

# Thiadiazine Derivatives as Antiprotozoal New Drugs

Julieta Coro Bermello<sup>a</sup>, Rolando Pérez Piñero<sup>b</sup>, Lianet Monzote Fidalgo<sup>c</sup>, Hortensia Rodríguez Cabrera<sup>\*a</sup> and Margarita Suárez Navarro<sup>a</sup>

<sup>a</sup>Laboratorio de Síntesis Orgánica, Facultad de Química, Universidad de La Habana 10400-Ciudad Habana, Cuba; <sup>b</sup>National Institute of Nanotechnology, National Research Council (NINT-NRC), and Department of Chemistry, University of Alberta, 11421 Saskatchewan Drive, Edmonton, AB T6G 2M9, Canada; <sup>c</sup>Departamento de Parasitología, Instituto de Medicina Tropical “Pedro Kouri”, Ciudad Habana, Cuba

**Abstract:** The 3,5-disubstituted tetrahydro-2H-1,3,5-thiadiazine-2-thione scaffold have found many applications in recent years. This review is aimed at highlighting the most important aspects about these compounds: synthesis, spectroscopic characterization and antiprotozoan activities. How the chemical nature of N-substituents influences the overall activity / cytotoxicity profile will also be discussed.

**Keywords:** Tetrahydro-2H-1,3,5-thiadiazine-2-thione, antiprotozoan activity.

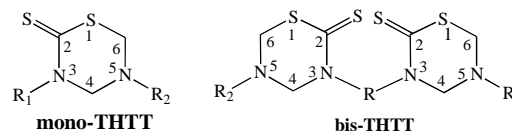
## 1. INTRODUCTION

Trypanosomatids are parasitic protozoa responsible for several tropical diseases of which African sleeping sickness (*Trypanosoma brucei gambiense* and *T. b. rhodesiense*), Chagas disease (*T. cruzi*), and the different forms of leishmaniasis (*Leishmania donovani* and *L. infantum*) are considered the most lethal [1]. At present, the chemotherapies associated with all forms of trypanosomiasis have many deficiencies, among them, poor efficacy, toxicity to humans, high cost, and the emergence of drug-resistant parasitic strains [2]. Unfortunately, because of the low profit margins associated with developing improved therapies for tropical diseases, there is no motivation for the pharmaceutical industry to develop better tropical disease drugs. Accordingly, many agencies, including the Special Program for Research and Training in Tropical Diseases at the World Health Organization (WHO/TDR), numerous academic research groups, various international/national bodies, and philanthropic foundations, have been sponsoring the discovery of new drug candidates for a number of tropical diseases [3].

Recently, the tetrahydro-2H-1,3,5-thiadiazine-2-thione (THTT) derivatives have received special attention due to the pharmacological properties that they display like anti-parasitic agents. Although the 3,5-disubstituted tetrahydro-2H-1,3,5-thiadiazine-2-thione (mono-THTT) scaffold (Fig. 1) was synthesized for the first time in 1848 [4], its accurate structure was established in 1944 [5]. The first publications of these compounds pointed out the significance of the nature of the substituent at N-3 on antimicrobial activity [6], and toxicity of the substituent on N-5 has been shown [7].

Optimum activities had been obtained in compounds bearing lipophilic groups at the N-3 and hydrophilic ones at 5<sup>th</sup> position [8].

The principal studies on the THTT derivatives [9-26] take into account the molecular structure of these molecules to obtain the best activity/cytotoxicity relation. In this context, we found in the bibliography the reports on many compounds with one THTT ring as the central core with a great variety of substituents in N-3 and N-5 position (mono-THTT) [9-18]. In the last years, to enhance the antiprotozoal effect two THTT rings were incorporated into the same molecular structure, connected to each other via their N-3 atom by a linear or branch aliphatic backbone and bearing alkyl or carboxyalkyl residues at N-5 (bis-THTT)[19-26] (Fig. 1).



**Fig. (1).** 3,5-disubstituted tetrahydro-2H-1,3,5-thiadiazine-2-thione scaffold (mono-THTT) and alkyl-linked bis-(2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids (bis-THTT).

Therefore this article will review the bibliography in order to show the development of new antiprotozoal agents using the THTT ring in their structure in the last ten years.

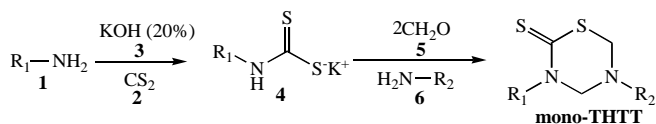
## 2. CHEMISTRY CONSIDERATIONS

### 2.1. 3,5 disubstituted-tetrahydro-2H-1,3,5-thiadiazine-2-thione (Mono-THTT)

The most used procedure to obtain the THTT derivatives is the reaction of the appropriate amine **1** with carbon disulfide **2** and potassium hydroxide **3**, to give the dithiocarba-

\*Address correspondence to this autor at the Laboratorio de Síntesis Orgánica, Facultad de Química, Universidad de La Habana 10400-Ciudad Habana, Cuba; Tel 878-1398; E-mails: hortensia@fq.uh.cu; hortensia\_cu@yahoo.es

mate potassium salt **4** (which was not isolated), followed by cyclocondensation with formaldehyde **5** and the selected amino acids [9-11, 13-15], pseudo peptides [9-11, 13-18], and amines or amino ester (**6**) [12] able to provide the nitrogen at 5th position of the thiadiazine ring in fair to moderate or good yields. In the first step of these synthetic procedures, water is employed as a protic polar solvent in order to stabilize the dithiocarbamate intermediate **4**, in the second step pH 7-8 phosphate buffer was used (Scheme 1) [9-18].

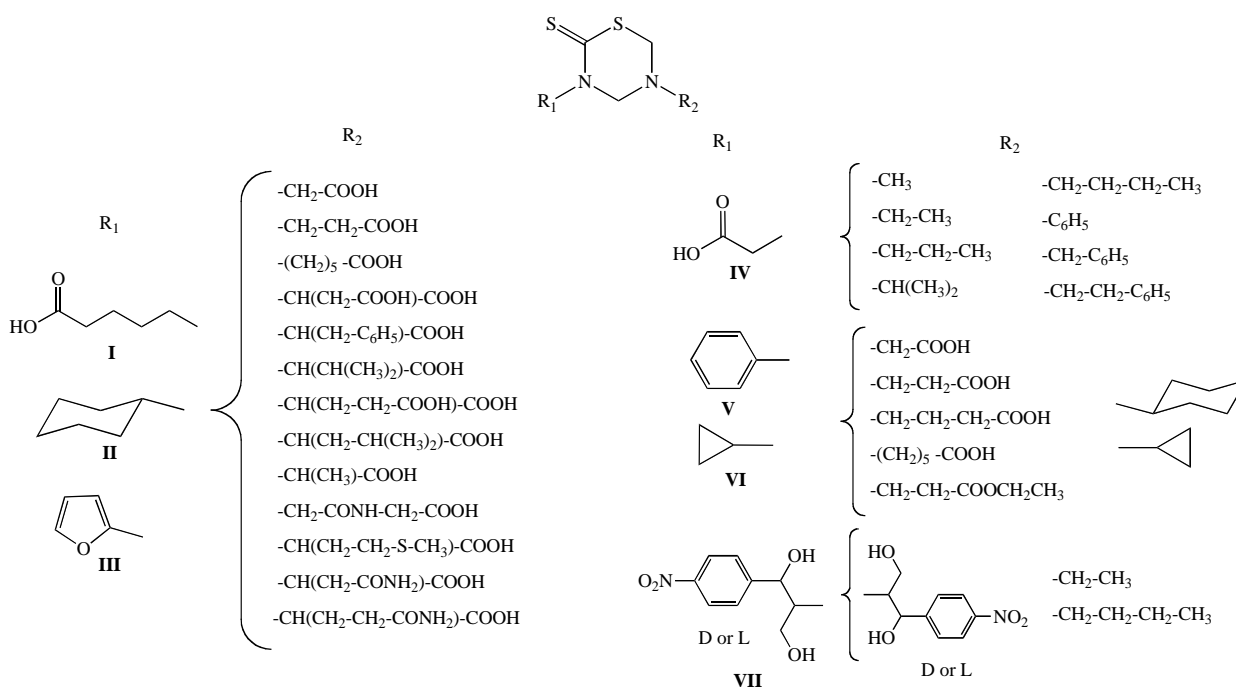


**Scheme 1.** General procedure for obtaining mono THTT.

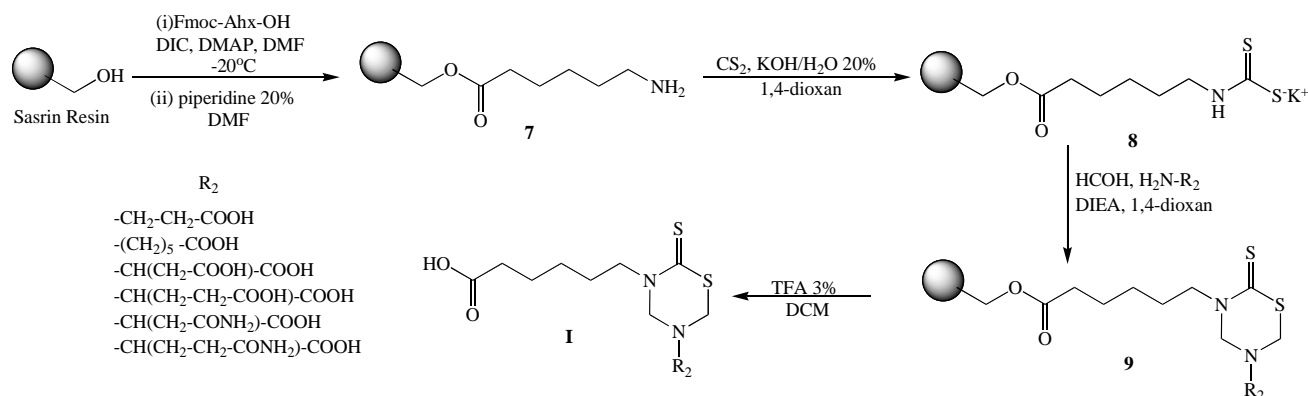
This methodology allowed the synthesis of 78 new mono-THTT derivatives to bunch up in at least seven series

(Fig. 2). The series synthesized differ as to the nature (lipophilic or hydrophilic) of substituent at 3rd position. Some series bear an aromatic (furfuryl [9,11], benzyl [11] and D or L deacetylated chloramphenicol [12]) and alkyl or cycloalkyl (ethyl, butyl, octyl, dodecyl, cyclopropyl and cyclohexyl) [9-11, 13-18] moiety, all of them lipophilic group; for other series the starting amines were hydrophilic (carboxyalkyl) group [9].

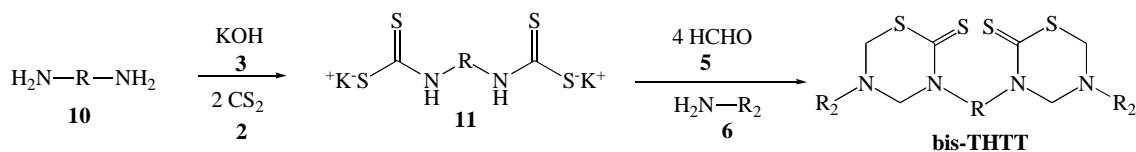
Another synthetic method used to obtain these compounds was the solid phase synthesis of 3-(5-carboxypentyl)-5-substituted tetrahydro-2H-1,3,5-thiadiazin-2-thione derivatives (**Serie I**). 6-Amino-*n*-hexanoic acid was attached via its C-terminal to hydroxymethyl polystyrene using a 'SASRIN' linker. The bound amino acid was transformed into the corresponding dithiocarbamate **8** followed by cyclization in the presence of formaldehyde and the corresponding free amino acids to afford 3-(5'-carboxypentyl)-5-substituted tetrahydro-2H-1,3,5-thiadiazin-2-thiones (**9**). The final products **I** were cleaved from the resin and obtained in moderate yields



**Fig. (2).** Representative mono-THTT synthesized.



**Scheme 2.** Solid Phase Synthesis of mono-THTT derivatives (**I**).



**Scheme 3.** General procedure for obtaining bis-THTT.

due to low solubility of the corresponding free amino acids in 1,4 dioxan (Scheme 2).

Although its use is limited by two factors: the possibility for the starting amines to be properly functionalized for the efficient coupling to the resin and the low solubility of the amino acids in the solvent employed. The development of this methodology would allow the generation of combinatorial library for THTT compounds.

### 2.2. Alkyl-linked bis-(2-thioxo-[1,3,5] thiadiazinan-3-yl) carboxylic acids (bis-THTT)

The synthetic methods for obtaining bis-THTT have been much less reported in the bibliography than the synthetic route for mono-THTT [19-22]. The general procedure is very similar to the one used for mono-THTT. The bis-THTTs were always obtained using diamines **10** and the amounts of all reagents were duplicated. In the first step the diamine reacts with carbon disulfide **2** in the presence of potassium hydroxide **3** to obtain the expected bisdithiocarbamate salt **11**. The addition of formaldehyde **5** and the corresponding amine or aminoacid (**6**) to **11** resulted in the cyclocondensation in a slightly alkaline medium (phosphate buffer, pH 7-8) to generate, after treatment with HCl 15%, the desired bis-THTT (Scheme 3) [19-21].

This procedure allowed the synthesis of 43 new bis-THTT derivatives to bunch up in at least four series (Fig. 3). All compounds were obtained in moderate to good yields except those of series **IX**, probably due to the use of a bulky

diamine (2,2-dimethyl-1,3-propanediamine) and the resulting steric hindrance at the cyclization stage.

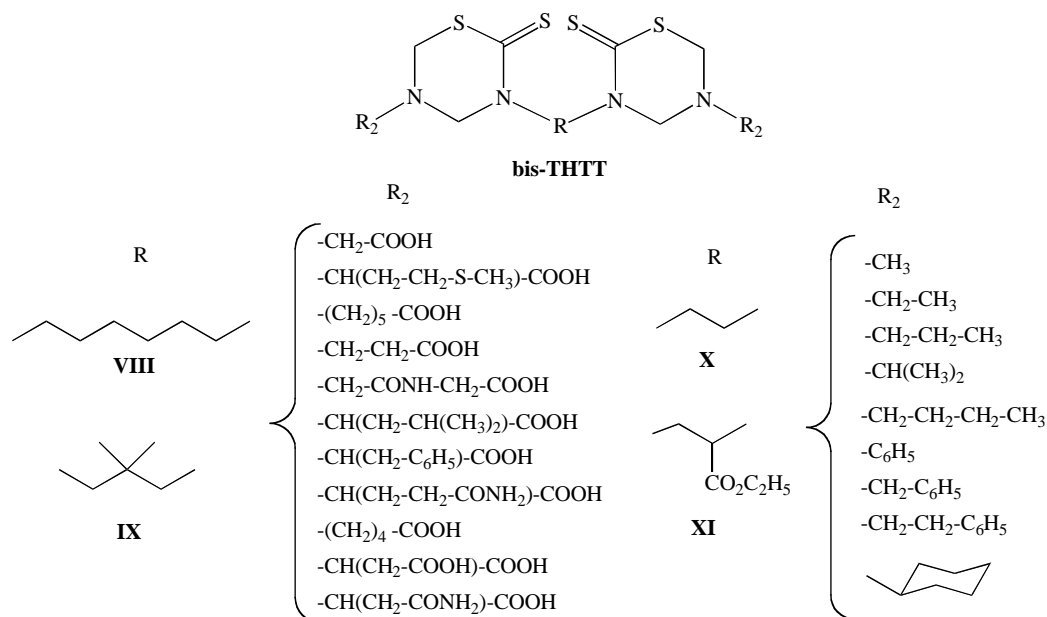
Recently, our group explored the feasibility of synthesis of new bis-THTT using more complex polyamines as linkage other than the initially reported diamines [22]. The N4-benzyl polyamine **14** was previously synthesized according to a methodology reported by O'Sullivan *et al.* [23] via a protection-deprotection strategy using ethyl trifluoroacetate as the selective protective group for primary amines in the presence of secondary amines. The synthetic route leading to derivatives of serie **XII** from the benzylated spermidine was similar to the one described above (Scheme 4).

The spermidyl linked bis-THTT series (**XII**) were obtained as solids in moderate yields.

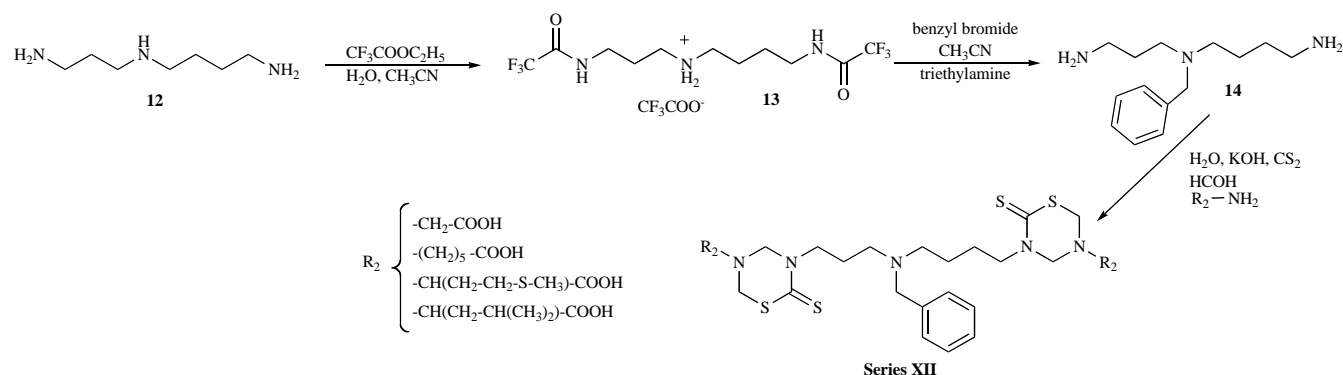
The experimental procedure used to obtain the THTT derivatives (mono and bis-THTT) is simple, allowing a wealth of molecular diversity depending on the nature of groups attached to both nitrogens (N-3 and N-5) of the heterocycle.

### 2.3. Proposed Reaction Mechanism for the Generation of the Thiadiazine Ring

According to the authors, the formation of the thiadiazinane ring is performed via a one pot domino reaction between the pre-formed DTC, formaldehyde and the amino acid component. Despite being considered a multi-component reaction, the reactants are added in a stepwise

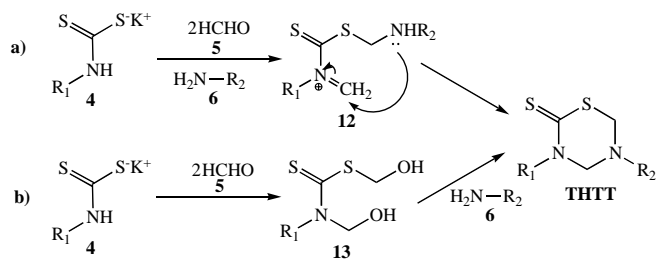


**Fig. (3).** General structures of bis-THTT synthesized.



**Scheme 4.** Synthetic route leading to bis-THTT (**XII**) from the benzylated spermidine.

fashion. Undoubtedly, one of the least explored aspects regarding the synthesis of THTT has been the study of the reaction pathway from the corresponding DTC. In one approach (Scheme 5a) the preformed DTC **4** is allowed to react simultaneously with formaldehyde and the corresponding amine to produce [substituted(aminomethyl)methanethionyl]methylidiazanium **12** [20]. This species possesses two different reactive centers in the same molecular backbone, a protonated imine and a thiosulfanyl methylamino group. The intramolecular addition of the secondary amino group to the carbon atom of the methylenide moiety leads to the THTT ring. The formation of the THTT ring via a {[hydroxymethyl (substituted) carbamothioyl] sulfanyl}methanol intermediate **13** [13] was proposed (Scheme 5b). This process involves in situ generation of **13** from the corresponding DTC **4** and formaldehyde (**5**) followed by condensation with a primary amine. The isolation and characterization of an analogue of **13**, via a crystallization process induced by the presence of KOH, was reported [13].



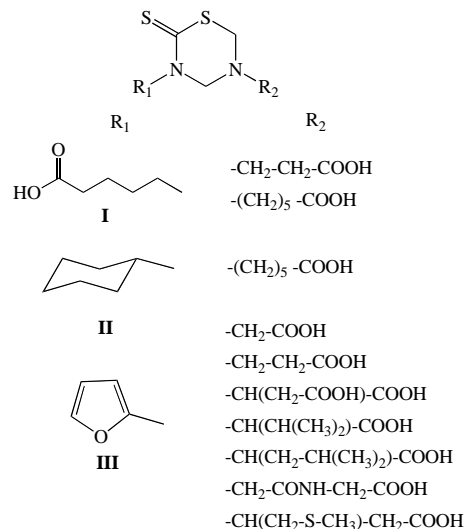
**Scheme 5.** Proposed reaction mechanism for the generation of the THTT ring.

Recently, we have reported a preliminary DFT study aimed at predicting the probable cyclization mechanism of the thiadiazinane-2-thione from an intermediate of type **13** [24]. Based on experimental facts and DFT studies, a probable cyclization route to the THTT ring from the corresponding {[hydroxymethyl(substituted) carbamothioyl] sulfanyl}methanol intermediate **13** in aqueous medium is proposed. Notably, water not only contributes to the reaction mechanism, but also plays an active role in the reaction mechanism. As far as we know, this is the first computational study on the formation of the thiadiazinane-2-thione ring.

### 3. STRUCTURAL CHARACTERIZATION OF MONO AND BIS-THTT DERIVATIVES

Structures of all the compounds were established on the basis of spectroscopic data and microanalysis. IR spectroscopy of the compounds showed absorption bands ranging from 3060 to 3450  $\text{cm}^{-1}$  for (OH and NH), 1690 to 1720  $\text{cm}^{-1}$  for (C=O) and from 1445 to 1508  $\text{cm}^{-1}$  for (C=S). Although, numerous studies have been published on the synthesis and characterization of these compounds [9-14], it was only in 2001 we published the first exhaustive structural characterization of mono-THTT derivatives [15-17]. These studies were considered an important structural data base to facilitate the characterization of novel compounds with the THTT ring in its structure.

The Nuclear Magnetic Resonance study deals with the complete  $^1\text{H}$  and  $^{13}\text{C}$  NMR assignments of a series of differently substituted THTT endowed with different organic addends on both heterocyclic nitrogen atoms (Fig. 4). The  $^1\text{H}$  NMR spectra at 300 MHz of THTT derivatives show, in general, two singlets corresponding to ring protons H-4 and H-6 around  $\delta$  4.50 and 4.40 respectively, besides other usual signals of the substituents. In order to assign unequivocally all NMR signals, 1D and 2D techniques such as DEPT (135), HMQC and HMBC were used [15].



**Fig. (4).** Compounds studies by NMR 1D and 2D techniques.

A structural study of 5-carboxy-ethyl-3-(2'-furfurylmethyl) tetrahydro-2*H*-1,3,5-thiadiazine-2-thione was made by means of X-ray crystallographic analysis determining the most stable conformation in the solid state. The theoretical calculations allowed to gain a better picture of the conformational profile of the given compound by means of the semiempirical AM1 method, as well as by ab-initio calculations at Hartree-Fock level using 3.21G\* and 6-31G\* basis sets. <sup>1</sup>H Noe experiments had also been carried out in order to obtain information about the conformational profile of this compound in solution [16].

The electrospray ionisation (ESI) in negative mode of the pharmacologically significant mono-THTT derivatives, and their subsequent fragmentations using an ion-trap mass spectrometer were investigated. Experiments on sequential product ion fragmentations (MS<sup>n</sup>) were performed in order to elucidate the degradation pathways for these compounds. The data reported show that the fragmentation of the even-electron [M-H]<sup>-</sup> ions could proceed through an internal nucleophilic substitution displacement. Decarboxylation and extrusion of carbon disulfide are other fragmentations observed [17].

On the other hand, the spectroscopic information gathered from previously synthesized mono-THTT derivatives [15-17] allowed to confirm the structure of the bis-THTT compounds. The structures of all the bis-THTT derivatives reported in the bibliography were established on the basis of spectroscopic data [19-22, 24, 25]. In general the <sup>1</sup>H and <sup>13</sup>C NMR signals of each THTT-ring were undistinguishable and all series, show a similar trend in the chemical shift of the common part of the molecular backbone. The <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data of alkyl, and polyamine-linked bis(2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids, pre-

pared from alkyl diamines and *N*4-(benzyl) spermidine, were fully assigned by the combination of one- and two-dimensional experiments (DEPT, HMBC, HMQC, COSY) [25]. In some cases, the <sup>13</sup>C NMR experiments could not be performed because the bis-THTT derivatives were poorly soluble in DMSO at the concentration required for NMR spectroscopy [19, 20].

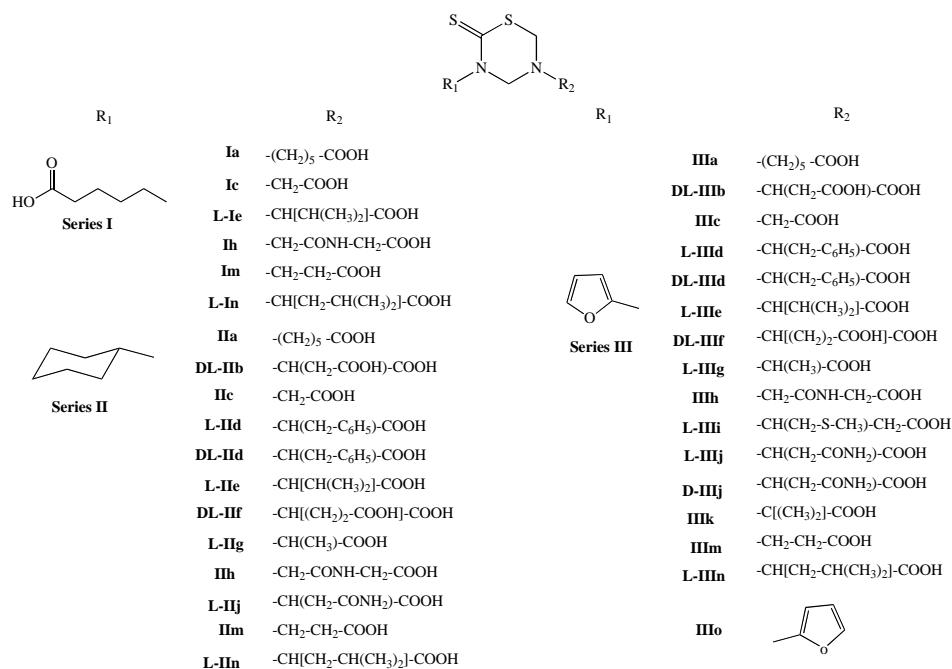
#### 4. BIOLOGICAL ACTIVITY OF THTT DERIVATIVES

The promising results of antiprotozoal activity achieved by reported THTT derivatives could be attributed to the interaction of cysteine proteinases, present in most groups of parasitic protozoa [26], with isothiocyanates [27] generated by hydrolysis of the THTT ring in a protic medium [28]. Notwithstanding it should not be excluded the possible interaction of the released aminoacids or dipeptides, attached to position 5 of the THTT ring, with other molecular targets, enhancing the antiparasitic activity observed of these derivatives.

On the other hand, the high lipid solubility and ease of enzymatic hydrolysis [29] generally associated with this heterocycle, has motivated its use as a biolabile prodrug in the design of drug-delivery systems (DDS). The aforementioned properties and the possibility to attach several structurally distinct substituents to the heterocycle ring to modify either the biological or physico-chemical properties of these compounds, have prompted us to use the THTT ring as a template in many research programs aimed at the development of new antiparasitic compounds [9, 11-14, 18-22].

##### 4.1. Biological Activity of Mono-THTT Derivatives

In 1999, we reported for the first time the antiprotozoal properties of 34 mono-THTT derivatives (Fig. 6) corre-



**Fig. (5).** General structure of mono-THTT derivatives (Series I, II and III) assays like antiprotozoan drugs against *Trichomonas vaginalis* and *Trypanosoma cruzi*; and like anticancer drugs against HeLa, HT-29 and Hep G2 cells.



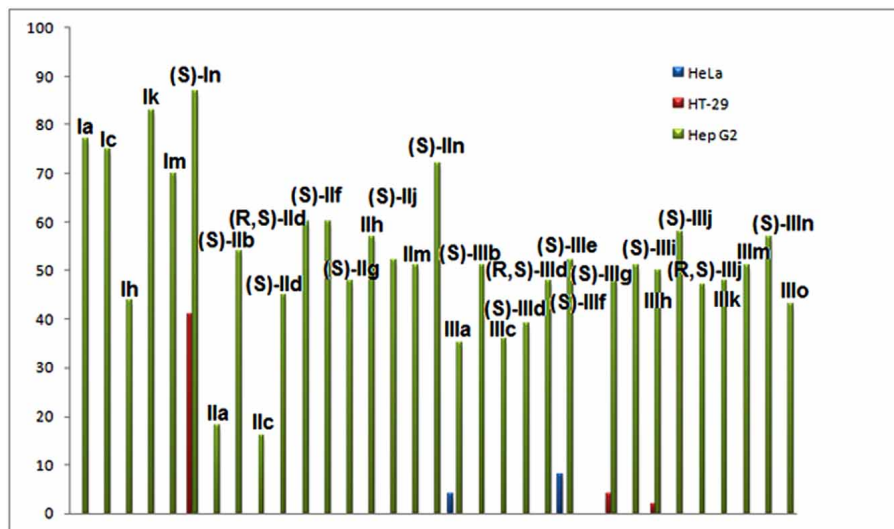


Fig. (7). Cytotoxicity ( $IC_{50}$  ( $\mu\text{mol/l}$ )) of compounds belonging to series **I**, **II** and **III** against HeLa, HT-29 and Hep G2 cells.

of them showed a  $IC_{50}$  lower than  $10 \mu\text{mol}$  against any cell line. Compound **IIIo**, detected in the decomposition of compound **IIIm** and presumably in one of the other thiadiazinones, were less cytotoxic than **IIIm**. The nature of  $R_1$  modulates the cytotoxicity of these compounds. Compounds bearing the aromatic furfuryl moiety (Series **III**) yielded the most interesting thiadiazinones. However, the nature of  $R_2$  influences the cytotoxic activity to a lesser grade. The (S) enantiomer showed more cytotoxic properties than its (R) isomer. Derivative (S) **IIIj**, bearing furfuryl and L-asparagine moieties, yielded the most interesting compound which is a candidate for a future anticancer study [10]. These results allowed the study through QSAR methodology of mono-THTT derivatives using the novel hybrid index  $pMR\chi$  [30].

With the aim to increase the antiprotozoan activity spectra, our group reported new biological assays to study the non-specific toxicity and anti-amastigote activity of 18 of these compounds [31]. Cytotoxicity assays of 24 mono-THTT were performed. The 17 compounds with higher anti-epimastigote activity and lower cytotoxicity were, thereafter, screened against amastigote of *Trypanosoma cruzi*. Out of these 17 derivatives, (S)-**IIId** was selected to be assayed *in vivo*, because of its remarkable trypanocidal properties. Nifurtimox and benzidazole were used as reference drugs. All of the compounds were highly toxic at  $100 \mu\text{g/mL}$  for macrophages and a few of them maintained this cytotoxicity even at  $10 \mu\text{g/mL}$ . Of the derivatives assayed against amastigotes, **Ik** and (S)-**IIId** showed an interesting activity, which was held even at  $1 \mu\text{g/mL}$ . It is demonstrated that the high anti-epimastigote activity previously reported is mainly due to the non-specific toxicity of these compounds. *In vivo* assays assessed a reduction of parasitemia after the administration of (S)-**IIId** to infected mice [31].

In 2004 and 2005 ten mono-THTT derivatives (Fig. 8) were tested *in vitro* for antiparasitic effects against both extracellular promastigotes and intracellular amastigotes of *Leishmania amazonensis* [32,33]. The compounds were found to be active against the amastigote form of the parasite, inhibiting parasite growing, from 10 to 89%, at a concentration of  $100 \mu\text{g/ml}$ . This activity suggests that thiadiaz-

ine derivatives could be considered as potential antileishmanial compounds (Fig. 9) [32].

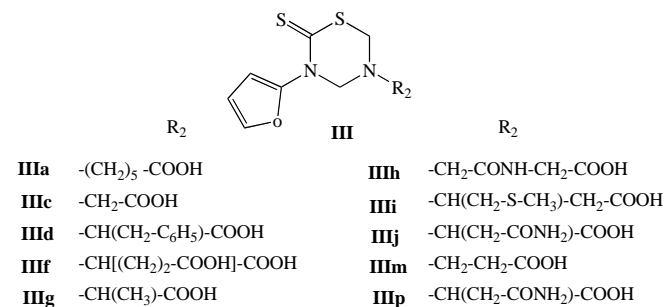


Fig. (8). General structure of the mono-THTT (Series **III**) tested *in vitro* for antiparasitic effects against both extracellular promastigotes and intracellular amastigotes of *Leishmania amazonensis*.

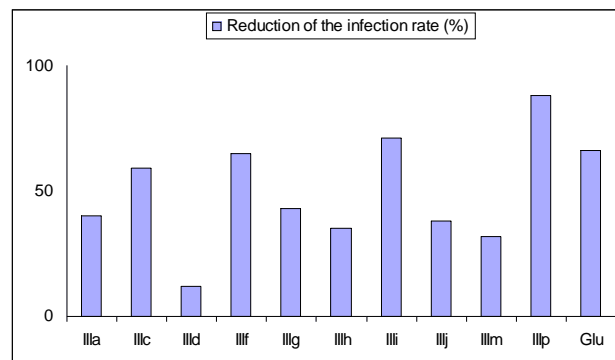


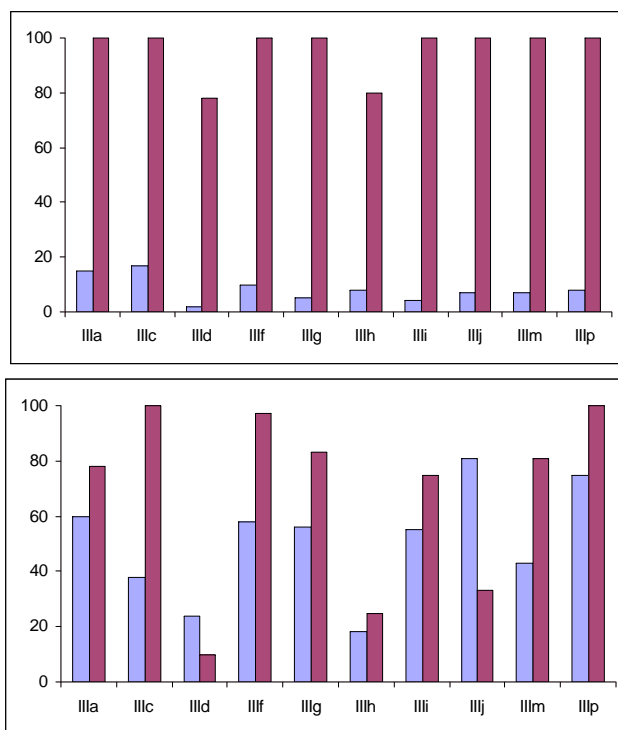
Fig. (9). Effect of mono-THTT derivatives and Glucantine (Glu) on intracellular amastigote.

These results confirmed that the THTT represent a group of compounds with significant *in vitro* activity against *L. amazonensis* and suggested that some of them could be considered for further study as new therapeutic alternatives [32,33].

All evaluated compounds caused an irreversible inhibition of the promastigote growth either after 1h of treatment



with 10  $\mu\text{g/mL}$  or after 24 h with 1  $\mu\text{g/mL}$  (Fig. 10). However, the compounds exhibited high toxicity and produced inhibition of the phagocytosis in the murine host cell [33]. The mono-THTT tested exhibited a strong activity against *L. amazonensis* at low concentrations.



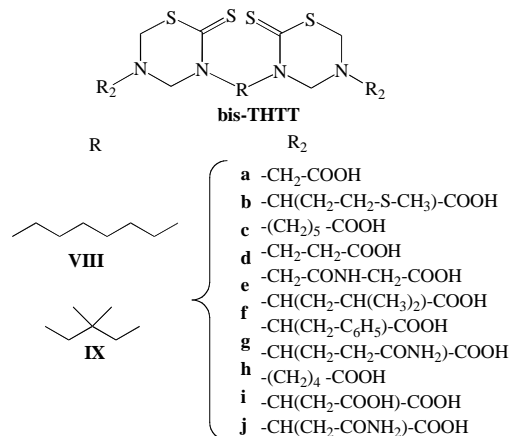
**Fig. (10).** Effects of mono-THTT derivatives **III** on the *L. amazonensis* growth inhibition. A. Promastigotes were incubated in the presence of 10  $\mu\text{g/mL}$ ; after 1h of treatment (blue column); incubated 5 days in fresh medium (violet columns). B.

#### 4.2. Biological Activity of Bis-THTT Derivatives

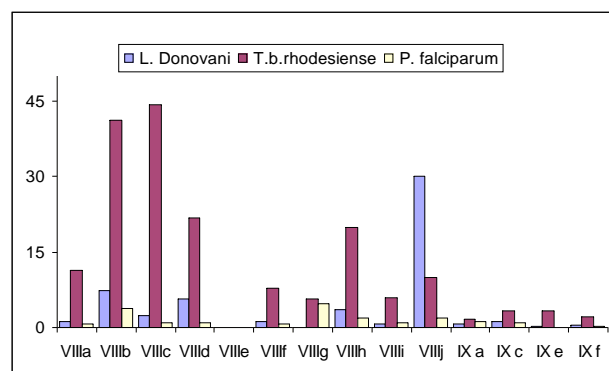
It has been reported that bis-thiadiazines have antifungal, antimicrobial and antiprotozoal activity [19-22, 34]. Specifically with antiprotozoal activities three series of bis-THTT had been reported [21, 22, 34]. The *in vitro* activity of compounds belonging to series **VIII** and **IX** (Fig. 11) against *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, and *Plasmodium falciparum* was studied. From these results it may be observed (Fig. 12) that the best activity profiles were found against *T. b. rhodesiense*. It is interesting that the activity against *T. b. rhodesiense* seems to be favoured for compounds having linear aminoacidic residues as substituents in N-5 of the THTT ring. Despite exerting a notable activity against *T. b. rhodesiense*, derivatives from series **VIII** appeared to be more cytotoxic than the analogs of series **IX** (Fig. 13) [34].

The *in-vitro* antiprotozoal evaluation of novel N4-(benzyl)spermidyl-linked bis-THTT derivatives from N4-(benzyl)spermidine (**XII**) was disclosed [22]. These compounds showed a potent protozoocidal activity against *T. cruzi* and *L. Donovanii* which turns to be comparable or greater than the currently employed chemotherapies. Despite this fact, the novel structures displayed a higher cytotoxicity than previously synthesized alkyl tethers analogs having the

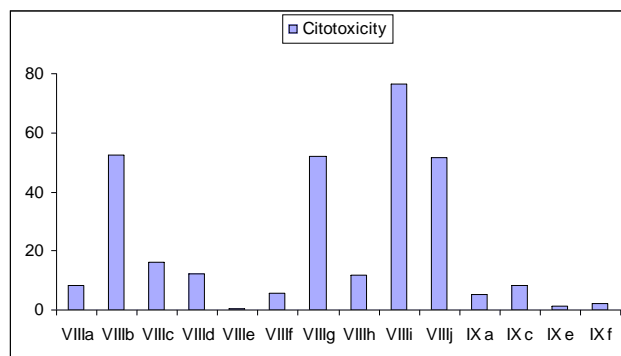
same amino acid residues attached to position N-5 of the heterocyclic ring. It has been hypothesized that increased cytotoxicity could be related with an interference of the polyamine metabolism in mammals [22].



**Fig. (11).** General structures of bis-THTT derivatives tested against *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, and *Plasmodium falciparum*.



**Fig. (12).** *In vitro* activity of compounds belonging to series **VIII** and **IX** against *Leishmania donovani*, *Trypanosoma brucei rhodesiense*, and *Plasmodium falciparum*.



**Fig. (13).** Cytotoxicity of bis-THTT of series **VIII** and **IX**.

The *in-vitro* antiprotozoal activity against *L. donovani*, *T. cruzi*, *T. b. rhodesiense*, and *P. falciparum* of some compounds of series **XII**, was compared with these activities for compounds of series **VIII** (Fig. 14 and Fig. 15) [22]. For



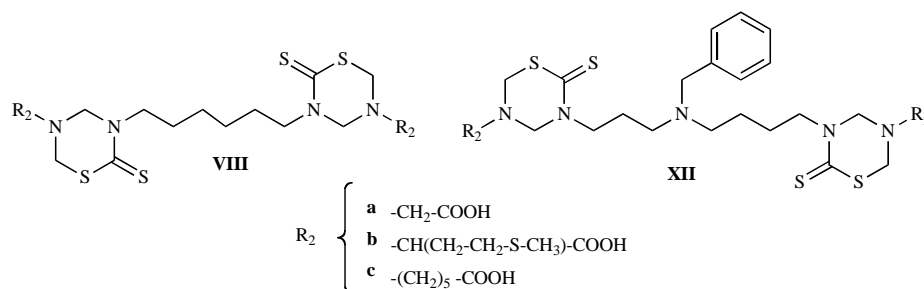


Fig. (14). Bis-THTT structures (VIII and XII).

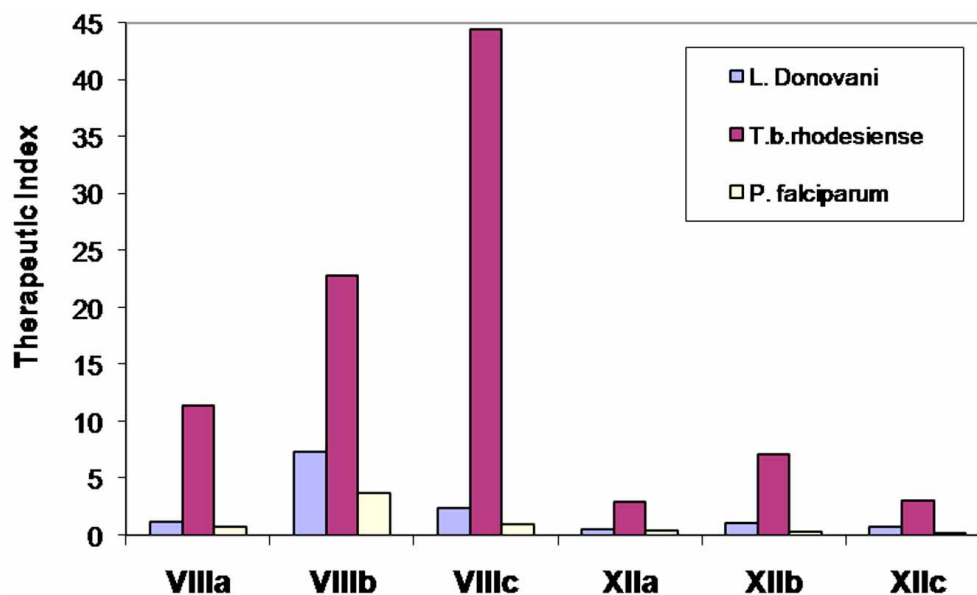


Fig. (15). Results for *in-vitro* anti-protozoal activity of N4-(benzyl)spermidinyl-linked bis-THTT and their hexyl-linked bis-THTT analogs.

comparative purposes, structures VIII and XII were changed only in the nature of their connective backbone. In the bis-THTT derivatives in both series that had the same aminoacid residue ( $R_2$ ), the displayed protozoocidal profile against the three parasitic lines assayed was almost the same. However, higher values of therapeutic indexes were found for compounds VIIIa–c. Considering that both series exerted a similar protozoal activity it is possible to assume that structures XII are more cytotoxic than their alkyl tethers analogs [22].

## 5. CONCLUSIONS

The most extensive method reported to obtain THTT derivatives proceed via a dithiocarbamate salt intermediary. This experimental procedure is simple and allows a wealth of molecular diversity depending on the nature of groups attached to both nitrogens of the heterocycle.

All THTT synthesized showed biological activity against some trypanosomatids at doses of 100  $\mu\text{g/mL}$ , at low doses some of them showed important activities. The results presented in this review made it possible to analyze how the chemical nature of N-3 and/or N-5-substituents in the THTT ring influences the overall activity/cytotoxicity profile against some protozoa parasites. The introduction of two THTT rings in the same molecule potentiated the antiprotozoan activity of these derivatives.

The excellent properties of the THTT scaffold made its use as the main core in many projects for the development of new antiparasitic agents and the synthesis of novel bis-THTT derivatives employing other polyamines as bridging moieties will be also the theme of future investigations.

## REFERENCES

- [1] Sachs, J. D.; Hotez, P. J. Fighting tropical diseases. *Science*, **2006**, *311*, 1521.
- [2] Renslo, A. R.; McKerrow, J. H. Drug discovery and development for neglected parasitic diseases. *Nat. Chem. Biol.*, **2006**, *2*, 701-10.
- [3] Nwaka, S.; Hudson, A. Innovative lead discovery strategies for tropical diseases. *Nat. Rev. Drug Discov.*, **2006**, *5*, 941-55.
- [4] Redtenbacher, J.; Liebig, J. Ueber das carbothildin. *Liebigs Ann. Chem.*, **1848**, *65*, 43-5.
- [5] Ainley, A.; Davies, D. The constitution of the so-called carbothialdines and the preparation of some homologous compounds. *J. Chem. Soc.*, **1944**, 147-9.
- [6] Rieche, A.; Hilgetg, G.; Martini A.; Nejedh, O.; Schlege, J. New compounds with bactericidal and fungicidal activity and which inhibit the growth of viruses. I. 2-thiotetrahydro-1,3,5-thiadiazine ("carbothialdine") and dithiocarbamic acid salts. *Arch. Pharm.*, **1960**, *293*, 957-67.
- [7] Zsolnai, T. Antimicrobial effects of thiocyanates, isothiocyanates and potential isothiocyanate forming substances. *Arzneim-Forsch. Drug Res.*, **1971**, *21*, 121-7.
- [8] Weaffen, W.; Martin, D.; Schade W. Relation between chemical structure and antimicrobial action. 1. Studies on the bacteriostatic properties of some 2-thiotetrahydro-1,3,5 thiadiazines. *Pharmazie*, **1963**, *18*, 420-6.

- [9] Ochoa, C.; Pérez, R.; Pérez, E.; Suárez, M.; Ochoa, E.; Rodríguez, H.; Gómez Barrio, A.; Muelas, S.; Nogal, J.J.; Martínez, R. A. Synthesis and Antiprotozoan properties of new 3,5-Disubstituted-Tetrahydro-2H-1,3,5-Thiadiazin-2-Thione derivatives. *Arzneimittel Forschung-Drug Res.*, **1999**, 49(II), 764-9.
- [10] Pérez, R.; Suárez, M. Rodríguez, H.; Ochoa, C. Study on the decomposition products thiadiazinethione and their anti-cancer properties. *Arzneimittel Forschung Drug Res.*, **2000**, 50 (II), 854-7.
- [11] Katiyar, D.; Tiwari, V. K.; Tripathi, R. P.; Srivastava, A.; Chaturvedi, V.; Srivastava, R.; Srivastava, B. S. Synthesis and antimycobacterial activity of 3,5-disubstituted thiadiazine thiones. *Bioorg. Med. Chem.*, **2003**, 11(20), 4369-75.
- [12] El-Shorbagi, A.-N. New tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives as potential antimicrobial agents. *Arch. Pharm. Pharm. Med. Chem.*, **2000**, 333, 281-6.
- [13] Aboul-Fadl, T.; Hussein, M.A.; El-Shorbagi, A.N.; Khallil, A.R. New 2H tetrahydro-1,3,5- thiadiazine-2-thiones incorporating glycine and glycnamide as potential antifungal agents. *Arch. Pharm. Pharm. Chem.*, **2002**, 335, 438-42.
- [14] Aboul-Fadl, T.; Khallil, A.R. Synthesis, degradation kinetics and *in vitro* antimicrobial activity of tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives of some beta-amino acids. *Arzneim- Forsch. Drug Res.*, **2003**, 53, 526-31.
- [15] Pérez, R.; Suárez, M.; Rodríguez, H.; Molero, D.; Martín, N.; Martínez, R.; Seoane, C. 1H and 13C Spectra assignment of 3,5-disubstituted tetrahydro-2H-1,3,5- Thiadiazin-2-thione derivatives. *Magn. Reson. Chem.*, **2001**, 39, 22-224.
- [16] Pérez, R.; Rodríguez, H.; Suárez, M.; Martín, N.; Seoane, C.; Novoa, H.; Blaton, N.; Peeters, O.; De Ranter C. A joint theoretical and experimental structural study of N, N-disubstituted tetrahydro-2H-1,3,5- thiadiazines. *Tetrahedron*, **2001**, 57, 9543-9.
- [17] Martínez, R.; Martín, N.; Seoane, C.; Suarez, M.; Perez, R.; Rodriguez, H.; Kayali, N. Study of the electrospray ionization and ion trapp fragmentation of negative ions of new 3,5-disubstituted tetrahydro-(2H)-1,3,5-thiadiazine-2-thione. *Rapid Commun. Mass Spectrom.*, **2001**, 15, 758-62.
- [18] Ozcelik, A.B.; Ersan, S.; Ural, A.U.; Ozkan, S.; Ertan, M. Synthesis of 3-substituted-5-(4- carboxycyclohexylmethyl)-tetrahydro-2H-1,3,5-thiadiazine-2-thione derivatives as antifibrinolytic and antimicrobial agents. *Arzneimittel Forschung.*, **2007**, 57(8), 554-9.
- [19] Hussein, M.A.; El-Shorbagi, A.N.; Khallil, A.R. Synthesis and antifungal activity of 3,3'-ethylenebis (5-alkyl-1,3,5-thiadiazine-2-thiones). *Arch. Pharm. Pharm. Med. Chem.*, **2001**, 334, 305-8.
- [20] El Bialy, S.A.A.; Abdelal, A.M.; El-Shorbagi, A.N.; Kheira, S.M.M. 2,3-Bis(5-alkyl-2-thiono-1,3,5- thiadiazin-3-yl) propionic acid: One-pot Domino synthesis and antimicrobial activity. *Arch. Pharm. Chem. Live Sci.*, **2005**, 338, 38-43.
- [21] Coro, J.; Pérez, R.; Rodríguez, H.; Suárez, M.; Vega, C.; Rolon M.; Montero, D.; Nogal J.J.; Gómez- Barrio A. Synthesis and antiprotozoan evaluation of new alkyl-linked bis(2-thioxo-[1,3,5]thiadiazin-3-yl) carboxylic acids. *Bioorg. Med. Chem.*, **2005**, 13, 3413-21.
- [22] Coro, J.; Little, S.; Yardley, V.; Suarez, M.; Rodríguez, H.; Martín, N.; Perez-Pineiro, R. Synthesis and Antiprotozoal evaluation of New N4-(benzyl)Spermidyl-linked bis(1,3,5-thiadiazinane-2-thione). *Archiv der Pharmacie.*, **2008**, 341, 318-29.
- [23] O'Sullivan, M.C.; Zhou, Q.; Li, Z.; Durham, T.B.; Rattendi, D.; Lane, S.; Bacchi, C.J. Polyamine derivatives as inhibitors of Trypanothione reductase and assessment of their tripanocidal activities. *Bioorg. Med. Chem.*, **1997**, 5, 2145-55.
- [24] Coro, J.; Álvarez, R.; Montero, A. L.; Suárez, M.; Martín, N.; Perez-Pineiro, R. A computational approach to the synthesis of 1,3,5-thiadiazinane-2 thiones in aqueous medium: theoretical evidence for water-promoted heterocyclization. *J. Mol. Model.*, **2008**, 14, 641-7.
- [25] Molero, D.; Coro, J.; Pérez, R.; Suárez, M.; Martínez, R.; Herrera, A.; Martín N. 1H and 13C NMR spectral Assignment of Alkyl and Polyamine-linked Bis (2-thioxo-[1,3,5]thiadiazin-3-yl) carboxylic acids. *Magn. Reson. Chem.*, **2007**, 45, 93-8.
- [26] North, M. J.; Mottran, J. C.; Coombs, G. H.M. J. Cysteine proteinases of parasitic protozoa. *Parasitol. Today*, **1990**, 6, 270-5.
- [27] Goksoyr, J. Chemical and fungicidal reactions of 3,5-dimethyl-tetrahydro-1,3,5-thiadiazine-2-thione. *Acta Chem. Scand.*, **1964**, 18, 1341-52.
- [28] Aboul-Fadl, T.; El-Shorbagi, A. New prodrug approach for amino acids and amino-acid-like drugs. *Eur. J. Med. Chem.*, **1996**, 31, 165-9.
- [29] El-Shorbagi, A.N. Model for delivery of amines through incorporation into a tetrahydro-2H-1,3,5- thiadiazine-2-thione structure. *Eur. J. Med. Chem.*, **1994**, 29, 11-5.
- [30] Carrasco, R.; Padrón, J.A.; Pérez, R.; Rodríguez, H.; Suárez, M.; Ochoa, C. Quantitative structure antitumoral-activity relationships of thiadiazinethione derivatives using the novel hybrid molecular index. *J. Pharm. Pharm. Sci.*, **2005**, 8(3), 586-92.
- [31] Muelas, S.; Suárez, M.; Pérez, R.; Rodríguez, H.; Ochoa, C.; Escario, J.A.; Gómez-Barrio, A. In Vitro and *in vivo* assays of 3,5-disubstituted-tetrahydro-2H-1,3,5-thiadiazin-2-thione derivatives against *Trypanosoma cruzi*. *Mem. Inst. Oswaldo Cruz Rio de Janeiro.*, **2002**, 97, 269-72.
- [32] Monzote, L; Montalvo, A. M.; Fonseca, L.; Pérez, R.; Suárez, M.; Rodríguez, H. Effect of thiadiazine derivatives on intracellular amastigotes of *Leishmania amazonensis*. *Mem. Inst. Oswaldo Cruz Rio de Janeiro.*, **2004**, 99(3), 329-30.
- [33] Monzote, L; Montalvo, A. M.; Fonseca, L.; Pérez, R.; Suárez, M.; Rodríguez, H. *In vitro* activities of thiadiazine derivatives against *Leishmania amazonensis*. *Arzneim-Forst. Drug Res.*, **2005**, 55, 4, 232-8.
- [34] Coro, J.; Atherton, R.; Little, S.; Wharton, H.; Yardeley, V.; Alvarez, A.; Suárez, M.; Pérez, R.; Rodríguez H. Alkyl-linked bis-THTT derivatives as potent *in vitro* trypanocidal agents. *Bioorg. Med. Chem. Lett.*, **2006**, 16, 1312-5.

Received: February 02, 2010

Revised: April 29, 2010

Accepted: June 15, 2010

© Bermello et al.; Licensee Bentham Open.

This is an open access article licensed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted, non-commercial use, distribution and reproduction in any medium, provided the work is properly cited.