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Design, Synthesis, and Pharmacological Evaluation of Nitrogen-Containing Tetrahydro-2H-1,3,5-thiadiazine-2-thione (THTT) Derivatives as Antileishmanial Agents

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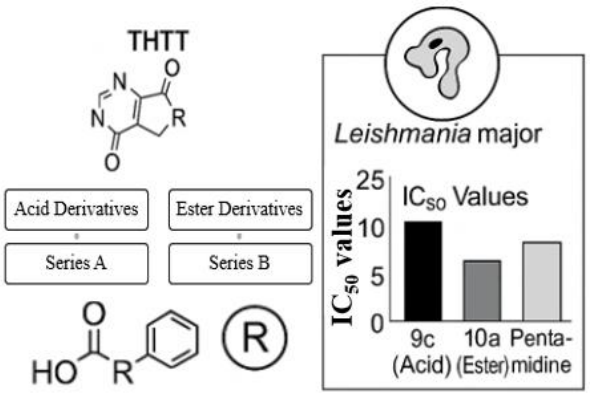
Abstract:
After synthesising four sets of heterocyclic compounds, namely tetrahydro-2H-1,3,5-thiadiazine thione derivatives[1], with yields ranging from fair to exceptional, the in vitro antileishmanial activity against Leishmania major (promastigotes) were assessed. [2] With IC_{50} values ranging from 15.4 to 39.3 µM, most of these drugs demonstrated notable antileishmanial efficacy when contrasted with the reference medicine pentamidine, which has an IC_{50} of 14.5 µM [3]. Investigation of the structure-activity relationship highlighted the significance of the N-3 and N-5 substituents in the leishmanicidal action. It is noteworthy that series B[4] containing the ester variants exhibited 1.5 to 5 times less activity than the acidic versions. Furthermore, the acid and its ester equivalents were compared based on their cytotoxic effects on the mammalian mouse fibroblast 3T3 cells. Due to the newly produced THTT ester derivatives' reduced toxicity and antileishmanial activity, they may serve as promising models for future antileishmanial ester prodrugs.

Graphical Abstract:



Keywords: *Antileishmanial, Cytotoxicity, Ester Derivatives, Leishmania major, Prodrug, Tetrahydro-2H-1,3,5-thiadiazine thione (THTT), Thiadiazine*

I. INTRODUCTION:

We successfully synthesized and evaluated four classes of heterocyclic compounds for their antileishmanial activity against the promastigote stage of *Leishmania major*. These compounds are tetrahydro-2H-1,3,5-thiadiazine thione (THTT) derivatives.[5] This analysis showed that several substances had strong antileishmanial effects, with half-maximal inhibitory concentration (IC_{50}) values ranging from 15.4 to 39.3 µM.[6] Some of the produced compounds seem to be on par with the effectiveness of well-established therapies since this performance stands out when contrasted with pentamidine, the standard reference medication with an IC_{50} value of 14.9 µM.[7]

Further analysis into these compounds' structure-activity relationships underscored the substituents' critical roles at the N-3 and N-5 positions within the heterocyclic ring structure. [8]It was observed that

modifications at these positions significantly influence the leishmanicidal effectiveness of the derivatives. Among the different series synthesized, the ester variants, designated as series B, were found to exhibit diminished antileishmanial activity, ranging from 1.5 to 5 times less potent than their corresponding acidic forms. [9] This reduction in efficacy suggests a nuanced interplay between molecular structure and biological activity, which could guide the optimization of these compounds.

Furthermore, the cytotoxicity of such compounds was measured with the mammalian mouse fibroblast 3T3 cells to compare the safety profiles of the acidic forms versus their ester analogues. The results showed that although the THTT ester derivatives had less antileishmanial activity, they also showed less cytotoxicity than the corresponding acids. Thus, their balance of efficacy and toxicity suggests their developmental potential as ester prodrugs. The ester derivatives may be suitable as safer foundational models for synthesising more potent antileishmanial agents to support ongoing efforts to develop more effective and less toxic treatments for leishmaniasis.

Prodrug engineering aims to harness these specific pharmacological properties to optimize drug performance by minimizing activity at non-target sites, improving pharmacokinetics and reducing toxicity and its adverse effects. The concept of prodrugs is as old as 1876, and since that time, it has been considerably developed. With advancement in drug design and development research, the use of prodrugs has become an integral part of drug delivery systems among the pharmaceuticals used over the years.

This is because prodrugs of active drugs can be used to overcome several limitations of parent compounds (*viz.* poor bioavailability, lack of selectivity, and high systemic toxicity). Often, the chemical modification itself is a simple but clever way of transforming active functional groups into less reactive forms such as esters, amides, carbonates, carbamates, oximes, phosphates, and N-M Mannich bases that will be reduced in the body at a given point.

Alternatively, oseltamivir (Figure 1), a prime example of an extensively implemented prodrug, is a widely known antiviral drug. Hepatic esterases convert the inactive ethyl ester prodrug oseltamivir to its proper form, which is active oseltamivir carboxylate. The key to this process is that the drug can still carry its potent antiviral effects at the sites it should and avoid being predigested to non-target tissues to reduce its chances of side effects.

However, careful planning of the creation of a prodrug is required to ensure that the conversion to the active form of the drug happens precisely and efficiently. Such conversion can be triggered by some events in the body, including changes in pH, specific enzyme interactions, or exposure to specific chemical mediators. These triggers will ensure that the prodrug is inactive until it is activated in the body, avoiding side effects by reducing the interaction with non-target tissues as it is through.

It is an important part of drug discovery and development, which in part involves the creation of prodrugs of drugs with the intent to improve their drug pharmacokinetics. Because prodrugs enhance ADME characteristics of the drug to enhance its solubility, permeability, and half-life, they have the signal to decrease the drug dose. The ongoing advancements in prodrug technology underscore the dynamic nature of drug delivery research and highlight the continual need for innovative approaches to improve drug efficacy, safety, and patient compliance. As this field evolves, it promises to bring forward new solutions to meet modern medicine's increasingly complex demands.

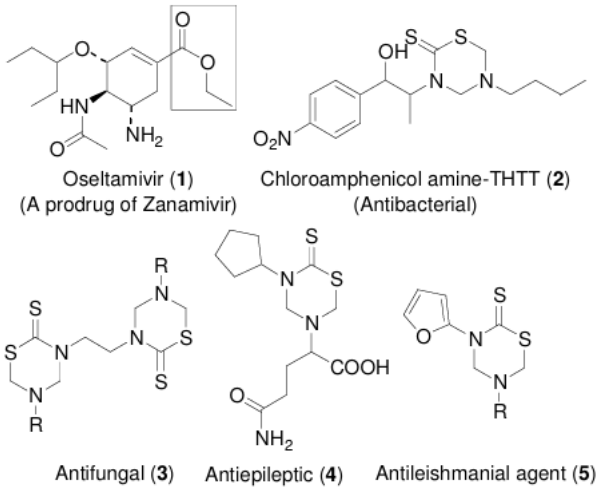


Figure 1. Biologically Active Compounds and Prodrug Candidates Incorporating the THTT Framework[10]

The biological effects of thiadiazine derivatives as prodrugs have been thoroughly investigated. These effects include antibacterial (compound 2, Fig. 1), antifungal (compound 3, Fig. 1), antiepileptic (compound 4, Fig. 1),

antileishmanial (compound 5, Fig. 1), anticancer, and antitubercular potential. Adding a THTT nucleus has also improved the prodrug properties of well-established drugs like ampicillin, cephalexin, and amoxicillin.

II. RESULTS AND DISCUSSION:

We set out to synthesise tetrahydro-2H-1,3,5-thiadiazine thiones and study their conversion into ester analogues to find prodrug candidates for antileishmanial use. This was driven by the increasing use of prodrug strategies in contemporary drug development and the remarkable effectiveness of thiadiazole derivatives.

We describe the synthesis of thiadiazine thione analogues spanning four distinct series, continuing our quest to produce physiologically active molecules and recognising the relevance of the thiadiazine thione core. Specific examples of these compounds are 1,3,5-thiadiazine-2-thione with N-3 and N-5 alkyl groups (series C), 3,5-disubstituted tetrahydro-2H-1,3,5-thiadiazine-6-thione with N-5 alkyl and N-3 carboxylic groups (series A), and variants with carboxylic groups at N-3 and alkyl groups at N-5 (series D). The DESTO test was also used to compare their antileishmanial potential.

At the 10 mmol scale, using known techniques, 3,5-disubstituted tetrahydro-2H-1,3,5-thiadiazine thiones in series A, C, and D were synthesized. A suitable alkylamine or arylalkylamine was first reacted with carbon disulphide and potassium hydroxide to produce dithiocarbamate salts; further treatment with formaldehyde produced the intermediates. With yields ranging from 65 to 86%, the thiadiazine thiones were synthesized by reacting these intermediates with glycine or amines.

Afterwards, in series B, the thiadiazine-2-thiones from series A were effectively transformed into ester forms by reacting with 2.0 equiv. Of thionyl chloride in different alcohols for 2 hours under ice chilling conditions (0 – 4 ºC), excellent isolated yields were achieved at the one mmol scale. Several spectroscopic techniques, such as EI, ~1H NMR, ~13C NMR, DEPT–135, DEPT–90, FT–IR, and HRMS, were used to confirm the structural integrity of all newly produced compounds.

The antileishmanial activity of these compounds was tested *in vitro* against *Leishmania major* using the DESTO assay. The findings were compared to amphotericin B and pentamidine, which were used as reference medicines. This evaluation was done throughout all four series. Most compounds showed strong antileishmanial effects, with IC_{50} values ranging from 15.48 to 39.36 µM, similar to pentamidine's IC50 of 14.95 µM.

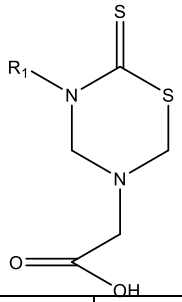
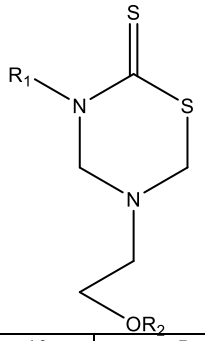
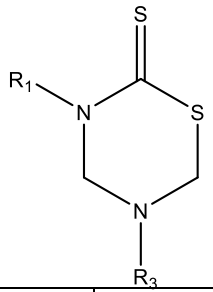
The structure-activity study of all four series shows that the kind of substituent at the N-3 and N-5 positions within the thiadiazine framework mainly determines the antileishmanial efficiency, in contrast to the benchmark medication pentamidine (IC_{50} = 14.95 µM, Table 1). The activity was increased, with IC_{50} values ranging from 15.48 to 136.98 µM, as shown in Table 1, when lipophilic substituents such as alkyl or aryl groups, were added at the N-5 position and hydrophilic groups, particularly carboxylic groups, at the N-3 position (compounds 9a-f, Series A). With an IC50 of 15.48 µM, compound 9e was the most effective, including a veratyl group at N-5 and a carboxylic group at N-3. On the other hand, as shown in Table 1, the activities were reduced when the substituents at these locations were changed, as in compounds 12a-d (Series D). The IC_{50} values ranged from 36.64 to 69.56 µM. For example, compound 9d from Series A demonstrated a significant activity level with an IC_{50} value of 24.56 µM.

In contrast, compound 12d, which had identical functions but different substituent locations, had a lower activity level with an IC_{50} value of 36.64 µM. The same pattern emerged when comparing compounds 9a and 12a and 9c and 12b. Curiously, with IC_{50} values ranging from 21.39 to 46.17 µM, compounds 11a-f from Series C, which include lipophilic groups at both the N-3 and N-5 positions, had activity similar to Series A. This highlights the importance of lipophilic groups in mediating the antileishmanial effects."

Comparing the activity of compounds in Series B, which contain ester groups at the N-3 position (compounds 10a-d), to their parent compounds in Series A, such as compound 9c (IC50 = 39.26 µM), a significant decrease was seen (IC50 values ranging from 36.54 to 202.52 µM, Table 1). Based on the IC_{50} values (202.52, 107.47, and 93.08 µM, respectively) in Table 1, the antileishmanial potential was 1.5 to 4-fold lower in the methyl, ethyl, and benzyl ester versions 10a, 10b, and 10c, respectively. Esters are engineered to activate in biological contexts by reverting to carboxylic forms via esterase activity, fitting the characteristics of a prodrug. Therefore, this decrease in ester activity is expected. [11] [12]

In addition, we used the 3T3 cell line model and the MTT test to assess comparative cytotoxicity, with cycloheximide serving as the control medication, to confirm that the prodrug met its decreased toxicity requirement. We started by looking at the cytotoxicity of compound 9c and its methyl ester counterpart, 10a, which are known for having a vigorous antileishmanial activity (IC50 = 39.26 µM). [13]

"Table 1: Physicochemical parameters and antileishmanial activity of various THTT scaffolds 9-12 (series A-D)[a,b]

Entry	Comp.	R^1	R^2	R^3	MW[c]	Log P[c]	PSA[c]	HBD[c]	HBA[c]	IC50[d]SEM[d] (µM)
										
1	9a	Et	–	–	220.3	0.93 ± 0.56	101.1	1	4	33.14 ± 10.44
2	9b	*i*-Pr	–	–	234.3	1.28 ± 0.56	101.1	1	4	136.98 ± 8.96
3	9c	*n*-Pr	–	–	234.3	1.46 ± 0.56	101.1	1	4	39.26 ± 9.39
4	9d	*n*-Bu	–	–	248.3	1.99 ± 0.56	101.1	1	4	24.56 ± 8.46
5	9e	3,4-(OMe)2.Bzl	–	–	342.4	2.01 ± 0.60	119.6	1	6	15.48 ± 9.64
6	9f	CH(Ph)Me	–	–	296.4	2.62 ± 0.57	101.1	1	4	30.70 ± 11.13
										
7	10a	*n*-Pr	Me	–	248.3	1.92 ± 0.57	90.1	0	4	202.52 ± 8.86
8	10b	*n*-Pr	Et	–	262.3	2.45±0.57	90.1	0	4	107.47 ± 12.20
9	10c	*n*-Pr	Bz	–	324.4	3.41 ± 0.61	90.1	0	4	93.08 ± 9.55
10	10d	*n*-Bu	Et	–	276.4	2.99 ± 0.57	90.1	0	4	36.54 ± 11.21
11	11a	CH(Ph)Me	-	Et	266.4	2.91 ± 0.50	63.8	0	2	46.17 ± 12.39
12	11b	CH(Ph)Me	-	*n*-Pr	280.4	3.44 ± 0.50	63.8	0	2	24.25 ± 7.49
13	11c	CH(Ph)Me	-	*i*-Pr	280.4	3.26 ± 0.51	63.8	0	2	21.39 ± 4.28
14	11d	CH(Ph)Me	-	*n*-Bu	294.4	3.98 ± 0.50	63.8	0	2	21.39 ± 11.21
15	11e	CH(Ph)Me	-	$(CH_2)_2OH$	282.4	2.45 ± 0.55	84.	1	3	32.22 ± 11.68
16	11f	Et	-	Et	190.3	1.23 ± 0.49	63.8	0	2	38.35 ± 16.29
										
17	12a	-	-	Et	220.3	0.63 ± 0.54	101.1	1	4	45.84 ± 10.44
18	12b	-	-	*n*-Pr	234.3	1.16 ± 0.54	101.1	1	4	69.56 ± 13.23
19	12c	-	-	$(CH_2)_2OH$	236.3	0.17 ± 0.59	121.1	2	5	39.36 ± 9.31
20	12d	-	-	*n*-Bu	248.3	1.69 ± 0.54	101.1	1	4	36.64 ± 4.83
21	Amphotericin B	-	-	-	-	-	-	-	-	0.31 ± 0.05
22	Pentamidine	-	-	-	-	-	-	-	-	14.95 ± 0.26

[a] Physicochemical parameter for the prediction of oral bioavailability.
[b] Antileishmanial results represented in IC50 as the mean of triplicate; testing incubation period of 72 h at 22 ± 1 ºC.

[c] MW: molecular weight; Log P: logarithm of the partition coefficient of the compound has been calculated for uncharged molecule; PSA: polar surface area; HBD: hydrogen bond donor; HBA: hydrogen bond acceptor.
[d] SEM = ± standard error of the mean.
[e] IC50 values for std. drugs amphotericin B = 0.31 □M and pentamidine = 14.95 □M."

With an IC_{50} of 202.52 µM, the THTT methyl ester analogue 10a was shown to have decreased antileishmanial activity and low cytotoxicity compared to its acidic precursor. This indicates that these ester derivatives might be good starting points for developing antileishmanial ester prodrugs. [14]

Predictions about the characteristics of agents with biological activity are crucial in the drug development process. Here, we employed Lipinski's rule of five, a method for estimating the oral bioavailability of medicines. Predictions of physical properties for these substances were made using ACD/Labs; [15] Table 1 displays the data. Every compound followed Lipinski's rule to a tee through all four seasons. All the chemicals examined had good oral bioavailability according to parameters including polar surface area (PSA), logP, and counts of hydrogen bond donors and acceptors. [16]

III. CONCLUSION:

The four THTT series have been synthesized and tested for antileishmanial activity in vitro, demonstrating that the 3,5-disubstituted tetrahydro-2h-1,3,5-thiadiazine thione derivatives are potent antileishmanial options. [17] [18] Compound 9e showed significant antileishmanial action at an IC_{50} of 15.48 µM. While the ester analogues showed decreased activity, their negligible cytotoxicity in comparative studies using 3T3 cell lines emphasises their potential as prodrug candidates. The absence of toxicity and significant antileishmanial efficacy suggests these compounds are promising templates for developing new antileishmanial prodrugs. Further in-depth studies are currently underway on these newly synthesized THTT analogues. [19] [20]

CONFLICT OF INTEREST:

The authors declare no conflict of interest.

REFERENCES:

[1]. Arshad, N., Hashim, J., Minhas, M.A., Aslam, J., Ashraf, T., Hamid, Z., Iqbal, T., and Javed, S. New Series of 3,5-Disubstituted Tetrahydro-2H-1,3,5-thiadiazine Thione (THTT) Derivatives: Synthesis and Potent Antileishmanial Activity, *Bioorganic & Medicinal Chemistry Letters*, 2018, 28, 3003-3009. doi:10.1016/j.bmcl.2018.07.045

[2]. Tamanna, Chaoping Fu, Meshil Qadir, Muhammad Ishaq Ali Shah, Amneh Shtaiwi, Rasool Khan, Shafiullah Khan, Thet Thet Htar, Amir Zada, M. A. Lodhi, Muhammad Ateeq, Arif Ali, Mohammad Naeem, Mohammad Ibrahim, Sher Wali Khan. Thiadiazine thione derivatives as anti-leishmanial agents: synthesis, biological evaluation, structure activity relationship, ADMET, molecular docking and molecular dynamics simulation studies. *Journal of Biomolecular Structure & Dynamics*, 07 Aug 2023, pp 1-15.

[3]. Özçelik AB, Ersan S, Ural AU, Özkan S, Ertan M. Synthesis of 3-substituted-5-(4-carboxycyclohexylmethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives as antifibrinolytic and antimicrobial agents. *Drug Res (Stuttg)*. 2011;61(12):669–76. doi:10.1055/S-0031-1296648.

[4]. El-Shorbagi AN. New tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives as potential antimicrobial agents. *Arch Pharm (Weinheim)*. 2000;333(9):281–6. doi:10.1002/1521-4184(20009)333:9<281::AID-ARDP281>3.0.CO;2-E

[5]. Sarwar S, Sarwar N, Ali H, Rafiullah R, Khan R, Ahmad A, et al. Targeting Cutaneous Leishmaniasis with Thiadiazine Thione Derivatives: An In Vivo Study of Its Anti-Inflammatory, Anti-Pyretic, Anti-Nociceptive, and Anti-Sedative Properties. *Adv Cardiovasc Dis*. 2025;13(1):93. doi:10.3390/biomedicines13010093

[6]. Moncorvo FMSU, Leon OLA, Curti C, Kabri Y, Redon S, Torres-Santos EC, Vanelle P. Enhancing Antileishmanial Activity of Amidoxime-Based Compounds Bearing a 4,5-Dihydrofuran Scaffold: In Vitro Screening Against *Leishmania amazonensis*. *Molecules*. 2024;29(22):5469. doi:10.3390/molecules29225469

[7]. Sundar S, Singh V, Agrawal N, Singh OP, Kumar R. Investigation new drugs for the treatment of leishmaniasis. *Expert Opin Investig Drugs*. 2024 Sep 3. doi:10.1080/13543784.2024.2400139.

[8]. Natarajan R, Natarajan G, Basak SC. Quantitative Structure–Activity Relationship (QSAR) Modeling of Chiral CCR2 Antagonists with a Multidimensional Space of Novel Chirality Descriptors. *Molecules*. 2025;30(2):307. doi:10.3390/molecules30020307

[9]. Barros AMC, Guilhon-Simplicio F, Pinto ACS, Oliveira MDL, Figueiredo VC, Sousa LYL, Franco AMR, Lima ÉS. Antileishmanial activity in silico and in vitro of semi-synthetic derivatives obtained from natural products. *Scientia Plena*. 2025;21(1):124501. doi:10.14808/sci.plena.2024.124501.

[10]. El-Shorbagi A. Disubstituted tetrahydro-2H-1,3,5-thiadiazine-2-thiones as lipophilic carriers for glutamine and glutamic acid. *Bull Fac Sci Assiut Univ*. 2000;29(1):1–9. doi:10.21608/BFSA.2000.66128

[11]. Rana NP, Patel SV, Patel A, Ahlawat P, Panjwani D. Recent Advances in Prodrug Approach over Conventional Drug Therapy. *Curr Drug Ther*. 2024;19(4):123–34. doi:10.2174/0115748855303949240827051824

[12]. Yang Z, Li X, Lü B, Wang G, Ma J, Jiang L, et al. One-Step Organic Synthesis of 18β-Glycyrrhetinic Acid–Anthraquinone Ester Products: Exploration of Antibacterial Activity and Structure–Activity Relationship, Toxicity Evaluation in Zebrafish. *Chem Biol Drug Des*. 2024;104(3):267–78. doi:10.1111/cbdd.14631

[13]. Pandya C, Sharma HR, Panicker RR, Samal S, Noble D, Gokulprasanth N, et al. Substituent Effect on Cytotoxicity and Interaction Profile of DNA/BSA With Selected Terpyridine Derivatives of Ester/Ferrocene and Their Fe(III) and Ru(III) Complexes—A Comparative Analysis. *Appl Organomet Chem*. 2025;39(1):e8001. doi:10.1002/aoc.8001

[14]. Román-Álamo L, Avalos-Padilla Y, Bouzón-Arnáiz I, Iglesias V, Fernández-Lajo J, Monteiro JM, et al. Effect of the aggregated protein dye YAT2150 on *Leishmania* parasite viability. *Antimicrob Agents Chemother*. 2024;68(2):e01127-23. doi:10.1128/aac.01127-23

[15]. Xie L, Yu H, Hu Y, Li H, Lou J, Zhou X. Prioritizing oral bioavailability in drug development strategies. *Future Med Chem*. 2024;16(12):2444871. doi:10.1080/17568919.2024.2444871

[16]. Caron G, Jiménez DG, Vallaro M, Vitagliano L, Lopez LL, Apprato G, Ermondi G. Molecular properties, including chameleonicity, as essential tools for designing the next generation of oral beyond rule of five drugs. *ADMET DMPK*. 2024;12(3):255–69. doi:10.5599/admet.2334

[17]. Park HS, Jung HJ, Park HS, Kim HJ, Park Y, Chun P, Chung HY, Moon HR. Design, synthesis, and anti-tyrosinase, anti-melanogenic, and antioxidant activities of novel (Z)-3-benzyl-5-benzylidene-2-thioxothiazolidin-4-one analogs. *Molecules*. 2025; 30(3): 517. doi:10.3390/molecules30030517.

[18]. Li Q, Xu Q, Wang Z, Niu QL, Ni XF, Jing X, et al. Design, synthesis, and biological evaluation of 3,4-dihydroisoquinolin-1(2H)-one derivatives as protein arginine methyltransferase 5 inhibitors for the treatment of non-Hodgkin's lymphoma. *J Med Chem*. 2024 Dec;67(26). doi:10.1021/acs.jmedchem.4c01548

[19]. Tisseur L, Cojean S, Gassama K, Logé C, Pagniez F, Cavé C, et al. Investigating the C2 modulation of the imidazo[1,2-a]pyrazine-based hit compound CTN1122: synthesis, in vitro antileishmanial activity, cytotoxicity and casein kinase 1 inhibition. *ChemMedChem*. 2024;19(12):e202400862. doi:10.1002/cmdc.202400862

[20]. Haddad H, Rodrigues KAF, Othman H, Veras LMC, Rodrigues RRL, Ouahchi I, et al. In vitro antileishmanial activity and in silico molecular modelling studies of novel analogs of Dermaseptins S4 and B2. *Curr Pharm Biotechnol*. 2024;25(9):792–803. doi:10.2174/0113892010296038240427050421