] The proposed mechanisms of action for these compounds was the inhibition of squalene epoxidase, which hampers the ergosterol production.[15] Other hydrazone-substituted thiazole derivatives were also active against the trypomastigote form of *T. cruzi,* with aryl groups (phenyl or dichlorophenyl) in position 4 of thiazole and pyridine-substitution on hydrazone moiety leading to the most promising results.[16] Additionally, in another library of thiazolylhydrazones, the presence of a biaryl unit bonded to the thiazole core led to higher antitrypanosomal activity and better selectivity index (SI).[17] Analogously, the presence of naphtyl or biaryl groups in phtalimido-thiazoles enhanced the antitripanossomal activity.[18] It has been also reported that pyridyl groups bonded to either the thiazole core[19] or to the hydrazonic unit of thiazolylhydrazones[20] play a role in the generation of more active and selective derivatives.

Therefore, herein, the synthesis of novel aryl and biarylthiazoles and their *in vitro* toxicity against mammalian host cells and their respective activity against intracellular forms of *T. cruzi* is reported. These thiazoles are obtained in straightforward synthetic routes, exhibit simpler structure compared to previously reported thiazolyl derivatives and attained half maximal effective concentration (EC_{50}) in the order of 1-2 μM when displaying a pyridine-phenyl-thiazole (PPT) backbone.

Experimental

Materials and instruments

The solvents were purchased from Isofar (Duque de Caxias, RJ, Brazil) and other chemicals used in the synthesis were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). All chemicals and solvents were used as received, unless otherwise indicated. Compounds **2a-2b**, and **3a-3d** were synthesized following previously reported procedures.[21,22] For Suzuki coupling reactions, a mixture of 1,4-dioxane, water and ethanol was degassed before use. For Buchwald-Hartwig coupling reaction, toluene was dried over sodium/benzophenone and distilled before use. Compounds **4a-4h, 5** and **6a-6b** were characterized via ^{1}H and ^{13}C nuclear magnetic resonance (NMR) on an Advance III HD 400 MHz spectrometer (Bruker, Billerica, Massachusetts, USA) using $CDCl_3$ or dimethyl sulfoxide-d_6 (DMSO-d_6). High resolution mass spectra (HRMS) were obtained on an microTOF time-of-flight mass spectrometer with electrospray ionization (Bruker Daltonics, Billerica, Massachusetts, USA) using direct infusion of the sample in a solution of acetonitrile, methanol and formic acid (0.1%), in positive mode.

Synthesis

Synthesis of compounds **4a-4h** via Suzuki cross-coupling

For the synthesis of compounds **4a-4h**, an oven-dried screw-capped Schlenk flask was evacuated, back-filled with nitrogen, and loaded with **3** (0.25 mmol), aryl/pyridyl boronic acid (0.25 mmol), $Pd(OAc)_2$ (3.0 mol%, 1.7 mg), PPh_3 (6.0 mol%; 4.0 mg), K_2CO_3 (0.75 mmol, 104 mg) and a mixture of dioxane/water/ethanol 5:1:1 (1.0 mL). The reaction was stirred at 120 °C for 18 h and allowed to cool down to room temperature. The mixture was then diluted with ethyl acetate (10 mL) and washed with water (3 × 5 mL) and sodium hydroxide (1 mol L^{-1}, 3 × 5 mL). After, the organic phase was dried with anhydrous sodium sulfate, filtered, and concentrated under vacuum. Lastly, the crude product was purified by column chromatography on silica gel using hexane/ethyl acetate as mobile phase.

4-(4'-Methoxy-[1,1'-biphenyl]-4-yl)-2-methylthiazole (**4a**)

Yellow solid; yield 23%; mp172-176 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (d, *J* 8.3 Hz, 2H), 7.63 (d, *J* 8.3 Hz, 2H), 7.60 (d, *J* 8.7 Hz, 2H), 7.32 (s, 1H), 6.99 (d, *J* 8.7 Hz, 2H), 3.86 (s, 3H), 2.79 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.9, 159.3, 140.3, 133.2, 132.9, 128.0, 126.9, 126.7, 114.3, 112.0, 55.4, 19.4; HRMS (ESI) *m/z*, calcd. for $C_{17}H_{16}NOS$ $[M + H]^+$: 282.0953; found 282.0940.

4-(4'-Fluoro-(1,1'-biphenyl)-4-yl)-2-methylthiazole) (**4b**)

White solid; yield 65%; mp 167-171 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, *J* 8.6 Hz, 2H), 7.63-7.54 (m, 4H), 7.35 (s, 1H), 7.15 (t, *J* 8.6 Hz, 2H), 2.79 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.0, 163.8, 161.3, 139.7, 136.8, 133.5, 128.6, 127.2, 126.8, 115.8, 112.3, 19.4; HRMS (ESI) *m/z*, calcd. for $C_{16}H_{13}FNS$ $[M + H]^+$: 270.0753; found 270.0738.

2-Methyl-4-(4-(pyridin-4-yl)phenyl)thiazole) (**4c**)

White solid; yield 63%; mp 190-193 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (d, *J* 6.0 Hz, 2H), 8.00 (d, *J* 8.4 Hz, 2H), 7.70 (d, *J* 8.4 Hz, 2H), 7.55 (d, *J* 6.0 Hz, 2H), 7.40 (s, 1H), 2.80 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 154.3, 150.3, 147.8, 137.4, 135.3, 127.3, 127.0, 121.4, 113.1, 29.7, 19.4; HRMS (ESI) *m/z*, calcd. for $C_{15}H_{13}N_2S$ $[M + H]^+$: 253.0799; found 253.0793.

1-(4'-(2-Methylthiazol-4-yl)-[1,1'-biphenyl]-4-yl) ethan-1 one (**4d**)

White solid; yield 55%; mp 200-203 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04 (d, *J* 8.2 Hz, 2H), 7.98 (d, *J* 8.2 Hz, 2H), 7.73 (d, *J* 8.2 Hz, 2H), 7.69 (d, *J* 8.2 Hz,

2H), 7.38 (s, 1H), 2.80 (s, 3H), 2.64 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 197.8, 166.2, 154.5, 145.2, 139.2, 135.9, 134.4, 129.0, 127.6, 127.0, 126.9, 112.8, 26.7, 19.4; HRMS (ESI) *m/z*, calcd. for $C_{18}H_{16}NOS$ $[M + H]^+$: 294.0953; found 294.0966.

4-(2'-Methoxy-[1,1'-biphenyl]-4-yl)-2-methylthiazole (**4e**)

White solid; yield 88%; mp 85-88 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91 (d, *J* 8.2 Hz, 2H), 7.59 (d, *J* 8.2 Hz, 2H), 7.41-7.27 (m, 3H), 7.07-6.95 (m, 2H), 3.82 (s, 3H), 2.78 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 165.8, 156.5, 155.1, 138.2, 133.2, 130.8, 130.3, 129.9, 128.7, 126.0, 120.9, 112.1, 111.3, 55.6, 19.4; HRMS (ESI) *m/z*, calcd. for $C_{17}H_{16}NOS$ $[M + H]^+$: 282.0953; found 282.0949.

4-(4'-Fluoro-[1,1'-biphenyl]-4-yl)thiazol-2-amine (**4f**)

White solid; yield 51%; mp 121-123 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.87 (d, *J* 8.4 Hz, 2H), 7.76-7.70 (m, 2H), 7.65 (d, *J* 8.4 Hz, 2H), 7.28 (t, *J* 8.9 Hz, 2H), 7.06 (s, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.7, 163.5, 161.1, 149.8, 138.1, 136.7, 136.6, 134.4, 128.9, 128.8, 127.1, 126.6, 116.3, 116.1, 102.3; HRMS (ESI) *m/z*, calcd. for $C_{15}H_{12}FN_2S$ $[M + H]^+$: 271.0705; found 271.0699.

2-Methyl-4-(3-(pyridin-4-yl)phenyl)thiazole (**4g**)

Yellow oil; yield 30%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.68 (dd, *J* 4.5, 1.6 Hz, 2H), 8.19 (t, *J* 1.6 Hz, 1H), 7.94-7.90 (m, 1H), 7.61-7.50 (m, 4H), 7.40 (s, 1H), 2.80 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.2, 154.5, 150.2, 148.3, 138.7, 135.5, 129.5, 126.9, 126.5, 125.1, 121.8, 112.9, 19.3; HRMS (ESI) *m/z*, calcd. for $C_{15}H_{13}N_2S$ $[M + H]^+$: 253.0799; found 253.0811.

4-(3-(Pyridin-4-yl)phenyl)thiazol-2-amine (**4h**)

Yellow solid; yield 25%; mp 199-200 ºC; ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.66 (d, *J* 6.0 Hz, 2H), 8.20 (s, 1H), 7.90 (d, *J* 7.7 Hz, 1H), 7.73 (d, *J* 6.0 Hz, 2H), 7.67 (d, *J* 7.7 Hz, 1H), 7.52 (t, *J* 7.7 Hz, 1H), 7.21 (s, 1H), 7.14 (s, 2H); ^{13}C NMR (101 MHz, DMSO-d_6) δ 168.8, 150.7, 149.8, 147.6, 137.9, 136.3, 129.9, 126.8, 126.1, 124.3, 121.7, 102.9; HRMS (ESI) *m/z*, calcd. for $C_{15}H_{12}N_3S$ $[M + H]^+$: 254.0752; found 254.0762.

Synthesis of compound **5** via Buchwald-Hartwig amination

For the synthesis of compound **5**, an oven-dried screw-capped Schlenk flask was evacuated, back-filled with nitrogen, and loaded with **3a** (0.40 mmol, 101 mg), phenoxazine (0.6 mmol, 110.6 mg), $Pd(OAc)_2$ (2.0 mol%, 1.9 mg), $HP(t\text{-}Bu)_3BF_4$ (6.0 mol%, 8.4 mg), sodium *tert*-butoxide (0.60 mmol, 58 mg) and toluene (5.0 mL). The reaction was stirred at 110 °C for 24 h and allowed to cool-down to room temperature. The mixture was then filtered and concentrated under vacuum. The resulting crude product was purified by column chromatography on silica gel using hexane/ethyl acetate as mobile phase.

10-(4-(2-Methylthiazol-4-yl)phenyl)-10*H*-phenoxazine (**5**)

Brown solid; yield 31%; mp 196-198 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (d, *J* 8.3 Hz, 2H), 7.39 (d, *J* 7.0 Hz, 3H), 6.71-6.55 (m, 6H), 5.98 (d, *J* 7.6 Hz, 2H), 2.82 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 166.6, 153.9, 143.9, 138.7, 134.4, 134.3, 131.2, 129.0, 123.2, 121.3, 115.4, 113.3, 113.2, 19.2; HRMS (ESI) *m/z*, calcd. for $C_{22}H_{16}N_2OSNa$ $[M + Na]^+$: 379.0881; found 379.0875.

Synthesis of compounds **6a** and **6b**

An oven-dried screw-capped Schlenk flask was evacuated and loaded with **3b** (0.30 mmol; 76.5 mg), appropriate aldehyde (0.375 mmol) and ethanol (2.5 mL). The reaction was stirred at 80 °C for 4 h and allowed to cool-down to room temperature. The mixture was then filtered and the yellow solids were dried at room temperature.

(*E*)-*N*-(4-(4-Bromophenyl)thiazol-2-yl)-1-(4-methoxyphenyl) methanimine (**6a**)

Yellow solid; yield 33%; mp 144-148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 7.97 (d, *J* 8.4 Hz, 2H), 7.83 (d, *J* 8.2 Hz, 2H), 7.56 (d, *J* 8.2 Hz, 2H), 7.34 (s, 1H), 7.02 (d, *J* 8.4 Hz, 2H), 3.90 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 163.5, 163.2, 152.3, 133.4, 131.9, 131.7, 127.8, 122.1, 114.4, 111.4, 55.5; HRMS (ESI) *m/z*, calcd. for $C_{17}H_{14}BrN_2OS$ $[M + H]^+$: 373.0010; found 373.0004.

(*E*)-4-(((4-(4-Bromophenyl)thiazol-2-yl)imino)methyl)-*N*,*N*-dimethylaniline (**6b**)

Yellow solid; yield 19%; mp 185-190 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.81 (s, 1H), 7.88 (d, *J* 8.8 Hz, 2H), 7.83 (d, *J* 8.4 Hz, 2H), 7.53 (d, *J* 8.4 Hz, 2H), 7.26 (s, 1H), 6.73 (d, *J* 8.8 Hz, 2H), 3.09 (s, 6H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.0, 163.7, 163.7, 153.5, 152.1, 133.7, 132.2, 132.2, 131.8, 131.7, 127.8, 127.8, 122.6, 121.9, 111.5, 111.5, 110.2, 40.1; HRMS (ESI) *m/z*, calcd. for $C_{18}H_{17}BrN_3S$ $[M + H]^+$: 386.0327; found 386.0313.

Compounds preparation for biological tests

For the *in vitro* analysis of the compounds against *T. cruzi*, stock solutions were prepared in dimethyl sulfoxide (DMSO) with the final concentration of the solvent never exceeding 0.6%, which did not exert any toxicity towards mammalian cells.[23] Benznidazole (Bz)

(2-nitroimidazole; Laboratório Farmacêutico do Estado de Pernambuco (LAFEPE), Brazil) was used as reference drug, and aliquots were stored at –20 ºC.

Mammalian cell cultures

For the *in vitro* analysis of compound toxicity against host mammalian cells, monolayers of mouse L929 fibroblasts were cultivated (4×10^3 cell *per* well into 96-well microplates) at 37 °C in RPMI-1640 medium (pH 7.2-7.4) without phenol red (Gibco, Waltham, USA) supplemented with 10% fetal bovine serum (FBS) and 2 mM glutamine (RPMIS), as reported.[24,25] All studies were carried out in strict accordance with the guidelines established by the FIOCRUZ Committee of Ethics for the Use of Animals (CEUA L038-2017).

Parasites and infection of the cell cultures

Tissue culture derived trypomastigotes (Tulahuen strain expressing the *E. coli* β-galactosidase gene) were maintained in L929 cell lines and collected from the supernatant after 96 h of parasite infection, following previously established protocols.[25] Briefly, after 24 h of L929 platting (4×10^3 cell *per* well), the cultures were incubated with trypomastigotes (using a 10:1 ratio) for 24 h at 37 °C. Then, the cell cultures were rinsed to get rid of non-internalized parasites and then further incubated at 37 °C until the release of parasites into the cell culture supernatant.

Cytotoxicity *in vitro* tests

L929 cell cultures were incubated for 96 h at 37° C with different concentrations of each compound (up to 200 µM) diluted in Dulbecco's Modified Eagle Medium-DMEM (without phenol red), their morphology evaluated by light microscopy and then cellular viability determined by the AlamarBlue® assay. For this colorimetric bioassay, 10 µL AlamarBlue® (Invitrogen, Waltham, USA) were added to each well and the plate further incubated for 24 h, after which the absorbance at 570 and 600 nm were measured. As negative controls, AlamarBlue® assay was also performed in the lack of cells, running only DMEM and DMEM containing each tested compound (at higher concentration). The results were expressed as percent difference in reduction between compound treated and vehicle treated cells by following the manufacturer's instructions and the value of EC_{50} corresponds to the concentration that reduces in 50% the cellular viability. Triplicate samples were run in the same plate and at least two assays performed in each analysis.[23]

Anti-*T. cruzi* activity analysis

For the assay on intracellular forms in L929 cell cultures, after 2 h of interaction (ratio of 10 parasites *per* host cell), the non-internalized trypomastigotes were removed by replacing the RPMI culture medium. Then, after 48 h of incubation, the compounds were added to the infected cultures (first at a fixed concentration of 10 and 20 µM and secondly, those that reduced in ≥ 50% the parasite load, were further assessed under a dose-response curve at concentration up to 10 µM, serially diluted 1:2). The cultures were incubated for 96 h at 37 °C/5% CO_2. Bz and DMSO (solvent used for the compounds) were run in parallel as positive and negative controls, respectively. After the elapsed time, 50 µL *per* well of CPRG (chlorophenol red-β-D-galactopyranoside) were added and a reading was done in a spectrophotometer at 570 nm. The activity of the compounds was expressed by the EC_{50}, which represents the concentration capable of inducing a 50% loss of viability in the parasites.[24,25] Triplicate samples were run in the same plate and at least two assays performed in each analysis.

Data analysis and EC_{50} calculation

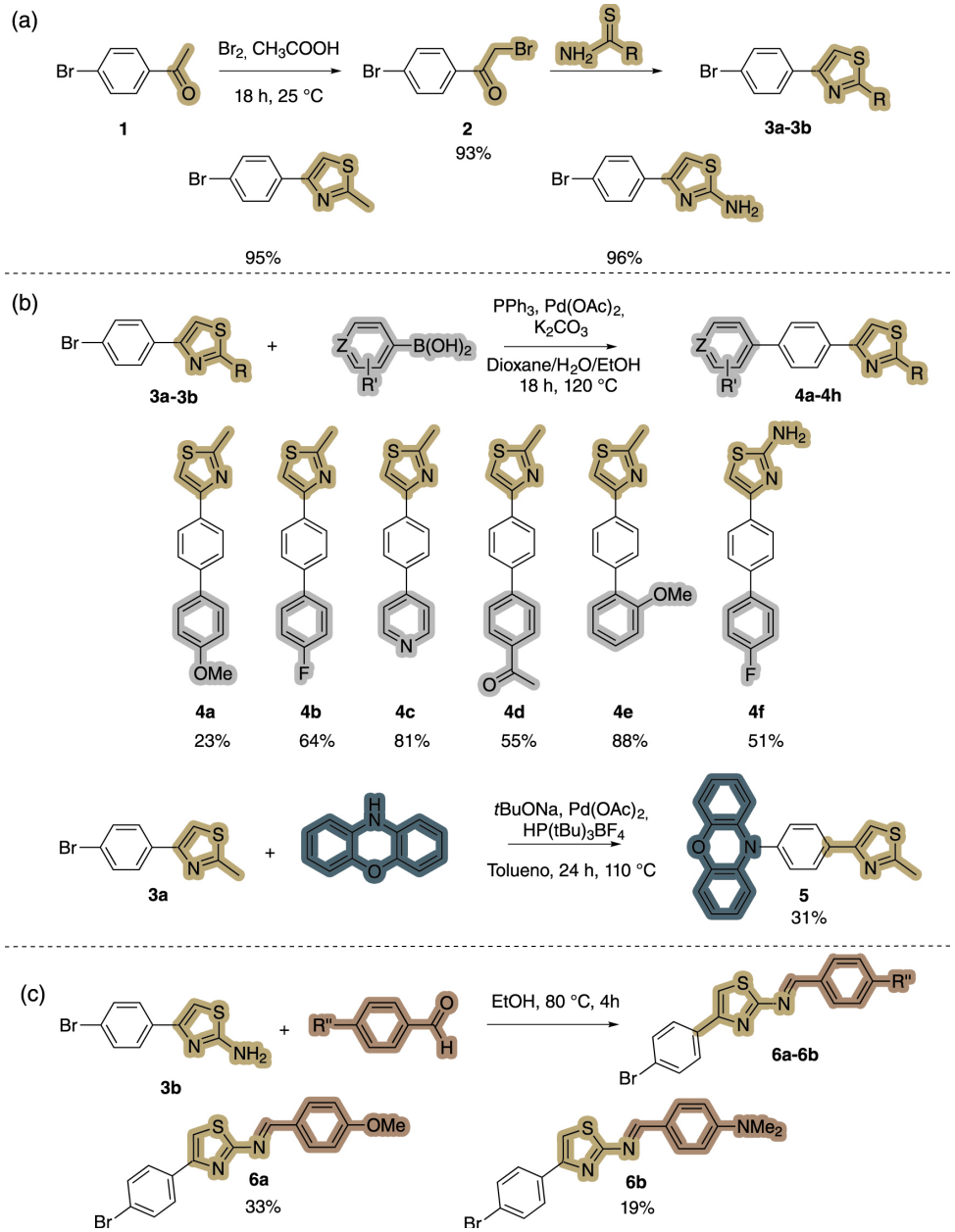
EC_{50} calculation as well as the 95% confidence interval presented in lieu of standard deviation, was performed by Prism Graphpad version 9.1.0[26] using nonlinear regression with the data obtained in at least two assays in triplicate.

Results and Discussion

For the synthesis of the targeted thiazoles, initially, 4-bromoacetophenone (**1**) was submitted to α-bromination followed by cyclization with thiourea or thiacetamide, following a Hantzch thiazole synthesis protocol.[22] These reactions afforded the brominated thiazole intermediates **3a** and **3b** in 95 and 96% yields, respectively, over two steps (Scheme 1a). Taking into account the good antitrypanosomal activity of previously reported biarylated thiazoles,[17,18] **3a** was then submitted to Suzuki cross-couplings using a system based on $Pd(OAc)_2$, PPh_3, K_2CO_3 and different aryl/heteroarylboronic acids. Under these conditions, the desired methyl-substituted biarylthiazoles **4a**-**4e** were obtained in yields ranging from 23 to 88% (Scheme 1b). Analogously, the NH_2-substituted intermediate **3b** was reacted with 4-fluorophenylboronic acid, leading the biarylthiazole **4f** in 51% yield. It is important to highlight that arylboronic acids displaying substitution a pattern similar to previously reported anti-*T. cruzi* active thiazoles were used, including halophenyl,[16] methoxyphenyl,[17] naphtyl,[18] and pyridyl.[19]

In addition to Suzuki arylation, other attempts were made to generate chemical diversity in both position 4 of aryl group and position 2 of thiazole ring. Therefore, **3a** was submitted to Buchwald-Hartwig conditions ($PdO(Ac)_2$, $HP(tBu)_3BF_4$, *t*-BuONa)[27] with phenoxazine leading to compound **5** in 31% yield (Scheme 1b). In respect to functionalization of position 2 of tiazole, the amino-substituted substrate **3b** was reacted with 4-methoxybenzaldehyde and 4-dimethylaminobenzaldehyde affording the target thiazolyl-imines **6a** and **6b** in 33 and 19% yields, respectively (Scheme 1c). In terms of molecular design, compound **5** was conceived considering the good activity of thiazole derivatives with planar groups bonded to aryl-thiazole unit.[18] On the other hand, imines **6a** and **6b** were designed considering the good activity of 2-hydrazolnyl thiazole analogs.[14,15]

The effect of compounds **4a-4f**, **5** and **6a-6b** on *in vitro* cultures of intracellular forms of *T. cruzi* were then evaluated using a fixed concentration (10 or 20 μM) and the results were expressed as percentage of infection reduction (Table 1). Benznidazole (Bz) was tested in the same conditions for sake of comparison. Compounds **4a** and **4b** did not present any reduction in the host cells infection, suggesting negative effect of both methoxy and fluorine substituents. In addition, the acetyl-substituted biarylthiazole **4d** presented only a moderate activity (11%



Scheme 1. Syntheses and structures of thiazoles **4a-4f**, **5** and **6a-6b**.

of infection reduction at 20 μM). The change in methoxy group position (**4a** *versus* **4e**) as well as the replacement of methyl-thiazole unit by a NH_2 analog (**4b** *versus* **4f**) did not afford significant improvement in the activity. In addition, the presence of planar phenoxazine group, as well as imine insertion on position 2 of thiazole did not lead to activity improvement, since compounds **5**, **6a** and **6b** provided only 0.7, 4.1 and 5.1% of reduction in the host cells infection, respectively. On the other hand, the presence of a 4-pyrydyl-group bonded to arylthiazole unit (**4c**) led to a considerable improvement in the trypanocidal activity, since 76% of infection reduction was achieved at 20 μM. This result reinforces the synergy in the combination of thiazole and pyridyl units in the development of anti-trypanosomal molecules.[19,20]

Table 1. *In vitro* effect of the studied compounds against intracellular forms of *Trypanosoma cruzi* (Tulahuen strain transfected with β-galactosidase) after treatment for 96 h at 37 °C using a fixed concentration of 10 or 20 μM

Compound	Reduction on the infection index of the host cells[a] / %
Bz	83 ± 0.4
4a[b]	0 ± 0
4b[c]	0 ± 0
4c[c]	76 ± 2.2
4d[c]	11 ± 1.6
4e[c]	6.0 ± 6.0
4f[c]	7.0 ± 7.0
5[b]	0.7 ± 0.5
6a[b]	4.1 ± 2.4
6b[b]	5.1 ± 1.6

[a]Mean ± standard deviation, two assays in triplicate; [b]10 μM; [c]20 μM. Bz: benznidazole.

The nonspecific cytotoxicity of the arylthiazoles against murine L929 fibroblasts was also evaluated (Table 2). LC_{50} values (lethal concentration at which 50% of the cells are killed) values higher than 200 μM were observed for **4a-4c**, **4e-4f**, **5**, **6a** and **6b**. The relatively higher toxicity observed for compound **4d** (LC_{50} = 175 μM) can be attributed to the presence of an electrophilic carbonyl group. In general, these findings indicated a low toxic potential for the arylthiazole/biarylthyazole scaffold.

Bearing in mind the low toxicity and good activity of compound **4c** at 20 μM, as well as the good antitrypanosomal activity of previously reported pyridyl-thiazoles,[19,20] two novel pyridine-phenyl-thiazole (PPT) analogs were synthetized: (*i*) compound **4g** in which the aryl *meta* substitution pattern was tested, and (*ii*) compound **4h**, also a *meta*-substituted analog, but with a NH_2 replacing the methyl group in position 2 of thiazole (Scheme 2a).

Table 2. *In vitro* toxicity (LC_{50}) of the studied compounds against mammalian host cells (L929 cell lines) after incubation for 96 h at 37 °C

Compound	LC_{50}[a] / μM
Benznidazol	> 200
4a	> 200
4b	> 200
4c	> 200
4d	175 ± 100
4e	> 200
4f	> 200
5	> 200
6a	> 200
6b	> 200

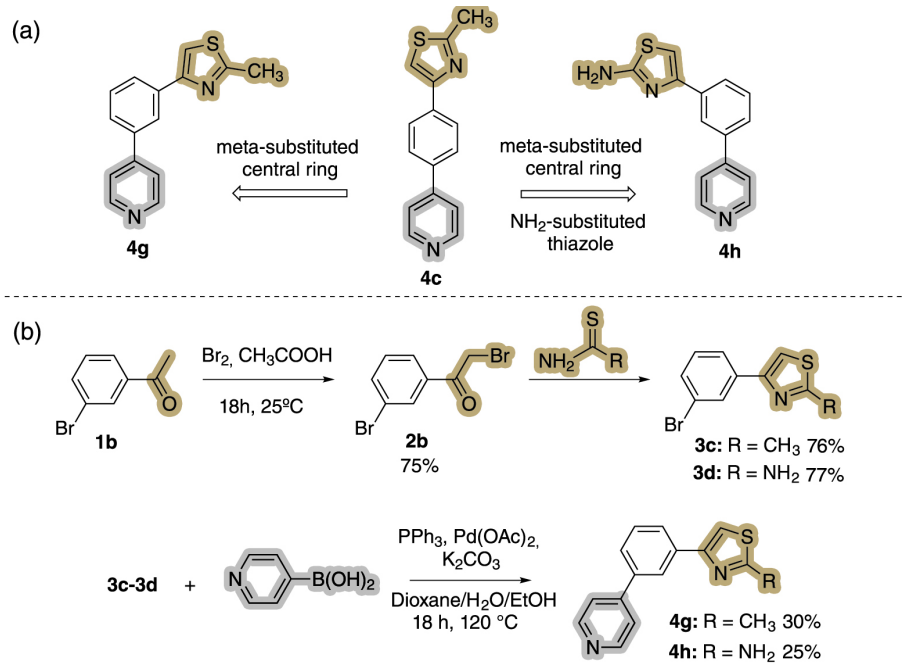
[a]Mean ± standard deviation, two assays in triplicate. LC_{50}: lethal concentration at which 50% of the cells are killed.

A similar straightforward synthetic route based on bromination, Hantzch thiazole synthesis, and Suzuki cross-coupling was employed and afforded **4g** and **4h** in 17 and 15% overall yield, respectively (Scheme 2b).

The dose-response (EC_{50}) of the PPT-based compounds (**4c**, **4g** and **4h)** against intracellular form of *T. cruzi* was further evaluated and their potency values ranged from 1.15 to 2.38 μM, being compound **4c** 2.6-fold more active than Bz (Table 3). These very good activities indicate the high potentiality of the PPT scaffold in the generation of anti-*T. cruzi* compounds. In addition, the two most active compounds (**4c** and **4g**) were also non-toxic to mammalian cells, leading to selectivity index (SI) (> 170 and > 97, respectively) higher than the observed for reference drug benznidazole (SI > 67).

Conclusions

In summary, we reported herein the synthesis of novel imine-, phenoxazine-, byaryl- and arylpyridyl-thiazole derivatives aiming antitrypanosomal activity. The compounds were obtained with reasonable to good yields in concise synthetic routes. All evaluated compounds presented low toxic profile against mammalian host cells. The 4-arylpyryl derivative **4c** which presented the highest antitrypanosomal activity in a fixed concentration was used as a model for the synthesis of other pyridyl analogues (**4g** and **4h**). The dose/response relationship of these three compounds was evaluated and they presented EC_{50} in the range of 1 to 2 μM (lower than benznidazole) besides presenting high selectivity, which are relevant characteristics for the development of a novel anti-*T. cruzi* hit compound.[9] In addition, we described here the pirydyl-phenyl-thiazole (PPT) unit as a new simple and privileged scaffold for antitrypanosomal activity. *In vivo* evaluation of these compounds, as well as the design of novel analogues, are undergoing in our group.



Scheme 2. (a) Design of *meta*-substituted **4c** analogues. (b) Synthetic route for *meta*-substituted compounds **4g** and **4h**.

Table 3. *In vitro* effect (EC_{50}) of the studied compounds against intracellular forms of *T. cruzi* (Tulahuen strain transfected with β-galactosidase) and L929 cell cultures (LC_{50}) after treatment for 96 h at 37 °C, and their corresponding selectivity index (SI)

Compound	EC_{50} / μM	LC_{50} / μM	SI
Bz	3 ± 1	> 200	> 67
4c	1.15 ± 0.070	> 200	> 170
4g	2.06 ± 1.73	> 200	> 97
4h	2.38 ± 1.46	124 ± 1.47	52

Bz: benznidazole. EC_{50}: half maximal effective concentration; LC_{50}: lethal concentration at which 50% of the cells are killed.

Supplementary Information

Supplementary data (NMR spectra, HRMS spectra, and dose-response curves) are available free of charge at http://jbcs.sbq.org.br as PDF file.

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Author Contributions

Kelly L. Figueira was responsible for investigation, writing original draft, data curation, formal analysis; Roberson D. Girão for investigation, formal analysis; Krislayne N. da Costa for investigation, formal analysis; Ana C. R. Barreto for investigation, formal analysis; Maria de Nazaré C. Soeiro for writing-review and editing, visualization, supervision, project administration, funding acquisition; Jones Limberger for conceptualization, writing-review and editing, visualization, supervision, project administration, funding acquisition.

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