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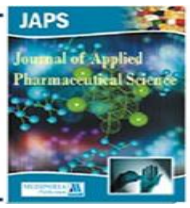

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Synthesis, characterization and anti-trypanosomal activity of R-(-)carvone and arylketones-thiosemi carbazones and toxicity against *Artemia salina* Leach.

Bienvenu Glinma, Salome D. S. Kpoviessi, Raymond H. Fatondji, Fernand A. Gbaguidi, Coco N. Kapanda, Joanne Bero, Didier M. Lambert, Veronique Hannaert, Joëlle Quetin-Leclercq, Mansourou Moudachirou, Jacques Poupaert and Georges C. Accrombessi

Bienvenu Glinma, Salome D. S. Kpoviessi, Raymond H. Fatondji, Fernand A. Gbaguidi, Mansourou Moudachirou and Georges C. Accrombessi
Department of Chemistry, Faculty of Sciences and Technologies,University of Abomey-Calavi, LACOPS, 01 PB : 4521 Cotonou, Benin.

Coco N. Kapanda, Joanne Bero, Didier M. Lambert, Joëlle Quetin-Leclercq and Jacques Poupaert
Louvain Drug Research Institute (LDRI), School of Pharmacy, Université catholique de Louvain, B1 7203 Avenue Emmanuel Mounier 72, B-1200 Brussels, Belgium.

Veronique Hannaert
Research Unit for Tropical Diseases (TROP), Christian de Duve Institute of Cellular Pathology (formerlyICP), Université catholique de Louvain, ICP-TROP 74.39,Avenue Hippocrate 74-75, B-1200 Brussels, Belgium

For Correspondence
Salome D. S. Kpoviessi
Department of Chemistry, Faculty of Sciences and Technologies,University of Abomey-Calavi, LACOPS, 01 PB : 4521 Cotonou, Benin.
Tel: +229 97883927

ABSTRACT

This work is focused on the synthesis and characterization of a series of N(4)-substituted thiosemicarbazones and the evaluation of their *in-vitro* anti-trypanosomal activity and toxicity. A series of thiosemicarbazones (**1**-**4**) and N(4)-phenyl-3-thiosemicarbazones (**5**-**8**) have been synthesized on R-(-)carvone, acetophenone, 4'-methylacetophenone and benzophenone by condensation reaction with good yields. All compounds were characterized by spectrometrical analysis methods infrared IR, nuclear magnetic resonance NMR (^{1}H &^{13}C) and mass spectrometry MS, confirming their structures respectively, and were evaluated for their *in-vitro* parasitic activity against the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* using the "LILIT, Alamar Blue" method (Baltz*et al*., 1985; Hirumi et al., 1994; Räz *et al*.,1997). Their toxicity against brine shrimp larvae (*Artemia salina* Leach) was studied, according to the method of Michael *et al.* (1956) resumed byVanhaecke *et al.* (1981) and bySleet and Brendel (1983). Some of them have exhibited a strong trypanocidal activity, especially compounds 8, 3, 1 and 4 with their half-inhibitory concentrations (IC_{50}) values equal to 8.48, 8.73, 39.71 and 67.17 micro-molar (µM) respectively. Except compounds **1** and **4**whose half-lethal concentration (LC_{50}) values were20.58 and 33.72 µM respectively and then toxics, all synthesized compounds showed negligible toxicity against *Artemia salina*L. (LC_{50}> 280 µM) and good selectivity (S) (SI "index" ≤1).

Key words: Thiosemicarbazones, 4-phenyl-3-thiosemicarbazones, spactrometrical analysis, Trypanocidal Activity, toxicity,Artemia salina L, selectivity index (SI).

INTRODUCTION

Parasitic diseases represent a major health problem worldwide with very limited treatment options, most treatments available dating back to decades and have limited effectiveness and / or undesirable side effects (Sanchez-Delgado and Anzellotti, 2004). Trypanosomes are known to be responsible for sicknesses presenting quite different clinical manifestations, geographical distribution, life cycle and insect vectors (Barrett *et al.*, 2003).Tse-tse flies are vectors of African trypanosomes, protozoa causing devastating diseases in humans and animals (Hu and Aksoy, 2005). An infectious disease of similar aetiology and epidemiology, the African trypanosomiasis is caused by the protozoan parasites agents of the genus *Trypanosoma* that live and multiply extracellularly in blood and tissue fluids of their mammalian hosts and are transmitted by the bite of infected tse-tse flies (*Glossinasp.*) (Sterverding, 2008). Human African

or sleeping sickness is caused by two subspecies of *T. brucei*: *T. brucei gambiense* and *T. brucei rhodesiense* which are found only in Africa (Wållbergand Harris, 2005), while the third subspecies, *T. brucei brucei*, is only infectious to animals (WHO, 2006).These diseases are responsible for considerable mortality and economic losses, and until now the drugs commonly used have often been very toxic and expensive, with no vaccine available (Courtin *et al*., 2008). They have been the subject of extensive investigations.The recent history of sleeping sickness, according to Steverding, reveals that trypanosomiasis can be controlled but probably not eradicated. The production of drugs against this evil is not always guaranteed; therefore, new, better and more affordable drugs are urgently required (Steverding *et al*., 2005; Steverding, 2008).

Recent studies have shown that thiosemicarbazones and their derivatives present a wide range of bioactivities (Beraldo *et al*., 2004; Graminha *et al*., 2008; Thota, 2010) and especially showed inhibitory effects against parasites (Du *et al*., 2002; Greenbaum *et al*., 2004 ;Fujii *et al*., 2005; Pérez-Rebolledo *et al*., 2008 ; Rosu *et al*., 2010 ; Fatondji *et al*., 2010; Glinma *et al*., 2011). In several cases, their pharmacological action is enhanced due to the presence of coordination metal ions (Chandra and Kumar, 2005; Rosu *et al*., 2010). It is well authenticated that a NS bidentate system is present in most of the thiosemicarbazones having carcinostatic potency and possessing substantial *in vitro* activity against various human tumor lines (Ferraz *et al*., 2009). Moreover, these properties are accentuated when these molecules are N(4)-substituted (Pérez-Rebolledo *et al*., 2008).

The aim of this work is to synthesize a series of thiosemicabazones and N(4)-phenyl-3-thiosemicarbazones, to evaluate their anti-trypanosomal activity on *Trypanosoma brucei brucei* and to test their toxicity against *Artemia salina* L.

MATERIAL AND METHODS

General techniques

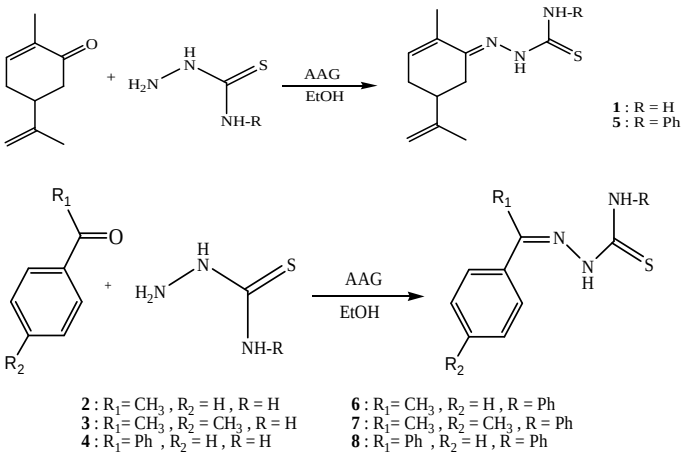
The melting points were taken on a fusionometer type *electrothermal 1A 9000* and are uncorrected. The IR spectra were recorded on a Perkin-Elmer FTIR 286. The frequencies of absorption bands are expressed in cm^{-1}. The NMR spectra were registered on a Brucker 500 in $CDCl_3$ (chloroform-d_6) or DMSO-d_6 (dimethylsulfoxide-d_6) which frequencies for ^1H and ^{13}C are 400 MHz and 100 MHz respectively. Chemical shifts are given in parts per million (ppm) relative to tetra-methyl silane as a benchmark. Multiplicity is designated as singlet (s), triplet (t), doublet (d) and multiplet (m).MS spectrometrical data of compounds were reported in APCI mode.

Chemistry

Reagents

Two reagents such as: thiosemicarbazide and 4-phenyl-3-thiosemicarbazide obtained from SIGMA-ALDRICH were used on carvone purchased from ACROS ORGANIQUE, acetophenone, 4'-methylacétophenone from FLUKA and benzophenone obtained from PROLABO. The glacial acetic acid used in the reaction is obtained from PROLABO. Ethanol 96° was used as recrystallization solvent products.

Method for the synthesis of Thiosemicarbazones (scheme 1)



Scheme1 : Synthetic routes of thiosemicarbazones (scaffold).

All compounds were synthesized through a similar method described by Aggarwal *et al*. (2008). Condensation reactions of these compounds are described in scheme 1. A solution of thiosemicarbazide or -phenyl-3-thiosemicarbazide (1mmol) in ethanol (5 mL) was added slowly to stirring solution of appropriate carbonyl compound (1 mmol) in 2.5 mL of ethanol containing glacial acetic acid, AAG, (1 mL). The solution was heated on a water bath for 10 minutes and cooled on an ice bath. The precipitate obtained on cooling was filtered, washed with cold distilled water until neutrality, dried and then recrystallized in ethanol 95° to give desired product.

After synthesizing, these compounds were submitted to their *in-vitro* trypanosomal activity against the bloodstream form of the strain 427 of *Trypanosoma brucei brucei*. They were also evaluated for their *in-vitro* toxicity against *Artemia salina* Leach followed performed methods.

Pharmacology

Anti-trypanosomal activity (LILIT, Alamar Blue^{TM})

The test is performed on the blood stream form of the strain 427 of *Trypanosoma brucei brucei* by the LILIT Alamar Blue^{TM} method (Baltz et al., 1985; Hirumi et al., 1994; Räz et al., 1997). The stock solutions of thiosemicarbazones have been prepared from an initial concentration of 10 mg/mL^{-1} in DMSO. The trypanosomes are grown in a medium containing 10% of heat-inactivated fetal calf serum and blood stream form supporting factor. The trypanosome suspensions were adjusted to $5x10^{-4}$ $tryp{\cdot}mL^{-1}$. In each well, 50 µL of different dilutions of the stock solution were added to 50 µL of suspension of trypanosomes. The plates were then incubated at 37°C for 72 hours in an atmosphere with 5% CO_2. 10 µL of dye "Alamar Blue^{TM}" is added to each well and then incubated for 4 hours. The dye "Alamar Blue^{TM}" is a reagent for detecting enzymatic activity. The wells in which the

concentration of compound is insufficient to inhibit the proliferation of trypanosomes are stained. The MIC is the concentration of unstained wells in which there is the lowest amount of thiosemicarbazone. The plate reading is made in comparison with control wells on a fluorescence plate reader using an excitation wavelength of 530 nm and an emission wavelength 590 nm.

Toxicity against Artemia salina L.

The test is performed against larvae of brine shrimp (*Artemia salina* LEACH) by the method of Michael *et al*. (1956) resumed by Vanhaecke *et al*.(1981) and by Sleet and Brendel (1983). Thus, *Artemia salina* eggs are incubated in sea water until hatching of young larvae (48h). Then, series of solutions of test substances at increasing concentrations were prepared in DMSO (dimethyl sufoxide)/seawater. A determined number of larvae introduced into each solution. All solutions and control solutions containing no active substance were left stirring for 24hours.Counting under a microscope the number of death larvae in each solution used to evaluate the toxicity of the solution. In the case where there was death in the control medium, the data was corrected by Abbott's formula:

% death=[(test - control) /control)] x100(Abbott, 1925)

Data (dose-response) are transformed by logarithm and the half-lethal concentration LC_{50} is determined by linear regression (Hafner et al., 1977). Tests were carried out in triplicate.
All data were expressed as mean±standard deviation of triplicate measurements.

RESULTS AND DISCUSSIONS

Chemistry

Eight thiosemicarbazones substituted or not (**1-8**) were synthesized with good yields in range from 65 to 88%. The structures of synthesis compounds were characterized with spectrometrical analysis. IR spectra show the typical band frequencies of $-NH_2$ from 3415 to 3227,−NH− between 3151-3145 and C=N from 1608 to 1587 cm^{-1}in compounds **1-4** structures; and −NH− bands in the range from 3488 to 3302; C=N bands 1633-1588 cm^{-1} in compounds **5-8** structures. All the products show frequencies of the thioamide group (N-CS-N) bands present in each structure: 1123-845 cm^{-1},.

The analysis of the ^{13}C NMR spectra results confirms the presence of fundamentally functions. The C=S peaks appear in the range from 179.12 to 175.47 ppm, peaks of C=N between 150.21 and 146.84 ppm, aromatic carbons are shown from 123.05 to 140.29 ppm, the C=C carbons in the structures of products **1** and **5** are shown in 110, 128, 133, 135, 148 and 149 ppm and methyl carbons of compounds **2**, **3**, **6** and **7** in 12, 13, 21, 24 ppm. ^1H NMR spectra analysis gives the characteristic protons existing in each structure. δ: 6.3-6.62 ppm for protons signals in $\mathbf{H}_2$NCS (**1-4**). In =NN**H**−, the protons signals are shown from 10.3-8.6 ppm (**1-4**) and 9.35-9.45 ppm (**5-8)**; and in CSNH-Ph, from 8.75-8.80 ppm for compounds **5-8**.

The molar mass of each synthesized molecule given by mass spectrometry is consistent with theoretical mass found.
These various tests done on all compounds have really confirmed the presence of functional groups and various types of protons and carbons forming the sequences of each of their structure.

Characterization of synthesized compounds

Carvone thiosemicarbazone (1)

Yield: 65%;m.p: 111-112°C; **IR** (NaCl, cm^{-1}): 3415, 3259 ν(NH_2); 3145 ν(NH); 1598 ν(C=N). **^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 176.16, 148.29, 147.27, 133.28, 132.04, 110.32 30.50, 30.21, 21.41, 18.83 ; **^{1}H NMR** (DMSO-d_6, 400MHz) δ(ppm): 1.82 (s, 3H, CH_3); 1.9 (s, 3H, CH_3); 2.28 (m, 4H, $2CH_2$); 2.68 (m 1H, H_2C-CH-CH_2); 4.8 (s, 2H, =CH_2); 4.9 (t, 1H, CH=C); 6.3 (s, 2H, $CSNH_2$); 10.3 (s, 1H, =NNH−). **MS** (m/z): [MH^+] 224.12 ; [MH^+]found 224.11

Acetophenone thiosemicarbazone (2)

Yield: 85%;m.p: 120-121°C; **IR** (NaCl, cm^{-1}): 3408, broad ~3300, 3233 ν(NH_2); 3145 ν(NH); 1587 ν(C=N); 1074, 964, 845, ν(N-CS-N).**^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 177.41, 146.19, 135.07, 128.55, 127.13, 123.05, 12.28;**^{1}H NMR** (DMSO-d_6, 400MHz) δ(ppm): 2.3 (s, 3H, CH_3); 7.7-7.2 (m 5H, H-Ar); 6.5 (broad 2H, $CSNH_2$); 8.9 (s, 1H, =NNH−). **MS** (m/z): [MH^+] 194.10 ; [MH^+]found 194.07

4'-methyl acetophenone thiosemicarbazone (3)

Yield: 81% m.p:148-149°C; **IR** (NaCl, cm^{-1}): 3411, 3379, 3227 ν(NH_2); 3145 ν(NH); 1598 ν(C=N); 1093, 1016, 848 ν(N-CS-N);817, 717 ν(p-CH3-Ar). **^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 179.12, 148.30, 140.29, 134.38, 130.45, 129.32, 126.44, 126.33, 21.33, 13.62 ;**^{1}H NMR** ($CDCl_3$, 400MHz) δ(ppm): 2.25 (s, 3H, CH_3); 2.41 (s, 3H, CH_3); 6.62 (s, 2H, $CSNH_2$); 7.6-7.15 (m, 4H, H-Ar); 8.85 (s, 1H =NNH−). **MS** (m/z): [MH^+] 208.14 ; [MH+]found 208.08

Benzophenone thiosemicarbazone (4)

Yield: 84%;m.p: 167-168°C; **IR** (NaCl, cm^{-1}): 3410, 3346, 3248 ν(NH_2); 3151 ν(NH); 1608 ν(C=N); 1069, 1026, 846 ν(N-CS-N).**^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 179.05, 150.21, 136.50, 131.16, 128.54,127.88 ;**^{1}H NMR** ($CDCl_3$, 400MHz) δ(ppm): 6.42 (s, 2H, $CSNH_2$); 7.6-7.2 (m, 10H, H-Ar); 8.60 (s, 1H, =NNH−). **MS** (m/z): [MH^+] 255.07 ;[M]found 255.09

Carvone 4-phenyl-3-thiosemicarbazone (5)

Yield: 88%; m.p:147-148°C; **IR** (NaCl, cm^{-1}):3488, 3188 ν(NH); 1592 ν(C=N); 1123, 1046, 888 ν(N-CS-N);**^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 175.96, 149.122, 146.84, 138.01, 135.40, 131.92, 128.78, 125.92, 123.87, 110.89, 40.66, 30.13, 29.23, 20.62, 17.78;**^{1}H NMR** ($CDCl_3$, 400MHz) δ(ppm): 1.75 (s, 3H, CH_3); 1.91 (s, 3H, CH_3); 2.17 (d, 2H, C-CH_2C=N); 2.45-2.35 (d_d, 2H, =CH-CH_2-C),; 2.75 (m, 1H, H_2C-CH-CH_2); 4.85 (s,2H,

=CH_2); 6.25 (t, 1H, CH=C); 7.69-7.25 (m, 5H, H-Ar); 8.77 (s, 1H, CSNH-Ph); 9.35 (s, 1H, =NNH−). **MS** m/z : [MH^+] 300.16 ; [MH^+]found 300.15

Acetophenone 4-phenyl-3-thiosemicarbazone (6)

Yield: 86%; m.p:191-192°C; **IR** (NaCl, cm^{-1}): 3302, 3248 ν(NH); 1588 ν(C=N); 1100, 1026, 847 ν(N-CS-N);**^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 176.33, 147.29, 137.92, 137.28, 130.18, 129.88, 128.82, 128.72, 126.57, 124.22, 13.85;**^{1}H NMR** ($CDCl_3$, 400MHz) δ(ppm): 2.35 (s, 3H, CH_3); 7.8-7.2 (m, 10H, H-Ar); 8.8 (s, 1H, CSNH-Ph); 9.40 (s, 1H, =NNH−). **MS** (m/z): [MH^+] 270.10 ; [MH^+]found 270.08

4'-methylacetophenone4-phenyl-3-thiosemicarbazone (7)

Yield:77%m.p: 175-176°C; **IR** (NaCl, cm^{-1}): broad 3398 ν(NH); 1633 ν(C=N); 1100, 1027, 835 ν(N-CS-N); 815, 748 ν(p-CH3-Ar); **^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 176.25, 147.32, 140.33, 137.94, 134.44, 129.43, 128.89, 126.33, 126.13, 124.22, 21.36, 13.71;**^{1}H NMR** ($CDCl_3$, 400MHz) δ(ppm): 2.35 (s, 3H, CH_3); 2.42 (s, 3H, CH_3); 7.7-7.25 (9H, H-Ar); 8.75 (s, 1H, CSNH-Ph); 9.45 (s, 1H, =NNH−). **MS** (m/z): [MH^+] 284.07 ; [MH^+]found284.11

Benzophenone4-phenyl-3-thiosemicarbazone (8)

Yield: 82%m.p: 153-154°C; **IR** (NaCl, cm^{-1}): 3338, 3304 ν(NH); 1594 ν(C=N); 1071, 1031, 855 ν(N-CS-N);**^{13}C NMR** (DMSO-d_6, 100MHz) δ(ppm): 175.47, 149.22, 137.11, 135.73, 130.49, 129.65, 129.15, 128.02, 127.75,125.38 ;**^{1}H NMR** ($CDCl_3$, 400MHz) δ (ppm): 76-7.25 (m, 15H, H-Ar); 8.75 (s, 1H, CSNH-Ph); 9.45 (s, 1H, =NNH−). **MS** (m/z): [MH^+] 332.02 ; [M]found 332.11

The scaffold (scheme 1) has advantageous properties: low molecular weight, reasonable *C*log *P*, good hydrogen bond donating and accepting capabilities (table 1), easy, and economical synthetic routes (Lipinski *et al*., 1997).

Table 1 : Synthesized compounds have Physical Properties Compatible with Reasonable Pharmacokinetics and Drug Availability.

	Molecular weight	*C*log *P*	No. of H bond donors	No. of H bond acceptors	No. of criteria met
Rule	< 500	< 5	< 5	< 10	at least 3
1	223	3.703	3	3	all
2	193	2.401	3	3	all
3	207	2.900	3	3	all
4	255	4.102	3	3	all
5	299	4.988	2	3	all
6	269	4.071	2	3	all
7	283	4.570	2	3	all
8	331	5.400	2	3	3

Pharmacology

All the molecules were tested for the anti-parasitic analysis against *T. b. brucei*. According to the works of Fujii *et al*. (2005) and Du *et al*.(2002) in their discovery, synthesis and potent inhibitors of *Rhodesain* and *Cruzain* of thiosemicarbazones and derivatives study, thiosemicarbazones compounds are trypanocidal when their half-inhibitory concentrations IC_{50} values are lower than 10 μM, and are regarded as moderate anti-trypanosomal agents if these values are between 10 and 100 μM, and have little or no activity when IC_{50} are higher than 100 μM. In our work, products have showed interesting anti-trypanosomal activities (table 2).

Table 2 : Anti-trypanosomal activities of synthesized thiosemicarbazones on *Trypanosoma brucei brucei*

No	Compounds	Half-inhibition concentration IC_{50} (μM)	Anti-trypanosomal activities
1	Thiosemicarbazonecarvone	39.71±10.20	Moderate
2	Thiosemicarbazoneacetophenone	212.15±6.17	Little or no
3	Thiosemicarbazone4-methylacetophenone	8.73±0.63	Trypanocidal
4	Thiosemicarbazonebenzophenone	67.17±3.15	Moderate
5	4-phenyl-3-thiosemicarbazone carvone	>334.44 *	None
6	4-phenyl-3-thiosemicarbazone acetophenone	>371.74 *	None
7	4-phenyl-3-thiosemicarbazone 4-methylacetophenone	>353.35 *	None
8	4-phenyl-3-thiosemicarbazone benzophenone	8.48±0.89	Trypanocidal

* precipitate in the diluted solution

Among them, compounds **8** and **3** have exhibited a strong activity with IC_{50}<10μM, 8.48 and 8.73 respectively. The products **1** and **4** showed moderate anti-trypanosomal activity (IC_{50} = 39.71 and 67.17 μM respectively). Compound **2** show low activity against parasites with IC_{50} = 212.15 μM. Molecules **5**, **6** and **7**, N(4)-phenyl-3-thiosemicarbazones, are sensitive to solvent of the culture medium of the parasites. Thus, the mixture becomes heterogeneous and they precipitate (the products stay insoluble) in the diluted solutions of the different wells. Therefore, they exhibit no significant activity on parasites. Their half-inhibitory concentrations are over than 300 μM (more than 334.44, 371.74 and 353.35 μM respectively). All synthesized compounds are also subjected to their toxic activities on *Artemia salina* L. The results are summarized in table 3. To assess the toxicity from the LC_{50} values of compounds, we have referred to the LC_{50} value of lapachol (281 μM) which is known as reference compound (Santos *et al*., 2003 ;Graminha *et al*., 2008).

Table 3: Cytotoxic activities of thiosemicarbazones on *Artemia salina*.

No	Compounds	Half-lethal concentration LC_{50} (μM)	Cytotoxic Activities
1	Thiosemicarbazonecarvone	20.58±0.07	Toxic
2	Thiosemicarbazoneacetophenone	363.74±0.04	No toxic
3	Thiosemicarbazone4-methylacetophenone	317.52±0.13	No toxic
4	Thiosemicarbazonebenzophenone	33.72±0.04	Toxic
5	4-phenyl-3-thiosemicarbazone carvone	430.10±0.02	No toxic
6	4-phenyl-3-thiosemicarbazone acetophenone	297.45±0.12	No toxic
7	4-phenyl-3-thiosemicarbazone 4-methylacetophenone	897.17±0.03	No toxic
8	4-phenyl-3-thiosemicarbazone benzophenone	366.76±0.02	No toxic

Analysis of the results reveals that only two products among them, **1** and **4**, have exhibited toxic activity on the larvae with LC_{50} values equal to 20.58 and 33.72 μM respectively. Other

compounds **2**, **3**, **5**, **6**, **7** and **8** have negligible toxicity (table 3). These tests that are a summary assessment of the toxicity of products reflects the sensitivity of shrimp larvae to the thiosemicarbazone and by extension that of the human species. Indeed, there is a correlation between toxicity on shrimp larvae and cytotoxicity on cells 9KB and 9PS (human carcinoma nasopharygien) a part (Pelka *et al*., 2000), cells A-549 lung carcinoma and HT-29 cells of carcinoma of the colon on the other (Carballo *et al*., 2002). Consequently, molecules**1** and **4**which exhibit both the anti-trypanosomal activities and toxic activity could open a promising avenue in the fighting against tumors diseases and anti-neoplastic activity (Graminha *et al*., 2008).

The analysis of the toxicity of shrimp larvae also allows us to determine the selectivity of studied molecules by calculating their selectivity index (SI = LC_{50} larvae / IC_{50} parasite). If the IS value obtained is greater than unity, the test compound is considered to be selective on the parasites. However, if IS is less than unity, the test compound is more toxic than anti-parasitic (Tiuman *et al*., 2005). Therefore, the index of selectivity of all synthesized compounds was calculated (Table 4) and showed that the compounds **1**, **4** and **6** (with their SI> 1) turn out quite selective on the parasite *Trypanosoma brucei brucei*.

Table 4: Selectivity index of synthesized compounds.

Compounds	LC_{50} µM	IC_{50} µM	Selectivity index ($SI = LC_{50}/IC_{50}$)
1	20.58±0.07	39.71±10.20	1.92
2	363.74±0.04	212.15±6.17	0.58
3	317.52±0.13	8.73±0.63	0.02
4	33.72±0.04	67.17±3.15	1.99
5	430.10±0.02	>334.44	0.77
6	297.45±0.12	>371.74	1.24
7	897.17±0.03	>353.35	0.39
8	366.76±0.02	8.48±0.89	0.02

It is noted that among these molecules, **1** and **4** in addition to their moderate anti-trypanosomal activity (IC_{50} = 39.71 µM and 67.17 respectively) show good selectivity (SI = 1.92 and 1.99 respectively) of *T.b. brucei*. The compounds **2**, **3**, **5**, **7** and **8** showed a selectivity index less than unity (Table 4) and, therefore, have a good selectivity on cells of shrimp larvae *Artemia salina*. Among these products, we have **8** and **3** (SI = 0.02) which possess interesting trypanocidal activity (IC_{50}<10 µM) and could, for this purpose, be a better way of treatment of trypanosomes.

CONCLUSION

A series of thiosemicabazones N(4)-substituted or not of four ketones were synthesized by condensation reactions and completely characterized. Biological activities for all compounds have been tested on *Trypanosoma brucei brucei* and *Artemia salina* L. Four molecules such as **1**, **3**, **4** and **8** have shown great activities on *T.b.brucei*with significant IC_{50} values less than 100 µM on parasites. Among them, products **1** and **4** have indicated toxic activity on *Artemia* and would be able to open a promising future in the fighting against tumors diseases and anti-neoplastic activity (Graminha *et al*., 2008). Other, generally, have not exhibited toxic properties.

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