Synthesis of New Pyridyl-Phenyl-Thiazoles and *In Vitro* Evaluation of Their Toxicity and Anti-*Trypanosoma Cruzi* Activity

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Introduction

Despite affecting more than one billion vulnerable people, particularly in Latin America, and causing some severe symptoms that can lead to permanent disability in those infected, Neglected Tropical Diseases (NTDs) such as Chagas disease remain unaddressed and without significant investment in the production and development of new drugs that promote cure or reduce side effects during treatment.(ENGELS; ZHOU, 2020; MOLYNEUX *et al.*, 2005)

In Brazil, the only available treatment to this illness is benznidazole (Bz), which has limited efficacy in the chronic phase and is associated with significant toxicity. Adverse effects related to its use are frequently documented in the literature. The most severe of these include allergic dermatitis, bone marrow suppression, and peripheral polyneuropathy, which can sometimes necessitate the discontinuation of treatment.(FERREIRA *et al.*, 2019; GONZAGA *et al.*, 2023; RIBEIRO *et al.*, 2020) This highlights a pressing need for new medications that are more effective and have fewer side effects, ultimately improving patient outcomes and quality of life.

Therefore, thiazole derivatives could serve as essential building blocks in the development of new therapeutic agents to their exceptional ability to undergo modifications resulting in diverse chemical properties and low or no side effects for therapeutic purposes.(DOS SANTOS *et al.*, 2021; HAUPTMANN, 2003; LI, 2013; OBAID *et al.*, 2022)

In recent decades, some studies using a thiazole portion have shown good results in preliminary tests against *T. cruzi*. Some of them are shown in Figure 1. Alvarez and collaborators,(ÁLVAREZ *et al.*, 2015, 2017) identified that the presence of one or more thiazole rings associated with furan shows excellent trypanocidal activity, the best compounds with IC_{50} 1.2 µM and selectivity index greater than 400 against intracellular amastigotes of Sylvio X-10 strain infecting Vero cellsa, but these compounds showed major solubility problems. Cardoso and coworkers,(Cardoso et al., 2014; Cardoso, 2008) identified that the bioisosteric exchange of a thiazolidin-4-one ring for the thiazole heterocycle, with the insertion

of 2-pyridyl at the C3 position of the thiazole ring increases the trypanocidal potential of the compounds, and it is capable of inducing parasite cell death by apoptosis.

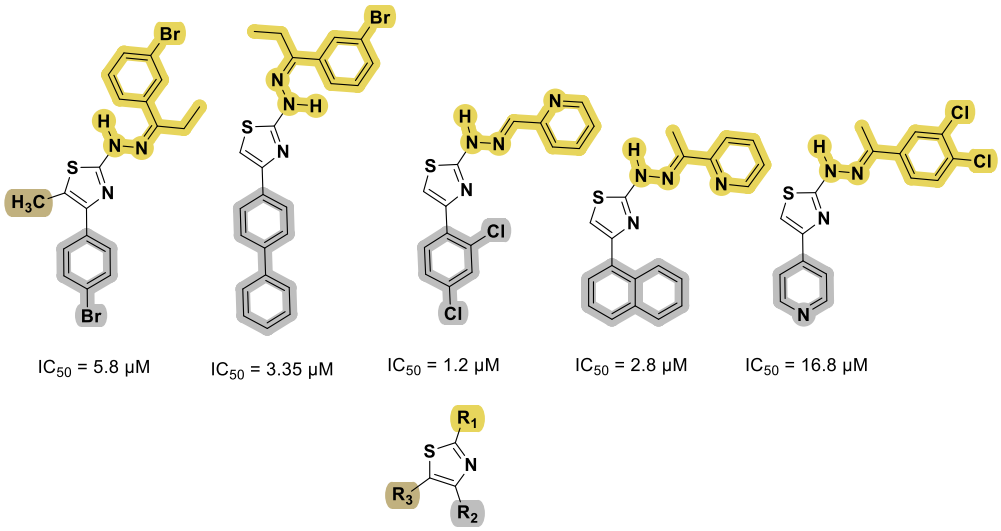


Figure 1. Some thiazoles structures evaluated against *T. cruzi* reported in the literature

In search to obtain potent trypanocidal compounds Gomes and collaborators,(GOMES *et al.*, 2016) found that the presence of a biaryl linked to the thiazole ring leads to a good trypanocidal activity and a better selectivity index. Based on extensive literature reports and additional research conducted by our group,(FIGUEIRA *et al.*, 2024) we developed a series of biarylthiazoles and heteroarylthiazoles. Our study involved testing many substitutions, including imine, methyl and amine groups at the C2 position of the thiazole ring, as well as exploring phenoxazine, aryl groups with different branches, and 4-pyridyl groups at the C4 position of the thiazole phenyl ring, all with the goal of enhancing antitrypanosomal activity.

The results revealed that all compounds exhibited a low toxicity profile against mammalian host cells. Notably, three compounds (Scheme 1a) containing the 4-pyridyl ring in C4 aryl position as well as methyl and amine groups at the C2 position of the thiazole ring demonstrated EC_{50} values in the range of 1 to 2,4 µM with high selectivity index. Consequently, we propose the pyridyl-phenyl-thiazole (PPT) structure as a promising new scaffold for antitrypanosomal agents.

Considering the studies and relevant results presented thus far, this research focused on the functionalization of pyridyl-phenyl-thiazoles with different substitution at C2 position of the thiazole ring, while incorporating the 3-pyridyl or 4-pyridyl group into the *meta* and *para* positions of the thiazole phenyl ring. These modifications were intended to assess their impact on antiparasitic activity and cytotoxicity in preliminary trials. The antiparasitic activity was evaluated using an *in vitro* model targeting intracellular trypomastigotes of the Tulahuen strain.

The new compounds were synthesized through efficient routes involving up to three steps, resulting in compounds with simple structures and low molecular weights. In the biological assays, the most effective compound exhibited an EC_{50} of 1.8 µM and a selectivity index (SI) greater than 111, demonstrating efficacy comparable to benznidazole (EC_{50} = 3 µM, SI > 103). Therefore, it shows promising potential for in vivo studies.

Material and Methods

The compounds **ABr01** and **ABr02** (Scheme 1b) were synthesized by bromination of bromoacetophenone (3-bromoacetophenone or 4-bromoacetophenone) using acetic acid and Br_2 in equimolar amounts, as previously described. (TSEITLER *et al.*, 2020) In the next step, **ABr01** and **ABr02** were reacted with the corresponding thio-compound (pyridine-3-carbothioamide, thioacetamide, or thiourea) in a 1:1 ratio and subjected to the Hantzsch reaction under ethanol reflux for 4 hours, producing intermediates **AT01-AT05** (Scheme 1b). For the synthesis of **AT03-AT05**, containing a basic portion, an additional step involved adding cold water and 1 $mol.L^{-1}$ Na_2CO_3 solution until pH 10 was reached, followed by placing the reaction in an ice bath for 30 minutes. (GODUGU *et al.*, 2021; KASPADY *et al.*, 2009; RAN *et al.*, 2016)

For the synthesis of **PPT04-PPT08** (Scheme 1c), a reaction mixture containing **AT01-AT05** (0.5 mmol), with 4-pyridinyl or 3-pyridinyl boronic acid (0.6 mmol), $Pd(OAc)_2$ (3.0 mol%, 3 mg), PPh_3 (6.0 mol%, 8.0 mg), and K_2CO_3 (1.5 mmol, 208 mg) was employed in a Suzuki coupling reaction.(FIGUEIRA *et al.*, 2024)

All PPTs were characterized via ^{1}H and ^{13}C (APT), by nuclear magnetic resonance (NMR) on an Advance III HD 400 MHz spectrometer (Bruker, Billerica, Massachusetts, USA) using $CDCl_3$ or dimethyl sulfoxide-d6 (DMSO-d6). 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.

To analyze the efficacy against *T. cruzi* and assess mammalian host cytotoxicity, stock solutions of **PPT04-PPT08** (standard from Farmanguinhos, Brazil) were prepared in dimethyl sulfoxide (DMSO). The final concentration of DMSO never exceeded 0.6%, a level that does not induce toxicity. Bz (from Laboratório Farmacêutico do Estado de Pernambuco [LAFEPE], Brazil) served as the trypanocidal reference drug control, and aliquots were stored at −20°C.(ARAUJO-LIMA *et al.*, 2023)

For the assays on intracellular forms in L929 cell cultures, cells were infected with trypomastigotes at a 10:1 parasite-to-host ratio and incubated for 2 hours. Afterward, non-

internalized trypomastigotes were removed by replacing the RPMI medium. Infected cultures were incubated for 48 hours, then treated with compounds at 10 µM. Compounds that reduced parasite load by ≥50% underwent further dose-response evaluation, with concentrations up to 10 µM, diluted 1:2. Cultures were incubated for 96 hours at 37°C with 5% CO_2. Bz and DMSO served as controls. After incubation, 50 µL of CPRG was added, and absorbance was measured at 570 nm. The efficacy was expressed as EC_{50}. Assays were performed in triplicate and repeated twice.(ROMANHA *et al.*, 2010)

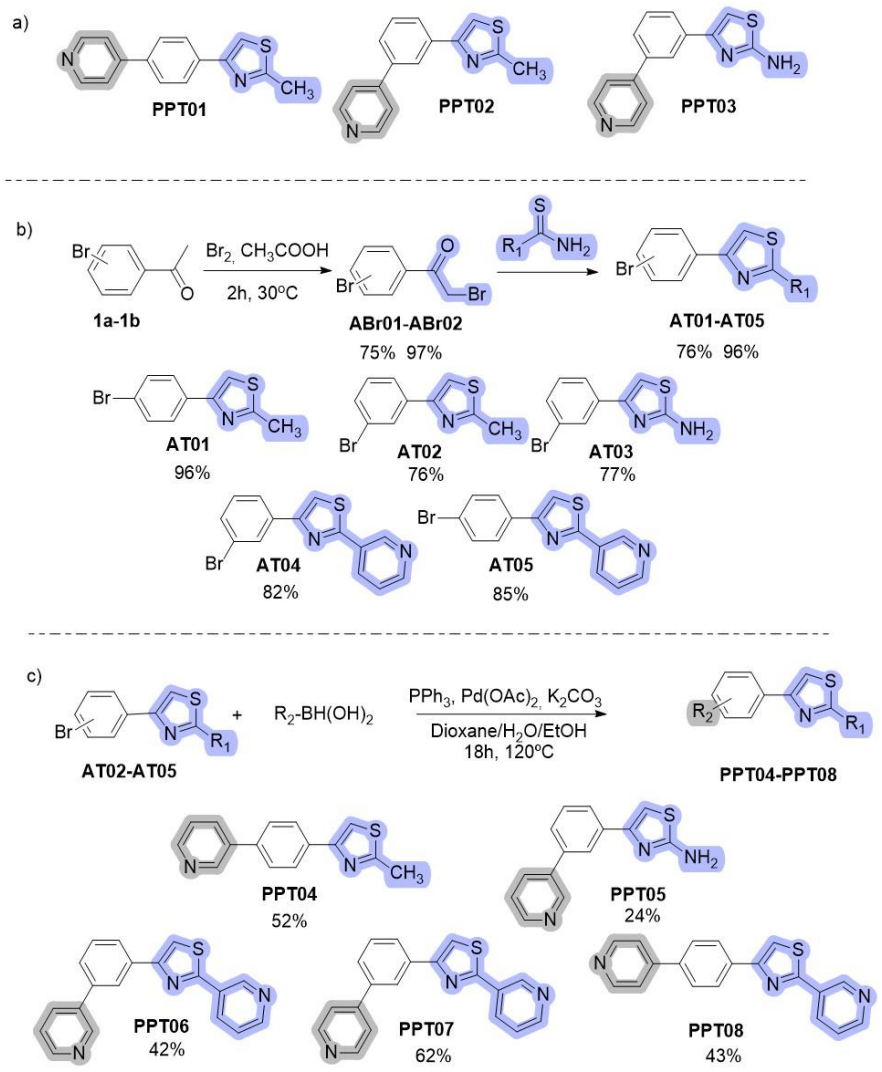
To evaluate cytotoxicity, L929 cell cultures were incubated for 96 hours at 37°C with different concentrations of each compound (up to 200 µM) diluted in DMEM (without phenol red). Cell morphology was assessed using light microscopy, and cell viability was determined using the AlamarBlue® assay. For the assay, 10 µL of AlamarBlue® (Invitrogen, USA) were added to each well, and the plate was incubated for 24 hours before measuring absorbance at 570 and 600 nm. Negative controls included DMEM without cells and DMEM containing each compound at the highest concentration. Results were expressed as the percent difference in reduction between compound-treated and vehicle-treated cells, following the manufacturer's instructions. The EC_{50} value represents the concentration that reduces cell viability by 50%. Assays were performed in triplicate, with at least two independent experiments.(FIGUEIRA *et al.*, 2024; ROMANHA *et al.*, 2010)

Results and Discussion

Following evidence from the literature, cited here previously, certain compounds with a thiazole ring, have shown activity against both *T. cruzi*. In a previous study,(FIGUEIRA *et al.*, 2024) eleven compounds were obtained through diverse substitutions at the C2 position (methyl, amine and imine) of the thiazole ring and diverse aryl and heteroaryl substitution in the C4 position of its phenyl group, yielding three highly potent trypanocidal agents, **PPT01-PPT03** (Scheme 1a.), exhibiting exceptional selectivity indices at a particular fixed concentration (Table 1). These molecules have in common the presence of the 4-pyridyl ring (Scheme 1a). Taking with base these results, new units of pyridyl-phenyl-thiazole were planned.

For the synthesis of the targeted PPTs, initially, 4-bromoacetophenone or 3-bromoacetophenone was submitted to α-bromination(TSEITLER *et al.*, 2020) in 96 and 75% in yields, followed by cyclization with the appropriated thiocompound (pyridine-3-carbothioamide, thiourea or thioacetamide) following a Hantzsch reaction protocol. These reactions culminated the brominated thiazole intermediates **AT01-AT05** in 76 until 96% yields. Taking into account the good antitrypanosomal activity of pyridyl-phenyl-thiazoles previously

reported,(GOMES *et al.*, 2016; FIGUEIRA *et al.*, 2024) **AT01**-**AT05** were submitted to Suzuki cross-couplings using a system based on $Pd(OAc)_2$, PPh_3, K_2CO_3 and 4-pyridinylboronic acid or 3-pyridinylboronic acid, previously reported.(FIGUEIRA *et al.*, 2024) Under these conditions, the desired PPTs (**PPT04-PPT08**) were obtained in yields ranging from 24 to 62% (Scheme 1c).



Scheme 1 a) PPTs with activity against *T. cruzi* reported previously by our group.(FIGUEIRA *et al.*, 2024) b) Syntheses and structures of intermediaries **AT01**-**AT05** c) Syntheses and structures of pyridyl-phenyl-thiazoles **PPT04**-**PPT08**

The anti-*T. cruzi* activity of compounds **PPT04-PPT08** was evaluated against intracellular forms of trypomastigotes (Tulahuen strain transfected with β-galactosidase) on *in vitro* tests (EC_{50}), as well the cytotoxicity on *in vitro* tests L929 cell cultures (LC_{50}) after treatment for 96 h at 37 °C. These data and the selectivity index (SI) of the most effective compounds are shown in Table 1. Benznidazole (Bz) was tested in the same conditions for the sake of comparison. The intermediates with 3-pyridyl substitution in R_1, **AT04** and **AT05**, also

participated of the biological assays, to compare the effect on parasitic activity in the absence of the phenyl-linked pyridine ring in R_2.

Table 1. In vitro effect (EC_{50}) of the studied compounds against intracellular forms of *Trypanosoma 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	1.94 ± 0.46	> 200	>103
AT04	>10	> 200	-
AT05	>10	> 200	-
PPT01[a]	1.15 ± 0.070	> 200	> 170
PPT02[a]	2.06 ± 1.73	> 200	> 97
PPT03[a]	2.38 ± 1.46	124 ± 1.47	52
PPT04	>10	> 200	-
PPT05	>10	> 200	-
PPT06	8.49 ± 4.9	76 ± 3	9
PPT07	4.29 ± 2.17	> 200	>47
PPT08	1.8 ± 1.5	> 200	>111

[a] The synthesize and data evaluate for compounds PPT01-PPT03 were described in a previous work.(FIGUEIRA *et al.*, 2024). The EC_{50} and LC_{50} values were calculated along with their standard deviations (SD).

The standout compound, **PPT08**, exhibited an EC_{50} of 1.8 µM and a selectivity index (SI) > 111, indicating highly selective and potent antitrypanosomal activity. This EC_{50} is lower than that of the reference drug benznidazole (EC_{50} = 1.94 µM, SI > 103) in the same test. These findings strongly suggest that the 4-pyridyl substitution at the R_2 position is critical for optimizing antitrypanosomal potential. The significant reduction in parasite load observed for **PPT08** aligns with previous literature that emphasizes some thiazole rings with 4-pyridyl substitutions in enhancing trypanocidal activity.

PPT07 also displayed promising antitrypanosomal activity, with an EC_{50} of 4.29 µM and SI > 47. Although slightly less potent than **PPT08**, **PPT07** still outperforms the others analogous that exceed 10 µM. This indicates that the 4-pyridyl group in R_2, in diverse orientations, contributes substantially to the efficacy of these derivatives.

Interestingly, **PPT06**, which contained 3-pyridyl substitution, demonstrated low activity (EC_{50} = 8.49 µM), with a much lower selectivity index (SI = 9), indicating limited specificity for *T. cruzi* relative to host cells. This suggests that subtle modifications in the substitution pattern can have pronounced effects on both efficacy and selectivity. The relatively high EC_{50} values for **PPT04** and **PPT05** (>10 µM) further confirm that the 3-pyridyl group does not

provide sufficient trypanocidal potency, emphasizing the importance of the 4-pyridyl group for optimal bioactivity.

The intermediates **AT04** and **AT05**, which also possess 3-pyridyl substitutions at R_1, showed no significant trypanocidal activity (EC_{50} > 10 µM). This lack of activity reinforces the critical role of the R_2 substitution in driving the biological activity of these derivatives. The comparison between these inactive intermediates and the highly active **PPT08** underscores the importance of the molecular framework and substitution pattern for tuning the biological properties of PPTs.

Talking about the cytotoxicity of all compounds was assessed using mammalian L929 cells, revealing that most derivatives exhibit low toxicity, with LC_{50} values exceeding 200 µM. This low toxicity is a crucial advantage, as it indicates that these compounds selectively target the parasite while sparing host cells, which is a key characteristic of any promising drug candidate. In contrast, **PPT06**, despite its moderate antitrypanosomal activity, had a lower LC_{50} of 76 µM, indicating some degree of toxicity to mammalian cells. This lower selectivity index (SI = 9) limits the therapeutic potential of **PPT06** compared to its counterparts. These results suggest that while **PPT06** has room for further optimization, the primary focus should remain on derivatives like **PPT08** that combine both efficacy and low toxicity.

Conclusion

In summary, five novel PPT derivatives were successfully synthesized, thoroughly characterized, and subsequently evaluated through *in vitro* assays. Despite structural modifications aimed at enhancing trypanocidal activity, only **PPT08** demonstrated outstanding efficacy against *Trypanosoma cruzi*, with an impressive EC_{50} of 1.8 µM and a high selectivity index (> 111). These findings underscore the crucial role of the 4-pyridyl substitution in conferring antitrypanosomal activity to this class of compounds. It is also noteworthy that all tested compounds exhibited low toxicity profiles. Consequently, **PPT01**, **PPT02**, and **PPT08** have been selected for further evaluation on *in vivo* assays.

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References

ÁLVAREZ, G.; PERDOMO, C.; CORONEL, C.; AGUILERA, E.; VARELA, J.; APARICIO, G.; ZOLESSI, F. R.; CABRERA, N.; VEGA, C.; ROLÓN, M.; DE ARIAS, A. R.; PÉREZ-MONTFORT, R.; CERECETTO, H.; GONZÁLEZ, M. Multi-anti-parasitic activity of arylidene ketones and thiazolidene hydrazines against Trypanosoma cruzi and Leishmania spp. Molecules, 22(5), 2017.

ÁLVAREZ, G.; VARELA, J.; CRUCES, E.; FERNÁNDEZ, M.; GABAY, M.; LEAL, S. M.; ESCOBAR, P.; SANABRIA, L.; SERNA, E.; TORRES, S.; THIEL, S. J. F.; YALUFF, G.; DE BILBAO, N. I. V.; CERECETTO,

H.; GONZÁLEZ, M. Identification of a new amide-containing thiazole as a drug candidate for treatment of chagas' disease. Antimicrobial Agents and Chemotherapy, 59(3), 1398–1404, 2015.

ARAUJO-LIMA, C. F.; CSRVALHO, R. de C. C.; PERES, R. B.; FIUZA, L. F. de A.; GALVÃO, B. V. D.; CASTELO-BRANCO, F. S.; BASTOS, M. M.; BOECHAT, N.; FELZENSZWALB, I.; SOEIRO, M. D. N. C. Acta Tropica In silico and in vitro assessment of anti-Trypanosoma cruzi efficacy , genotoxicity and pharmacokinetics of pentasubstituted pyrrolic Atorvastatin-aminoquinoline hybrid compounds. [*s. l.*], v. 242, n. February, 2023.

CARDOSO, M. V. de O. Síntese e Avaliação de Tiossemicarbazonas e Tiazolinonas como Inibidores da Protease Cruzaína do Trypanosoma cruzi (TCC). 104, 2008.

CARDOSO, M. V. D. O.; SIQUEIRA, L. R. P. De; SILVA, E. B. Da; COSTA, L. B.; HERNANDES, M. Z.; RABELLO, M. M.; FERREIRA, R. S.; DA CRUZ, L. F.; MAGALHÃES MOREIRA, D. R.; PEREIRA, V. R. A.; DE CASTRO, M. C. A. B.; BERNHARDT, P. V.; LEITE, A. C. L. 2-Pyridyl thiazoles as novel anti-Trypanosoma cruzi agents: Structural design, synthesis and pharmacological evaluation. European Journal of Medicinal Chemistry, 86, 48–59, 2014.

DE MORAES GOMES, P. A. T.; DE OLIVEIRA BARBOSA, M.; FARIAS SANTIAGO, E.; DE OLIVEIRA CARDOSO, M. V.; CAPISTRANO COSTA, N. T.; HERNANDES, M. Z.; MOREIRA, D. R. M.; DA SILVA, A. C.; DOS SANTOS, T. A. R.; PEREIRA, V. R. A.; BRAYNER DOS SANTOSD, F. A.; DO NASCIMENTO PEREIRA, G. A.; FERREIRA, R. S.; LEITE, A. C. L. New 1,3-thiazole derivatives and their biological and ultrastructural effects on Trypanosoma cruzi. European Journal of Medicinal Chemistry, 121, 387–398, 2016. Disponível em: http://dx.doi.org/10.1016/j.ejmech.2016.05.050.

DOS SANTOS, G. C.; MARTINS, L. M.; BREGADIOLLI, B. A.; MORENO, V. F.; DA SILVA-FILHO, L. C.; DA SILVA, B. H. S. T. Heterocyclic compounds as antiviral drugs: Synthesis, structure–activity relationship and traditional applications. Heterocyclic Chemistry. 58, 2226–2260. 2021.

ENGELS, D.; ZHOU, X. N. Neglected tropical diseases: An effective global response to local poverty-related disease priorities. Infectious Diseases of Poverty, 9(1), 1–9, 2020.

FERREIRA, A. M.; DAMASCENO, R. F.; MONTEIRO-JUNIOR, R. S.; OLIVEIRA, I. A. C. de; PRATES, T. E. C.; NUNES, M. C. P.; HAIKAL, D. S. Reações adversas ao benzonidazol no tratamento da Doença de Chagas: revisão sistemática de ensaios clínicos randomizados e controlados. Cadernos Saúde Coletiva, 27(3), 354–362, 2019.

FIGUEIRA, K.; GIRÃO, R.; DA COSTA, K.; BARRETO, A.; SOEIRO, M. de N.; LIMBERGER, J. Synthesis, in vitro Toxicity, and Antitrypanosomal Activity of Arylated and Diarylated Thiazoles. Journal of the Brazilian Chemical Society, 35(1), 1–8, 2024. Disponível em: https://jbcs.sbq.org.br/audiencia_pdf.asp?aid2=11522&nomeArquivo=2023-0069AR.pdf.

GODUGU, K.; SHAIK, S.; MOHINUDDIN PINJARI, M. K.; GUNDALA, T. R.; CHELLAPPA SUBRAMANYAM, D. V.; LOKA, S. S.; DIVI, H.; VEMULA, V.; REDDY NALLAGONDU, C. G. Solid state thiazole-based fluorophores: Promising materials for white organic light emitting devices. Dyes and Pigments, 187, 2020, 109077, 2021. Disponível em: https://doi.org/10.1016/j.dyepig.2020.109077.

GONZAGA, B. M. de S.; FERREIRA, R. R.; COELHO, L. L.; CARVALHO, A. C. C.; GARZONI, L. R.; ARAUJO-JORGE, T. C. Clinical trials for Chagas disease: etiological and pathophysiological treatment. Frontiers in Microbiology, 14, 2023.

HAUPTMANN, S.; THEOPHIL, E.; SPEICHER, A. The Chemistry of Heterocycles: structures, reactions, synthesis, and applications. WILEY-VCH, 646, 2003.

KASPADY, M.; NARAYANASWAMY, V.; RAJU, M.; RAO, G. Synthesis, Antibacterial Activity of 2,4-Disubstituted Oxazoles and Thiazoles as Bioisosteres. Letters in Drug Design & Discovery, 6(1), 21–28, 2009.

LI, J. J. Heterocyclic chemistry in drug discovery. WILEY, 697, 2013.

MOLYNEUX, D. H.; HOTEZ, P. J.; FENWICK, A. “Rapid-impact interventions”: How a policy of integrated control for Africa’s neglected tropical diseases could benefit the poor. PLoS Medicine, 2(11), 1064–1070, 2005.

OBAID, R. J.; ULLAH, E.; NAEEM, N.; AL-ROOQI, M. M.; SADIQ, A.; JASSAS, R. S.; MOUSSA, Z.; AHMED, S. A. Pharmacological significance of nitrogen-containing five and six-membered heterocyclic scaffolds as potent cholinesterase inhibitors for drug discovery. Process Biochemistry, 120, 250–259, 2022. Disponível em: https://doi.org/10.1016/j.procbio.2022.06.009.

RAN, K.; GAO, C.; DENG, H.; LEI, Q.; YOU, X.; WANG, N.; SHI, Y.; LIU, Z.; WEI, W.; PENG, C.; XIONG, L.; XIAO, K.; YU, L. Identification of novel 2-aminothiazole conjugated nitrofuran as antitubercular and antibacterial agents. Bioorganic and Medicinal Chemistry Letters, 26(15), 3669–3674, 2016. Disponível em: http://dx.doi.org/10.1016/j.bmcl.2016.05.088.

RIBEIRO, V.; DIAS, N.; PAIVA, T.; HAGSTRÖM-BEX, L.; NITZ, N.; PRATESI, R.; HECHT, M. Current trends in the pharmacological management of Chagas disease. International Journal for Parasitology: Drugs and Drug Resistance, 12, 7–17, 2020. Disponível em: https://doi.org/10.1016/j.ijpddr.2019.11.004.

ROMANHA, A. J.; DE CASTRO, S. L.; SOEIRO, M. de N. C.; LANNES-VIEIRA, J.; RIBEIRO, I.; TALVANI, A.; BOURDIN, B.; BLUM, B.; OLIVIERI, B.; ZANI, C.; SPADAFORA, C.; CHIARI, E.; CHATELAIN, E.; CHAVES, G.; CALZADA, J. E.; BUSTAMANTE, J. M.; FREITAS-JUNIOR, L. H.; ROMERO, L. I.; BAHIA, M. T.; LOTROWSKA, M.; SOARES, M.; ANDRADE, S. G.; ARMSTRONG, T.; DEGRAVE, W.; ANDRADE, Z. de A. In vitro and in vivo experimental models for drug screening and development for Chagas disease. Memorias do Instituto Oswaldo Cruz, 105(2), 233–238, 2010.

TSEITLER, T. A.; SIDOROVA, L. P.; EMEL’YANOV, V. V.; SAVATEEVA, E. A.; CHUPAKHIN, O. N. Synthesis and Antiglycating Activity of 2-Aminopropylmorpholino-5-Aryl-6H-1,3,4-Thiadiazine Dihydrobromides and 2-Aminopropylmopholino-5-Thienyl-6H-1,3,4-Thiadiazine Dihydrobromides. Pharmaceutical Chemistry Journal, 539(10), 890–894, 2020.