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Synthesis and antiprotozoan evaluation of new alkyl-linked bis(2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids

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Abstract—Two new series of several alkyl-linked bis(2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids were synthesized in a two step procedure from the corresponding alkyl bis-dithiocarbamic salt intermediary. The novel compounds were evaluated for their activity in vitro against *Trypanosoma cruzi* strain CL (clone CL B5) and *Trichomonas vaginalis* strain JH 31A.

1. Introduction

The antibacterial,[1,2] antifungal,[3,4] anthelmintic,[5] and tuberculostatic[6] properties of tetrahydro-(2*H*)-1,3,5-thiadiazine-2-thione derivatives (THTT) has been known for several decades. Besides its renowned antimicrobial activity this versatile heterocycle has found increased application in the drug research arena as a biolabile prodrug[7] in the design of drug delivery system (DDS) due to its high lipid solubility and enzymatic rate of hydrolysis. In this regard, several aminoacids,[8] peptides,[9,10] and primary-amine-containing drugs[11–13] have been successfully attached to the THTT moiety to enhance their cellular uptake by improving lipophilicity in the area where the drug molecule is released by the physiological and/or enzyme catalytic effects. Another important advantage of THTT derivatives is their stability in simulated gastric fluid (SGF), which facilitates their stomach absorption in a less ionized form in the case of oral administration.[8]

The excellent physico-chemical properties of this heterocycle have prompted us to use it as the main core in our integral project for the development of new antiparasitic agents. In a previous work we have described the synthesis of several tetrahydro-(2*H*)-1,3,5-thiadiazine 2-thiones bearing furfuryl, cyclohexyl, and carboxypentyl radicals at N-3 and carboxyalkyl residues at N-5[14] (Fig. 1).

The synthesized molecules were evaluated in vitro and in vivo against *Trypanosoma cruzi* and *Trichomonas vaginalis* showing significant antiprotozoal activity that, in some cases, was further correlated to a non-specific toxicity effect.[15] In another study the remarkable cytotoxicity properties of these derivatives against HeLa and HT-29 cells were also tested.[16]

Recently, we reported the antiparasitic properties of some 3-furfuryl-5-carboxyalkyl tetrahydro-(2*H*)-1,3,5-thiadiazine-2-thione against both extracellular

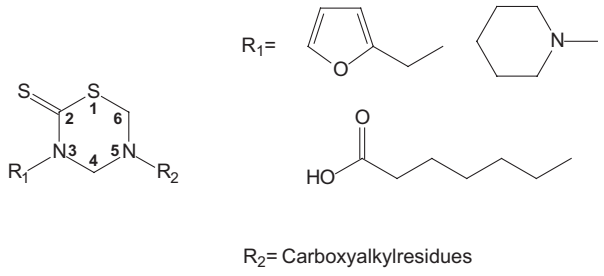

Figure 1.

Keywords: Chemotherapy; Antiparasitics; Thiadiazine-2-thione.
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promastigotes and intracellular amastigotes of *Leishmania amazonensis*.[17,18] The results showed that the evaluated compounds exhibited a strong antiproliferative activity on all developmental stages of the parasite.

The promising results of antiprotozoal activity achieved by previously reported THTT derivatives could be attributed to the interaction of cysteine proteinases, present in most groups of parasitic protozoa,[19] with isothiocyanates[20] generated by hydrolysis of the thiadiazine-2-thione ring in a protic medium.[8] 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 observed antiparasitic activity of these derivatives.

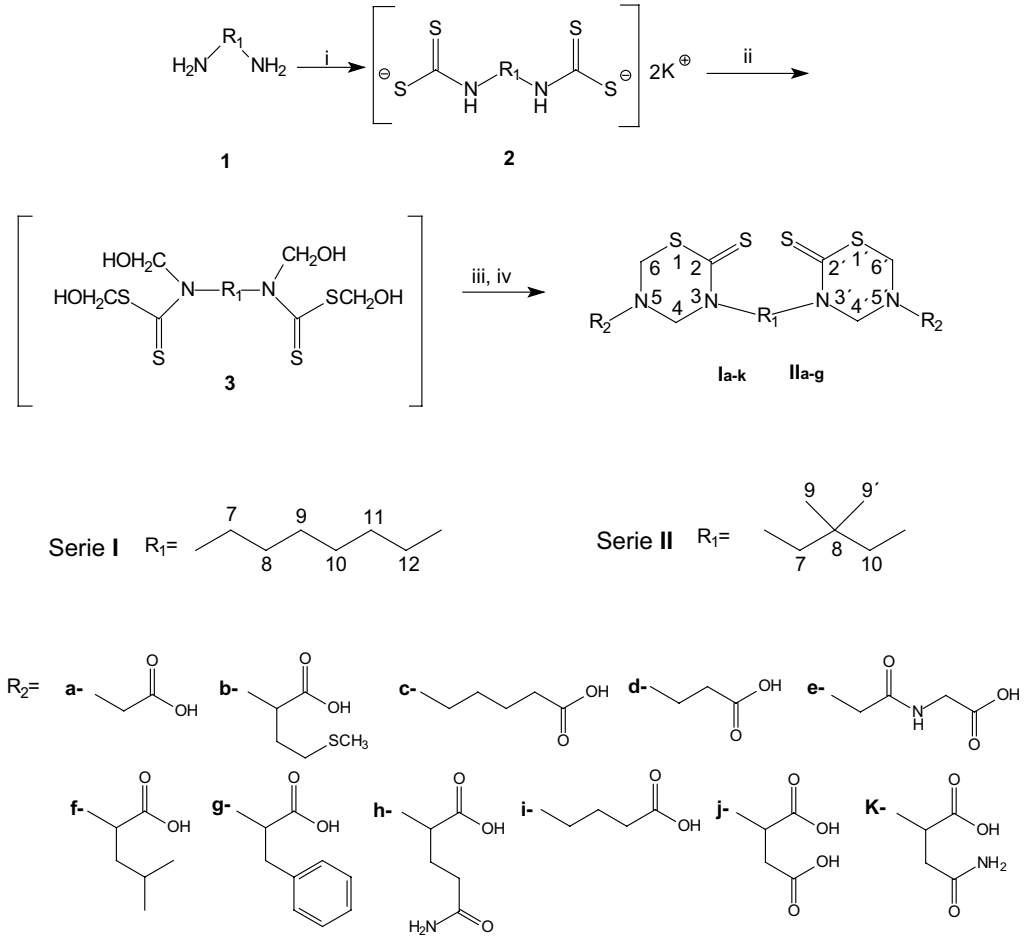
Following our interest in antiprotozoal drugs, we report the synthesis and biological evaluation of new alkyl-linked bis(2-thioxo-[1,3,5] thiadiazinan-3-yl) carboxylic acids with the aim they could act as prodrugs able to inhibit the cysteine proteinase of some protozoan. To enhance the antiprotozoal effect of the novel compounds 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 carboxyalkyl residues at N-5. In this way, the structural requirements for optimum antimicrobial activity, as previously reported for tetrahydro-(2*H*)-1,3,5-thiadiazine-2-thione analogs,[4] were present in the new molecules. All the novel heterocycles described in this work were evaluated for their potential antiprotozoal activity.

2. Results and discussion

2.1. Synthetic approach

Several methods have been reported on the synthesis of THTT including the use of isothiocyanate[21] and solid phase organic synthesis (SPOS) methodology,[22] but the most extensive to date proceed via a dithiocarbamate salt intermediary.[4] This experimental procedure is simple allowing a wealth of molecular diversity depending on the nature of groups attached to both nitrogens of the heterocycle. The new compounds were obtained following the previously reported experimental procedure for the synthesis of 3,5-disubstituted tetrahydro thiadiazine-2-thiones derivatives[14] (Scheme 1).

To increase the power of our synthetic protocol to generate diversity and with the aim of incorporating two rings into the same molecular structure, we planned our synthetic strategy starting from commercially available diamines **1** (1,6-diaminohexane or 2,2-dimethyl-1,3-propanediamine), which in the first step it react with CS_2



Scheme 1. Reagents: (i) KOH (20%), CS_2, rt; (ii) HCHO; (iii) $H_2N–R_2$, rt; (iv) HCl (15%).

in the presence of KOH to afford the expected bis-dithiocarbamate salt **2.** Addition of formaldehyde to this intermediary resulted in the formation of the adduct **3** in situ,[7] which were allowed to react with the corresponding aminoacid or glycyl-glycine in a slightly alkaline medium (phosphate buffer, pH 7.8) to generate, after treatment with HCl 15%, the desired bis(2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids **I** or **II**.

All compounds were obtained in moderate to good yields except those of series **II**, probably due to the use of a bulky diamine (2,2-dimethyl-1,3-propanediamine) and the resulting steric hindrance at the cyclization stage.

The IR, ^{1}H, and ^{13}C NMR spectral data (chemical shifts and multiplicities, DEPT for ^{13}C, HMQC, and HMBC for ^{1}H–^{13}C NMR correlation experiments), microanalyses, and the spectroscopic information gathered from previously synthesized thiadiazin-2-thione derivatives[14,23,24] allowed us to confirm the structure of the new compounds. ^{13}C NMR experiments could not be performed for compounds **Ib**, **Id**, **If**, **Ig**, **Ih**, **Ik**, **Ij**, and **IId** because these derivatives were poorly soluble in DMSO at the concentration required for NMR spectroscopy.

Formation of the THTT ring for series **I** and **II** was proven by IR, ^{1}H NMR, and ^{13}C NMR. The presence of the characteristic band of the carbonyl (C=O) and thiocarbonyl (C=S) groups around 1720 cm^{-1} and 1480 cm^{-1}, respectively, was confirmed in the IR spectra of both series.

Ring protons 6, 6′-H and 4, 4′-H were assigned at δ 3.97–4.52 ppm and δ 4.08–4.69 ppm, respectively, according to NOE experiments. Due to the stereoheterotopic nature of these methylene protons they are observed either as two singlets or two doublets depending on the presence of a chiral center in the substituent attach to N-5. The ^{13}C NMR spectra of these compounds exhibited signals in the thiocarbonyl, carbonyl, aromatic, and aliphatic regions. The thiocarbonyl carbon (C-2, C-2′) was observed in the narrow range of δ 190.1–193.4 ppm and carbon C-6, C-6′ displayed a signal at δ 63.1–73.9 ppm downfield from the corresponding resonance of C-4, C-4′ δ 55.8–60.4 ppm. The COOH carbon appears at δ 170–174 ppm. Assignments for the ^{1}H NMR and ^{13}C NMR resonance corresponding to the aliphatic backbone, connecting both heterocycles in series **I** and **II**, were also achieved by means of HMQC, HMBC, and HH COSY experiments, substituent effects, and DEPT data. From the spectroscopy data collected we concluded that the signals corresponding to the thiadiazine-2-thione are relatively insensitive to the nature of the substituent attached to N-3 and N-5. In general the ^{1}H and ^{13}C NMR signals of each thiadiazine-2-thione rings were undistinguishable and both series, **I** and **II**, show a similar trend in the chemical shift of the common part of the molecular backbone.

2.2. Biological activity test

The antiprotozoal activity of the newly synthesized compounds was assessed against *T. cruzi* strain CL (clone CL B5) epimastigotes, and *T. vaginalis* strain JH 31A. Toxicity of the compounds to mammalian cells was also determined.

There are several compounds that apparently exhibit an important effect on parasite cultures, but it is not a specific activity against *Trichomonas* or *Trypanosoma* but a consequence of their toxicity. If more toxic compounds are discarded, compounds **Ib**, **Ii**, and **Ik** have important trypanocidal activities but only at concentrations of 10 μg/mL, because of its unspecific toxicity at higher concentrations (Table 1). Among series **II** compound **IIg** (Table 2) appears to be the most effective as well as **IIf** as they maintain its activity at non-toxic concentrations of 10 down to 1 μg/mL. Regarding anti-*Trichomonas* activity, the results were similar, although metabolic pathways of this parasite are quite different to *T. cruzi*. Again compounds **Ib**, and **IIg** were the most active against this parasite (Fig. 2).

With the three concentrations assayed, approximated 50% inhibitory concentrations (IC_{50}) for the five most active compounds were determined using a microcomputer log-probit analysis program SPSS ver.12.0S. (SPSS Inc., Chicago, Illinois). The results listed in Table 3 showed an important activity against *T. cruzi* of the deemed compounds compared to the reference drug Nifurtimox, on the other hand a lower inhibitory effect on *T. vaginalis* was observed in comparison to Metronidazole.

3. Conclusions

We have explored the ability of linear and branch alkyl diamines to act as synthetic precursors of heterocyclic compounds with biological activity.

This study describes the synthesis of two series of (alkyl-linked 2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids **Ia–k** and **IIa–g** obtained from 1,6-diaminohexane or 2,2-dimethyl-1,3-propanediamine. These molecules were prepared by interaction of the formed adduct between the bis-dithiocarbamate salt intermediary and formaldehyde with the corresponding aminoacid or glycyl-glycine. Preliminary biological evaluation demonstrated that some of the new compounds possess notable activity against *T. cruzi* and *T. vaginalis*, suggesting potential for the development of useful antiparasitic agents.

The possible improvement of the activity/cytotoxicity profile of these (alkyl-linked 2-thioxo-[1,3,5]thiadiazinan-3-yl) carboxylic acids by replacing the connective aliphatic backbone for a linear polyamine structure is currently under investigation.

4. Experimental

4.1. Materials

All reagents were of analytical grade, dried, and purified when necessary. Chiral aminoacids used in the synthesis of compounds **I** and **II** belonged to series L.

Table 1. Biological activity of series **I** against *Trypanosoma cruzi* and *Trichomonas vaginalis*

Compound	Concn (μg/mL)	Cytotoxicity (% *C*)	Antiparasitic activity		
			Trypanosoma (% AE)	*Trichomonas*[a]	
				24 h	48 h
Ia	100	100	92.5	100	100
	10	85.2	82.0	100	100
	1	4.9	73.7	(31.9)	(24.6)
Ib	100	100	95.0	100	100
	10	3.5	82.5	99.2	100
	1	0	39.9	(84.2)	(90.1)
Ic	100	100	90.1	100	100
	10	100	77.6	100	100
	1	0	12.8	(80.7)	(90.7)
Id	100	40.6	94.3	92.0	100
	10	11.4	79.5	(50.8)	(56.8)
	1	1.5	41.5	(0)	(1.6)
Ie	100	38.3	96.9	95.2	100
	10	3.8	55.7	(55.1)	(73.7)
	1	0	17.0	0	0
If	100	96.0	93.1	100	100
	10	50.8	85.3	(75.3)	93.2
	1	11.6	55.7	(0)	(17.9)
Ig	100	93.4	94.2	100	100
	10	60.3	77.3	88.6	100
	1	21.3	38.8	(2.2)	(7.4)
Ih	100	87.1	92.0	100	100
	10	27.7	80.4	(46.8)	(36.3)
	1	0	23.9	(0)	(1.6)
Ii	100	100	93.1	100	100
	10	64.2	80.0	100	100
	1	4.6	63.9	(64.8)	(23.2)
Ij	100	35.5	86.3	92.7	100
	10	7.5	56.4	(30.2)	(27.9)
	1	0	37.0	(0)	(0)
Ik	100	58.2	100	100	100
	10	9.4	64.4	(50.2)	(33.2)
	1	0	51.2	(15.6)	(7.9)
Nifurtimox	10		89.1		
	1		54.9		
	0.5		45.6		
Metronidazol	2			100	100
	1			97.9	100
	0.5			(83.5)	94.1

[a] Anti-*Trichomonas* activity is expressed as percentages of reduction and/or growth (parentheses), indicative of cytocidal or (cytostatic) activities, respectively, after 24 and 48 h post-incubation.

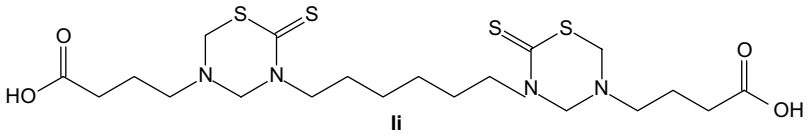
Melting points were determined in a Buchi 535 apparatus and were not corrected. The progress of the reaction and purity of compounds were monitored by TLC analytical silica gel plates (Merck F254). Spectra were obtained as follows: FTIR were recorded on a Bruker IRS48 spectrometer; ^{1}H NMR spectra were recorded at 300 MHz, and ^{13}C NMR at 75.5 MHz, on a Bruker Avance-300 instrument. The one-bond heteronuclear correlation (HMQC) and the long range ^{1}H–^{13}C correlation (HMBC) spectra were obtained using the inv4gs and the inv4gslplrnd programs, respectively, with the Bruker software; Microanalysis was performed in a Perkin–Elmer 2400 CHN by the *Servicio de Microanálisis de la Universidad Complutense de Madrid*. Silica gel used for column chromatography was 230–100 mesh.

4.1.1. General procedure for {5-[(carboxyalkyl)-methyl]-2-thioxo-[1,3,5]thiadiazinan-3-yl]-alkyl}-6-thioxo-[1,3,5]-thiadiazinan-3-yl]-carboxylic acids (Ia–k, IIa–g). To a stirred solution of the corresponding diamine (5 mmol) in 25 mL of water, potassium hydroxide (0.56 g, 10 mmol, as a 20% aqueous solution), and carbon disulfide (0.6 mL, 10 mmol) were added at room temperature. The mixture was subsequently stirred for 4 h. Then formaldehyde solution 37% (1.6 mL, 20 mmol) was added and the stirring continued for 1 h. The

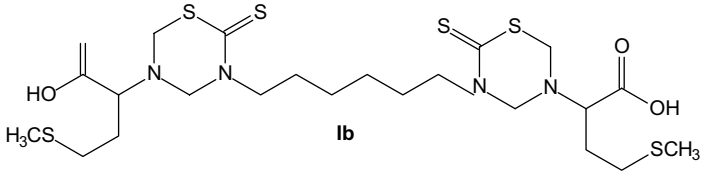
Table 2. Biological activity of series **II** against *Trypanosoma cruzi* and *Trichomonas vaginalis*

Compound	Concn (μg/mL)	Cytotoxicity (% *C*)	Antiparasitic activity		
			Trypanosoma (%AE)	*Trichomonas*[a]	
				24 h	48 h
IIa	100	76.6	94.0	100	100
	10	68.1	29.6	99.0	100
	1	13.3	16.6	(54.7)	(13.3)
IIb	100	67.8	53.2	100	100
	10	61.0	21.5	94.4	97.2
	1	4.2	16.1	(42.9)	(16.0)
IIc	100	65.6	77.6	100	100
	10	64.4	35.6	97.7	100
	1	0	21.5	56.7	(14.0)
IId	100	10.1	76.8	(11.8)	(9.9)
	10	7.7	28.9	(0)	(0)
	1	0	6.8	(0)	(0)
IIe	100	37.8	83.5	100	100
	10	31.1	59.3	(84.0)	(84.5)
	1	20.9	4.8	(27.1)	(20.3)
IIf	100	94.5	87.1	100	100
	10	50.7	60.4	(64.4)	(69.9)
	1	7.7	57.0	(0)	(6.5)
IIg	100	23.6	93.7	100	100
	10	12.1	93.6	(82.2)	(92.8)
	1	0	79.7	(38.8)	(9.4)
Nifurtimox	10		89.1		
	1		54.9		
	0.5		45.6		
Metronidazol	2			100	100
	1			97.9	100
	0.5			(83.5)	94.1

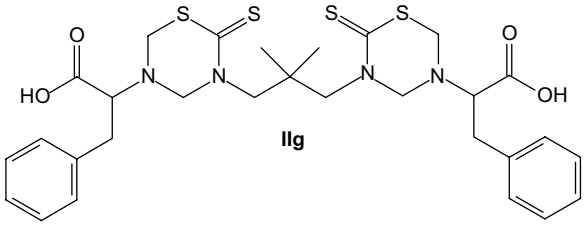
[a] Anti-*Trichomonas* activity is expressed as percentages of reduction and/or growth (parentheses), indicative of cytocidal or (cytostatic) activities, respectively, after 24 and 48 h post-incubation.



Ii



Ib



IIg

Figure 2.

Table 3. Trypanocidal and trichomonacidal activities expressed as approx. IC_{50} (μg/mL) for the five most active compounds and the reference drugs

Compound	Approx. IC_{50}	
	Trypanosoma	*Trichomonas*
Ib	1.6	0.2
Ii	0.2	0.8
Ik	1.4	6.5
IIf	0.7	7.3
IIg	<0.1	1.7
Nifurtimox	0.7	
Metronidazole		0.3

reaction mixture was added dropwise to a suspension of the corresponding aminoacid or glycyl-glycine (10 mmol) in a pH 7.8 buffer solution of phosphate (10 mL), stirred for 2 h and filtered off. The aqueous solution was cooled in an ice bath and acidified to pH 2 by 15% hydrochloric acid. In most cases the obtained precipitate was filtered and then kept in a vacuum drier overnight. The solid residue was crushed with cold ether and filtered. For compounds **Ij** and **Ik**, after acidification of the reaction mixture the resulted oil was extracted with ethyl acetate and dried over sodium sulfate. The solvent was removed and the oil was precipitated from cold ether. Purification was carried out by column chromatography on silica gel (cyclohexane/EtOH, 5/1 to 5/3).

4.1.2. {5-[6-(5-Carboxymethyl-2-thioxo-[1,3,5]thiadiazinan-3-yl)-hexyl]-6-thioxo-[1,3,5]thiadiazinan-3-yl}-acetic acid (Ia). From 1,6-diaminohexane (580 mg, 5 mmol) and glycine (950 mg, 10 mmol); yield **Ia** (1530 mg, 66%); mp 130–132 °C; IR (KBr) ν_{max} 2931 (νOH), 2854 (ν_{as} CH_2), 1712 (νC=O), 1506 (νC=S), 1332 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.27 (4H, m, 9-H, 10-H), 1.56 (4H, m, 8-H, 11-H), 3.50 (4H, s, 2 × CH_2CO_2H), 3.87 (4H, t, J = 7.6 Hz, 7-H, 12-H), 4.51 (4H, s, 6-H, 6′-H), 4.52 (4H, s, 4-H, 4′-H) ppm; ^{13}C NMR (DMSO-d_6) δ 26.0 (C-9, C-10), 26.1 (C-8, C-11), 51.1 (2 × CH_2CO_2H), 51.3 (C-7, C-12), 58.4 (C-6, C-6′), 69.8 (C-4, C-4′), 171.0 (2 × CO_2H), 190.3 (C-2, C-2′) ppm. Anal. Calcd for $C_{16}H_{26}N_4O_4S_4$: C 41.19, H 5.62, N 12.02; Found: C 41.31, H 5.29, N 12.19.

4.1.3. 2-(5-{6-[5-(1-Carboxy-3-methylsulfanyl-propyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-4-methyl sulfanyl-butyric acid (Ib). From 1,6-diaminohexane (580 mg, 5 mmol) and L-Methionine (1490 mg, 10 mmol); yield **Ib** (1470 mg, 48%); mp 85–87 °C; IR (KBr) ν_{max} 2929 (νOH), 2854 ($\nu_{as}CH_2$), 1718 (νC=O), 1469 (νC=S), 1330 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.13 (4H, m, 9-H, 10-H), 1.42 (4H, m, 8-H, 11-H), 1.82–1.88 (4H, m, 2 × NCH(CO_2H)CH_2–), 2.29 (6H, s, 2 × SCH_3), 2.34–2.39 (4H, m, 2 × –CH_2SCH_3), 3.38–3.40 (2H, m, 2 × NCH(CO_2H)–), 3.67 (4H, t, J = 7.6 Hz, 7-H, 12-H), 4.37 (4H, d, J = 11.2 Hz, 6-H, 6′-H), 4.46 (4H, d, J = 8.7 Hz, 4-H, 4′-H) ppm. Anal. Calcd for $C_{26}H_{36}N_4O_4S_6$: C 43.13, H 5.93, N 9.15; Found: C 43.34, H 5.71, N 9.23.

4.1.4. 6-(5-{6-[5-(5-Carboxy-pentyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-hexanoic acid (Ic). From 1,6-diaminohexane (580 mg, 5 mmol) and 6-aminohexanoic acid (1300 mg, 10 mmol); yield **Ic** (2080 mg, 72%); mp 110–112 °C; IR (KBr) ν_{max} 2937 (νOH), 2854 ($\nu_{as}CH_2$), 1693 (νC=O), 1500 (νC=S), 1326 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.29–1.34 (8H, m, 9-H, 10-H, 2 × N(CH$_2$)$_2$CH_2–), 1.47–1.59 (12H, m, 8-H, 11-H, 2 × NCH$_2$CH_2–, 2 × –CH_2CH$_2$-CO_2H), 2.20 (4H, t, J = 7.2 Hz, 2 × –CH_2CO_2H), 2.65 (4H, t, J = 7.0 Hz, 2 × NCH_2–), 3.89 (4H, t, J = 7.4 Hz, 7-H, 12-H), 4.46 (4H, s, 6-H, 6′-H), 4.48 (4H, s, 4-H, 4′-H) ppm; ^{13}C NMR (DMSO-d_6) δ 24.7 (2 × –$CH_2CH_2CO_2H$), 26.1, −26.8 (C-9, C-10, C-8, C-11, 2 × NCH$_2$CH_2–, 2 × N(CH$_2$)$_2$CH_2–), 34.1 (2 × –CH_2CO_2H), 49.4 (2 × NCH_2–), 51.4 (C-7, C-12), 57.6 (C-6, C-6′), 69.7 (C-4, C-4′), 174.9 (2 × CO_2H), 190.4 (C-2, C-2′) ppm. Anal. Calcd for $C_{24}H_{42}N_4O_4S_6$: C 49.80, H 7.31, N 9.68; Found: C 49,56; H 7.03, N 9.48.

4.1.5. 3-(5-{6-[5-(2-Carboxy-ethyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-propionic acid (Id). From 1,6-diaminohexane (580 mg, 5 mmol) and β-alanine (890 mg, 10 mmol); yield **Id** (1250 mg, 45%); mp 68–70 °C; IR (KBr) ν_{max} 2929 (νOH), 2854 ($\nu_{as}CH_2$), 1712 (νC=O), 1505 (νC=S), 1323 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.29 (4H, m, 9-H, 10-H), 1.51–1.56 (4H, m, 8-H, 11-H), 2.32 (4H, t, J = 6.5 Hz, 2 × –CH_2CO_2H), 2.66 (4H, t, J = 6.9 Hz, 2 × NCH_2–), 3.87 (4H, t, J = 7.4 Hz, 7-H, 12-H), 4.46 (4H, s, 6-H, 6′-H), 4.48 (4H, s, 4-H, 4′-H) ppm. Anal. Calcd for $C_{18}H_{30}N_4O_4S_4$: C 43.70, H 6.11, N 11.32; Found: C 43.91, H 6.37, N 11.43.

4.1.6. 2-[5-(6-{5-[(Carboxymethyl-carbamoyl)-methyl]-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl]-acetylamino)-acetic acid (Ie). From 1,6-diaminohexane (580 mg, 5 mmol) and glycyl-glycine (1320 mg, 10 mmol); yield **Ie** (1360 mg, 47%); mp 93–95 °C; IR (KBr) ν_{max} 3313 (νNH), 2935 (νOH), 2852 ($\nu_{as}CH_2$), 1718 (νC=O_{acid}), 1666 (νC=O_{amide}), 1508 (νC=S), 1319 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.27 (4H, m, 9-H, 10-H), 1.58 (4H, m, 8-H, 11-H), 3.42 (4H, s, 2 × NCH_2C(=O)–), 3.74 (4H, d, J = 5.8 Hz, 2 × –NHCH_2CO_2H), 3.89 (4H, t, J = 7.4 Hz, 7-H, 12-H), 4.50 (4H, s, 6-H, 6′-H), 4.52 (4H, s, 4-H, 4′-H), 8.27 (2H, t, J = 5.8 Hz, 2 × NH) ppm; ^{13}C NMR (DMSO-d_6) δ 26.0 (C-9, C-10), 26.2 (C-8, C-11), 41.3 (2 × –NHCH_2CO_2H), 51.6 (C-7, C-12), 53.3 (2 × NCH_2 C(=O)–), 58.7 (C-6, C-6′), 69.6 (C-4, C-4′), 168.7 (2 × $CONH$–), 171.6 (2 × CO_2H), 190.1 (C-2, C-2′) ppm. Anal. Calcd for $C_{20}H_{32}N_6O_6S_4$: C 41.36, H 5.55, N 14.47. Found: C 41.54, H 5.78, N 14.27.

4.1.7. 2-(5-{6-[5-(1-Carboxy-3-methyl-butyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-4-methyl pentanoic acid (If). From 1,6-diaminohexane (580 mg, 5 mmol) and L-leucine (830 mg, 10 mmol); yield **If** (1550 mg, 51%); mp 95–97 °C; IR (KBr) ν_{max} 2931 (νOH), 2858 ($\nu_{as}CH_2$), 1718 (νC=O), 1498 (νC=S), 1330 (νC–O), 927 (δOH) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.76–0.80 (12H, m, 2 ×

–CH(CH_3)$_2$), 1.20 (6H, m, 9-H, 10-H, 2 × –CH(CH$_3$)$_2$), 1.45–1.49 (8H, m, 8-H, 11-H, 2 × NCH(CO$_2$H)CH_2–), 3.38–3.40 (2H, m, 2 × NCH(CO$_2$H)–), 3.80 (4H, t, J = 7.2 Hz, 7-H, 12-H), 4.37 (4H, s, 6-H, 6′-H), 4.48 (4H, s, 4-H, 4′-H) ppm. Anal. Calcd for $C_{24}H_{42}N_4O_4S_4$: C 49.80, H 7.31, N 9.68. Found: C 49.65, H 7.57, N 9.78.

4.1.8. 2-(5-{6-[5-(1-Carboxy-2-phenyl-ethyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-3-phenyl propionic acid (Ig). From 1,6-diaminohexane (580 mg, 5 mmol) and L-phenylalanine (1650 mg, 10 mmol); yield **Ig** (1950 mg, 52%); mp 98–100 °C; IR (KBr) ν_{max} 2929 (νOH), 2854 ($\nu_{as}CH_2$), 1718 (νC=O), 1496 (νC=S), 1328 (νC–O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.27–1.61 (8H, m, 9-H, 10-H, 8-H, 11-H), 3.13–3.15 (2H, m, 2 × NCH(CO$_2$H)–), 3.80 (4H, t, J = 7.7 Hz 7-H, 12-H), 4.40–4.45 (4H, m, 2 × –CH_2Ph), 4.47 (4H, s, 6-H, 6′-H), 4.52 (4H, s, 4-H, 4′-H), 7.18–7.30 (10H, m, H–Ph) ppm. Anal. Calcd for $C_{30}H_{38}N_4O_4S_4$: C 55.70, H 5.92, N 8.66. Found: C 55.91, H 5.71, N 8.85.

4.1.9. 4-Carbamoyl-2-(5-{6-[5-(3-carbamoyl-1-carboxy-propyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-butyric acid (Ih). From 1,6-diaminohexane (580 mg, 5 mmol) and L-glutamine (1480 mg, 10 mmol); yield **Ih** (1820 mg, 60%); mp 78–81 °C; IR (KBr) ν_{max} 3406 (νNH), 2925 (νOH), 2852 ($\nu_{as}CH_2$), 1683 (νC=O$_{acid}$), 1635 (νC=O$_{amide}$), 1488 (νC=S), 1330 (νC–O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30–1.33 (4H, m, 9-H, 10-H), 1.53–1.56 (4H, m, 8-H, 11-H), 2.64–2.68 (4H, m, 2 × NCH(CO$_2$H)CH_2–), 2.77 (4H, t, J = 7.1 Hz, 2 × –CH_2CONH$_2$), 3.56–3.66 (2H, m, 2 × NCH(CO$_2$H)–), 3.90 (4H, t, J = 7.4 Hz, 7-H, 12-H), 4.47 (4H, s, 6-H, 6′-H), 4.49 (4H, s, 4-H, 4′-H) ppm. Anal. Calcd for $C_{22}H_{36}N_6O_6S_4$: C 43.40, H 5.96, N 13.80. Found: C 43.57, H 5.67, N 13.62.

4.1.10. 5-(5-{6-[5-(5-Carboxy-butyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-pentanoic acid (Ii). From 1,6-diaminohexane (580 mg, 5 mmol) and 4-aminobutyric acid (1170 mg, 10 mmol); yield **Ii** (1780 mg, 65%); mp 120–123 °C; IR (KBr) ν_{max} 2935 (νOH), 2852 ($\nu_{as}CH_2$), 1706 (νC=O), 1500 (νC=S), 1330 (νC–O) cm^{-1} ;^{1}H NMR (DMSO-d_6) δ 1.29–1.31 (4H, m, 9-H, 10-H), 1.58–1.60 (4H, m, 8-H, 11-H), 1.70–1.75 (4H, m, 2 × NCH$_2$CH_2), 2.26 (4H, t, J = 7.2 Hz, 2 × –CH_2CO$_2$H), 2.68 (4H, t, J = 7.0 Hz, 2 × NCH_2–), 3.89 (4H, t, J = 7.7 Hz, 7-H, 12-H), 4.45 (4H, s, 6-H, 6′-H), 4.47 (4H, s, 4-H, 4′-H) ppm; ^{13}C NMR (DMSO-d_6) δ 22.7 (2 × NCH$_2$CH_2), 26.1 (C-9, C-10, C-8, C-11), 31.6 (2 × –CH_2CO$_2$H), 49.1 (2 × NCH_2–), 51.5 (C-7, C-12) 57.9 (C-6, C-6′), 69.7 (C-4, C-4′), 174.6 (2 × CO_2H), 190.4 (C-2, C-2′) ppm. Anal. Calcd for $C_{20}H_{34}N_4O_4S_4$: C 45.95, H 6.56, N 10.72. Found: C 45.78, H 6.30, N 10.57.

4.1.11. 3-(5-{6-[5-(1-Carboxymethyl-2-oxo-propyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-4-oxo-pentanoic acid (Ij). From 1,6-diaminohexane (580 mg, 5 mmol) and L-aspartic (1330 mg, 10 mmol); yield **Ij** (1330 mg, 45%); mp 104–106 °C; IR (KBr) ν_{max} 2925 (νOH), 2852 ($\nu_{as}CH_2$), 1618 (νC=O), 1498 (νC=S), 1388 (νC–O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30–1.34 (4H, m, 9-H, 10-H), 1.51–1.56 (4H, m, 8-H, 11-H), 2.43 (4H, dd, J = 19.1 Hz, J = 3.2 Hz, 2 × –CH_2CO$_2$H), 3.77 (2H, dd, J = 3.5 Hz, J = 3.3 Hz, 2 × NCH(CO$_2$H)–), 3.89 (4H, t, J = 6.2 Hz, 7-H, 12-H), 4.47 (4H, s, 6-H, 6′-H), 4.49 (4H, s, 4-H, 4′-H) ppm. Anal. Calcd for $C_{20}H_{30}N_4O_8S_4$: C 41.22, H 5.19, N 9.61. Found: C 41.43, H 5.31, N 9.43.

4.1.12. 3-(5-{6-[5-(1-Carboxymethyl-2-amino-propyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-4-amino-pentanoic acid (Ik). From 1,6-diaminohexane (580 mg, 5 mmol) and L-Asparagine (1590 mg, 10 mmol); yield **Ik** (1290 mg, 63%); mp 102–105 °C; IR (KBr) ν_{max} 3382 (νNH), 2925 (νOH), 2852 ($\nu_{as}CH_2$), 1679 (νC=O$_{acid}$), 1647 (νC=O$_{amide}$), 1500 (νC=S), 1361 (νC–O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.30–1.34 (4H, m, 9-H, 10-H), 1.51–1.55 (4H, m, 8-H, 11-H), 2.64–2.69 (4H, m, 2 × –CH_2CONH$_2$), 2.75–2.79 (2H, m, 2 × NCH(CO$_2$H)–), 3.89 (4H, t, J = 7.2 Hz, 7-H, 12-H), 4.46 (4H, s, 6-H, 6′-H), 4.49 (4H, s, 4-H, 4′-H). Anal. Calcd for $C_{20}H_{32}N_6O_6S_4$: C 41.36, H 5.55, N 14.47. Found: C 41.54, H 5.23, N 14.58.

4.1.13. {5-[3-(5-Carboxymethyl-2-thioxo-[1,3,5]thiadiazinan-3-yl)-2,2-dimethyl-propyl]-6-thioxo-[1,3,5]thiadiazinan-3-yl}-acetic acid (IIa). From 2,2-dimethyl-1,3-propanediamine (0.43 mL, 5 mmol) and glycine (950 mg, 10 mmol); yield **IIa** (880 mg, 39%); mp 146–148 °C; IR (KBr) ν_{max} 2962 (νOH), 2925 ($\nu_{as}CH_2$), 1712 (νC=O), 1488 (νC=S), 1340 (νC–O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 1.06 (6H, s, 9-H, 9′-H), 3.56 (4H, s, 2 × –CH_2CO$_2$H), 4.16 (4H, s, 7-H, 10-H), 4.52 (4H, s, 6-H, 6′-H), 4.64 (4H, s, 4-H, 4′-H) ppm; ^{13}C NMR (DMSO-d_6) δ 25.8 (C-9, C-9′), 50.7 (C-7, C-10), 57.8 (2 × –CH_2CO$_2$H), 58.7 (C-6, C-6′), 72.0 (C-4, C-4′), 169.2 (2 × CO_2H), 191.3 (C-2, C-2′) ppm. Anal. Calcd for $C_{15}H_{25}N_4O_4S_4$: C 39.80, H 5.34, N 12.38. Found: C 39.69, H 5.43, N 12.20.

4.1.14. 2-(5-{3-[5-(1-Carboxy-3-methylsulfanyl-propyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-2,2-dimethyl-propyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-4-methylsulfanyl-butyricacid (IIb). From 2,2-dimethyl-1,3-propanediamine (0.43 mL, 5 mmol) and L-methionine (1490 mg, 10 mmol); yield **IIb** (1320 mg, 44%); mp 83–85 °C; IR (KBr) ν_{max} 2906 (νOH), 2914 ($\nu_{as}CH_2$), 1720 (νC=O), 1485 (νC=S), 1336 (νC–O) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.91 (6H, s, 9-H, 9′-H), 2.02–2.05 (4H, m, 2 × NCH(CO$_2$H)CH_2–), 2.45 (6H, s, 2 × –SCH_3), 2.54–2.56 (4H, m, 2 × –CH_2SCH$_3$), 3.55–3.58 (2H, m, 2 × NCH(CO$_2$H)–), 4.20 (4H, s, 7-H, 10-H), 4.41 (4H, d, J = 13.6 Hz, 6-H, 6′-H), 4.56 (4H, d, J = 13.7 Hz, 4-H, 4′-H) ppm; ^{13}C NMR (DMSO-d_6) δ 15.0 (2 × –SCH$_3$), 24.1 (C-9, C-9′), 29.2 (2 × NCH(CO$_2$H)CH_2–), 32.6 (2 × –CH_2SCH$_3$), 51.6 (C-7, C-10), 55.8 (C-6, C-6′), 59.2 (2 × NCH-(CO$_2$H)–), 63.1 (C-4, C-4′), 173.3 (2 × CO_2H), 190.7 (C-2, C-2′) ppm. Anal. Calcd for $C_{21}H_{34}N_4O_4S_6$: C 42.12, H 5.72, N 9.36. Found: C 42.36, H 5.97, N 9.51.

4.1.15. 6-(5-{3-[5-(5-Carboxy-pentyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-2,2-dimethyl-propyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-hexanoic acid (IIc). From 2,2-dimethyl-1,3-propanediamine (0.43 mL, 5 mmol) and 6-aminohexanoic acid (1300 mg, 10 mmol); yield **IIc** (1000 mg, 35%); mp 135–137 °C; IR (KBr) ν_{max} 2931 (νOH), 2860 ($\nu_{as}CH_2$), 1701 (νC=O), 1479 (νC=S), 1294 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.81 (6H, s, 9-H, 9′-H), 0.97–1.01 (12H, m, 2 × NCH_2 $(CH_2)_3CH_2$–), 1.71 (4H, t, J = 7.1 Hz, 2 × –CH_2CO_2H), 2.17 (4H, t, J = 7.0 Hz, 2 × NCH_2–), 3.67 (4H, s, 7-H, 10-H), 3.97 (4H, s, 6-H, 6′-H), 4.08 (4H, s, 4-H, 4′-H) ppm; ^{13}C NMR (DMSO-d_6) δ 24.1 (2 × $N(CH_2)_3$ CH_2–), 25.8 (C-9, C-9′), 26.4 (2 × NCH_2CH_2–), 26.8 (2 × $N(CH_2)_2CH_2$–), 34.0 (2 × –CH_2CO_2H), 49.0 (2 × NCH_2–), 57.9 (C-7, C-10), 60.4 (C-6, C-6′), 72.3 (C-4, C-4′), 174.8 (2 × CO_2H), 193.4 (C-2, C-2′) ppm. Anal. Calcd for $C_{23}H_{40}N_4O_4S_4$: C 48.91, H 7.14, N 9.92. Found: C 48.76, H 7.34, N 9.78.

4.1.16. 3-(5-{6-[5-(2-Carboxy-ethyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-hexyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-propionic acid (IId). From 2,2-dimethyl-1,3-propanediamine (0.43 mL, 5 mmol) and β-alanine (890 mg, 10 mmol); yield **IId** (820 mg, 34%); mp 65–67 °C; IR (KBr) ν_{max} 2956 (νOH), 2929 ($\nu_{as}CH_2$), 1700 (C=O), 1475 (νC=S), 1332 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.70 (6H, s, 9-H, 9′-H), 2.31 (4H, t, J = 6.5 Hz, 2 × –CH_2CO_2H), 2.80 (4H, t, J = 6.5 Hz, 2 × NCH_2–) 3.71 (4H, s, 7-H, 10-H), 4.00 (4H, s, 6-H, 6′-H), 4.19 (4H, s, 4-H, 4′-H) ppm. Anal. Calcd for $C_{17}H_{28}N_4O_4S_4$: C 42.48, H 5.87, N 11.66. Found: C 42.21, H 5.55, N 11.46.

4.1.17. 2-[5-(3-{5-[(Carboxymethyl-carbamoyl)-methyl]-2-thioxo-[1,3,5]thiadiazinan-3-yl]-2,2-dimethyl-propyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl]-acetylamino)-acetic acid (IIe). From 2,2-dimethyl-1,3-propanediamine (0.43 mL, 5 mmol) and glycyl-glycine (1320 mg, 10 mmol); yield **IIe** (1222 mg, 43%); mp 154–156 °C; IR (KBr) ν_{max} 3325 (νNH), 2972 (νOH), 2914 (νCH_2), 1741 (νC=O, ácido), 1636 (C=O, amida), 1483 (νC=S), 1309 (ν C–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.07 (6H, s, 9-H, 9′-H), 3.36 (4H, s, 2 × NCH_2C(=O)–), 3.77 (4H, d, J = 6.3 Hz, 2 × –$NHCH_2CO_2H$), 4.18 (4H, s, 7-H, 10-H), 4.48 (4H, s, 6-H, 6′-H), 4.66 (4H, s, 4-H, 4′-H), 8.37 (2H, t, J = 6.5 Hz, 2 × NH) ppm; ^{13}C NMR (DMSO-d_6) δ 25.8 (C-9, C-9′), 40.7 (2 × –$NHCH_2CO_2H$), 52.9 (2 × NCH_2C(=O)–), 59.0 (C-6, C-6′), 60.4 (C-7, C-10), 71.8 (C-4, C-4′), 168.8 (2 × –CONH), 171.5 (2 × –CO_2H), 193.1 (C-2, C-2′) ppm; Anal. Calcd for $C_{19}H_{30}N_6O_6S_4$: C 40.27, H 5.34, N 14.83. Found C 40.41, H 5.23, N 14.67.

4.1.18. 2-(5-{3-[5-(1-Carboxy-3-methyl-butyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-2,2-dimethyl-propyl}-6-thioxo-[1,3,5]thiadiazinan-3yl)-4-methylpentanoic acid (IIf). From 2,2-dimethyl-1,3-propanediamine (0.43 mL, 5 mmol) and L-leucine (830 mg, 10 mmol); yield **IIf** (1250 mg, 44%); mp 85–87 °C; IR (KBr) ν_{max} 2931 (νOH), 2869 ($\nu_{as}CH_2$), 1720 (νC=O), 1469 (νC=S), 1299 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.90 (12H, s, 2 × –$CH(CH_3)_2$), 0.93–0.99 (8H, m, 9-H, 9′-H, 2 × –$CH(CH_3)_2$), 1.51–1.54 (4H, m, 2 × $NCH(CO_2H)CH_2$–), 2.77–2.79 (2H, m, 2 × $NCH(CO_2H)$–), 4.17 (4H, s, 7-H, 10-H), 4.35 (4H, d, J = 13.8 Hz, 6-H, 6′-H), 4.58 (4H, d, J = 13.7 Hz, 4-H, 4′-H) ppm; ^{13}C NMR (DMSO-d_6) δ 21.9 (2 × –$CH(CH_3)_2$), 25.5 (C-9, C-9′), 24.3 (2 × –$CH(CH_3)_2$), 38.3 (2 × $NCH(CO_2H)CH_2$–), 51.6 (C-7, C-10), 55.8 (C-6, C-6′), 58.8 (2 × $NCH(CO_2H)$–), 66.4 (C-4, C-4′), 173.7 (2 × CO_2H), 192.4 (C-2, C-2′) ppm; Anal. Calcd for $C_{23}H_{40}N_4O_4S_4$: C 48.91, H 7.14, N 9.92. Found: C 48.70, H 7.01, N 9.79.

4.1.19. 2-(5-{3-[5-(1-Carboxy-2-phenyl-ethyl)-2-thioxo-[1,3,5]thiadiazinan-3-yl]-2,2-dimethyl-propyl}-6-thioxo-[1,3,5]thiadiazinan-3-yl)-3-phenyl propionic acid (IIg). From 2,2-dimethyl-1,3-propanediamine (0.43 mL, 5 mmol) and L-Phenylalanine (1650 mg, 10 mmol); Yield **IIg** (1350 mg, 43%); mp 100–102 °C; IR (KBr) ν_{max} 2962 (νOH), 2927 ($\nu_{as}CH_2$), 1724 (C=O), 1494 (νC=S), 1334 (νC–O) cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.97 (6H, s, 9-H, 9′-H), 3.25 (2H, dd, J = 4.4 Hz, J = 4.5 Hz, 2 × $NCH(CO_2H)$–), 4.21 (4H, s, 7-H, 10-H), 4.34 (4H, dd, J = 10.2 Hz, J = 4.5 Hz, 2 × –CH_2Ph), 4.45 (4H, d, J = 13.7 Hz, 6-H, 6′-H), 4.69 (4H, d, J = 13.8 Hz, 4-H, 4′-H), 7.12–7.31 (10H, m, H–Ph) ppm; ^{13}C NMR (DMSO-d_6) δ 24.1 (C-9, C-9′), 35.7 (2 × –CH_2Ph), 51.6 (C-7, C10), 55.9 (C-6, C-6′), 62.2 (2 × $NCH(CO_2H)$), 73.9 (C-4, C-4′), 126.8–137.7 (C-Ph), 173.3 (2 × CO_2H), 192.2 (C-2, C-2′) ppm; Anal. Calcd for $C_{29}H_{36}N_4O_4S_4$: C 55.04, H 5.73, N 8.85. Found: C 55.24, H 5.91, N 8.97.

4.2. Antiprotozoal in vitro assays

4.2.1. Trypanocidal in vitro test. Trypanocidal activity was determined by a colorimetric method that uses *T. cruzi* strain CL (clone CL B5) epimastigotes that were genetically engineered to express the *Escherichia coli* β-galactosidase gene, *lacZ*. The amount of β-galactosidase activity is directly proportional to the number of transfected parasites.[25] To perform this test, epimastigotes were grown in LIT medium supplemented with 10% heat-inactivated foetal calf serum at 28 °C. When the epimastigotes were in log phase 1×10^5/mL (200 μL/well) were seeded into microtiter plates. The parasites were exposed to the test compounds, initially dissolved in DMSO, at 100, 10, and 1 μg/mL in triplicate. After 72 h of incubation at 28 °C, 25 μL of CPRG (Chlorophenol red β-D-galactopyranoside) 100 μM in 0.1% Triton X-100 was added to each well and enzymatic reaction was allowed for 6 h at 37 °C. Optical densities (OD) were then read at 595 nm. Percentages of antiepimastigote activity (% AE) were determined with respect to controls, as

$$\%\ AE = [(OD_e - OD_{eb})/(OD_c - OD_{cb})] \times 100$$

Being OD_e and OD_c, the optical densities of experimental groups and controls, and OD_{eb} and OD_{cb}, the blanks of compounds and culture medium, respectively. Nifurtimox was the reference drug.

4.2.2. Trichomonacidal in vitro test. Trichomonacidal activity was assayed on *T. vaginalis* strain JH 31A

(ATTC, Maryland) in TYM medium supplemented with heat-inactivated horse serum as described.[26] By means of microscopic counts, cytostatic (percentage of growth inhibition) and cytocidal (percentage of reduction) activities were determined with respect to controls. Metronidazol was used as reference drug.

4.3. Cytotoxicity test

Toxicity of the compounds to mammalian cells was determined. J774 macrophages cultured in RPMI 1640 medium were seeded (70,000 cells/well) in 96-well flat-bottom microplates (NUNC) with 200 μL of medium. The cells were allowed to attach for 24 h at 37 °C and then exposed to the compounds for another 24 h. After addition of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], the amount of formazan was quantified by measurement of optical densities at 595 nm. Results are expressed as percentages of cytotoxicity.

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