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Alkyl-linked bis-THTT derivatives as potent in vitro trypanocidal agents

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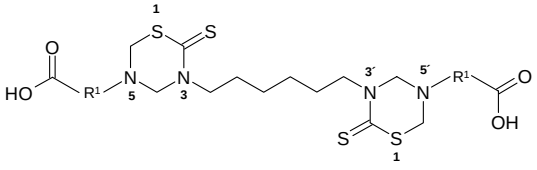
Abstract—The effect of several alkyl-linked bis tetrahydro-(2*H*)-1,3,5-thiadiazine-2-thione (bis-THTT) on *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, and *Plasmodium falciparum* is reported. Most of the compounds exhibited a potent activity against the three parasitic strains but the best in vitro activity profiles were found against *T. b. rhodesiense* with IC_{50} values ranging between 0.3 and 4 μM for the most active compounds.

Infectious diseases such as malaria, leishmaniasis, and human African trypanosomiasis (HAT) kill millions of people each year primarily in the poorest areas of the developing world.[1] The treatment options for such infections are inadequate or non-existent. The increasing costs for drug development and the need for high financial return on investment in the pharmaceutical industry further aggravate this situation.[2] Whilst there is no indication that drug development by pharmaceutical companies for the most neglected diseases will significantly improve in the near future, emerging international initiatives and more effective strategic research will open new horizons in the search for novel, potent, and cost-effective drugs.[3]

Following our ongoing project to develop new antiparasitic agents using the THTT ring as the central core,[4–6] we recently reported the synthesis and antiprotozoal evaluation of alkyl-linked bis-(2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids[7] (Fig. 1). The notable activity against *Trypanosoma cruzi* and *Trichomonas vaginalis* found in some of the evaluated compounds encouraged us to test the effect of the synthesized bis-THTT derivatives on other parasites.

In this paper, we discuss the in vitro activity of compounds belonging to series **I** and **II** against *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, and *Plasmodium falciparum*.



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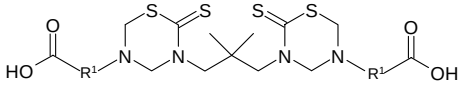

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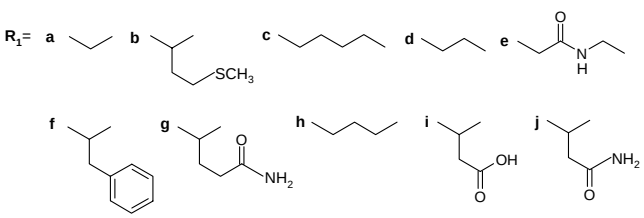

Figure 1. Alkyl-linked bis-(2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids **I** and **II**.

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Alkyl-linked bis-(2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids **I** and **II** (Fig. 1) were synthesized as previously described,[7] starting from commercially available 1,6-diaminohexane and 2,2-dimethyl-1,3-propane followed by addition of CS_2 in a basic medium affording the expected bis-dithiocarbamate salt which underwent further cyclization in the presence of formaldehyde and the corresponding amino acid in a buffer solution of pH 7.8. The structure of the synthesized compounds agreed with reported structural data.[7]

The in vitro anti-protozoal activity of the alkyl-linked bis-THTT was evaluated against *L. donovani*, *T. b rhodesiense STIB900*, and *P. falciparum 3D7*. Briefly, for the *L. donovani* assay, peritoneal exudate macrophages were infected with *L. donovani* amastigotes and exposed to the compounds for 5 days. IC_{50} values were determined by comparing the % infection of the test compounds to uninfected controls.[8] Anti-trypanosomal activity was evaluated by exposing bloodstream forms of the parasite to compound for 72 h[9] and anti-plasmodial activity was determined in a 72-h assay, exposing infected red blood cells to the compounds and evaluating via uptake of tritiated hypoxanthine [H][3].[10] Results are shown in Table 1.

From these results it may be observed that the best activity profiles were found against *T. b. rhodesiense* with IC_{50} values between 0.36 and 4.27 μM, except for **Ig** and **Ij**. For this set of activity, therapeutic indices (TI) were also calculated. Compounds **Ib** (TI-41.2), **Ic** (TI-44.2), **Id** (TI-21.8), and **Ih** (TI-19.9) had the most promising values (Fig. 2). Interestingly, activity against *T. b. rhodesiense* seems to be favored for compounds having linear aminoacidic residues as substituents in position 5 of the THTT ring.

Despite exerting a notable activity against *T. b. rhodesiense*, derivatives from series **II** appeared to be more cytotoxic than the analogs of series **I**. This fact could be related to the nature of the connective alkyl moiety since compounds in both series, with the same aminoacidic residues at N5, had different cytotoxicity values. Nevertheless, at this time we could not provide any conclusive data about this matter and it will be addressed in a further study.

In general, all the compounds were less effective against *L. donovani* and *P. falciparum.*

Derivatives **Ib, Ic** and **Ih** were selected for in vivo evaluation against *T. b. rhodesiense*. However, the compounds were insoluble in the testing medium (10% DMSO/PBS) and hence no conclusions could be drawn. The lack of solubility may be caused by the strong inter-molecular association via hydrogen bonding through the two terminal carboxylic residues. This fact has been previously confirmed by IR analysis through the presence of the νC=O band in the range between 1700 and 1720 cm^{-1} typical for associated aliphatic carboxylic acids and it also affected the structural elucidation of some bis-THTT due to their low solubility in DMSO-d_6 at the concentration required for ^{13}C NMR spectroscopy.[7] Inter-molecular bonding was also observed for previously synthesized mono-THTT derivatives as corroborated by X-ray diffraction experiments and IR spectroscopy[11] with lesser consequences for solubility than the observed for compounds of series **I** and **II**. Due to the high potential of bis-THTT as antiprotozoal agents and in order to disrupt hydrogen bonding to overcome intermolecular association, the synthesis of new THTT acid derivatives is currently under investigation.

For many years activity of THTT derivatives has been closely linked to the formation of highly reactive species (isothiocyanates and dithiocarbamic acid derivatives) originating from hydrolysis of the heterocycle ring in

Table 1. Results of the in vitro assays for compounds of series **I** and **II**

Compound	IC_{50} μM (therapeutic index)[a]			Cytotoxicity IC_{50} KB cells
	L. donovani	*T. b. rhodesiense*	*P. falciparum*	
Ia	7.07 (1.2)	0.73 (11.4)	10.97 (0.8)	8.33
Ib	7.26 (7.3)	2.30 (41.2)	14.19 (3.7)	52.54
Ic	6.74 (2.4)	0.36 (44.4)	16.95 (1.0)	16.09
Id	2.18 (5.6)	0.56 (21.8)	13.14 (0.9)	12.33
Ie	7.43 (0)	0.93 (0)	2.25 (0)	<0.52
If	5.42 (1.1)	0.73 (7.9)	9.13 (0.6)	5.73
Ig	>49 (—)	9.37 (5.8)	11.02 (4.7)	52
Ih	24.33 (3.5)	4.27 (19.9)	43.29 (2)	11.67
Ii	30.24 (0.7)	3.6 (5.9)	23.19 (0.9)	76.29
Ij	19.48 (>30)	5.34 (>10)	39.48 (>2)	>51.72
IIa	6.85 (0.7)	2.88 (1.7)	3.76 (1.3)	5.09
IIc	7.27 (1.1)	2.48 (3.4)	9.04 (0.9)	8.16
IIe	3.53 (0.3)	0.37 (3.2)	16.78 (0.1)	1.17
IIf	5.54 (0.4)	1.04 (2.1)	9.56 (0.2)	2.21
Pentostam	53.06			
Pentamidine		0.011		
Chloroquine			0.021	
Podophyllotoxin				0.0036

[a] Therapeutic index is the ratio of cytotoxicity IC_{50} to the anti-parasite IC_{50}.

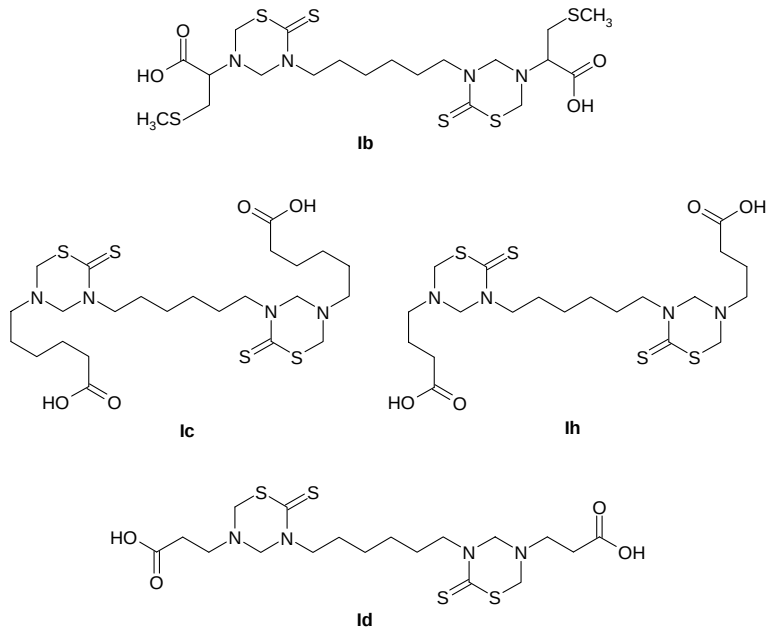

Figure 2. Compounds with the best profile of activity/cytotoxicity against *T. b. rhodesiense*.

protic media;[12] however, more accurate studies have recently questioned this theory.[4,13,14]

Previously we reported the anticancer properties of several THTT derivatives and studied the degradation pathway of one of the most interesting antiprotozoal agents in methanol and methanol/water solution by means of HPLC–MS techniques.[4] Interestingly, none of the major degradation products detected, *N*,*N*′-disubstituted thiourea or the symmetric 3,5-disubstituted-(2*H*)-1,3,5-thiadiazine-2-thione, showed a significant cytotoxicity activity compared to the parent compound. Furthermore, the THTT ring proved to be very stable in methanol rather than in methanol/water solution where its hydrolysis rate increased notably. In a similar study, other authors have confirmed the presence of decomposition products corresponding to the hydrolysis of C4–N5 bond[13] and other six- and five-membered heterocycles.[14]

Since the degradation pathway and decomposition products are greatly influenced by factors such as molecular structure, pH, and type of solvent among others, this issue should be addressed in particular for each family of THTT and not as a general trend. In this way, the possible mechanism of action as well as the elucidation of probable molecular targets would be accomplished with more precision. Nevertheless, the role of the intact THTT ring in biological activity should not be over-estimated, mainly in those cases where reasonable stability of the heterocycle ring could be assured. An earlier study by Aboul-Fadl and El-Shorbagi[15] concluded that the hydrolysis of several alkyl and aralkyl THTT-glycine derivatives could be described in terms of specific base-catalyzed reactions. It also demonstrated that the hydrolysis rate is tuned by the nature of the substituents at N3, rendering compounds with long alkyl side chains moderately stable against chemical and enzymatic degradation. Moreover, THTT derivatives have also shown great stability in simulated gastric fluid (SGF).

Whether bis-THTTs are stable or not in protic solvents will be addressed in further studies. In any case, acting as a drug delivering system for peptides, primary-amine containing drugs, and polyamines or mimicking hydrophobic polyamines, this structure offers great possibilities to the development of new parasitic agents and it is the ongoing theme of research in our group.

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