

Trypanocidal and cytotoxic evaluation of synthesized thiosemicarbazones as potential drug leads against sleeping sickness

Bienvenu Glinma · Sika D. S. Kpoviessi · Fernand A. Gbaguidi · Coco N. Kapanda · Joanne Bero · Joëlle Quetin-Leclercq · Mansourou Moudachirou · Jacques Poupaert · Georges C. Accrombessi · Emma W. Gachomo · Lamine Baba-Moussa · Simeon O. Kotchoni

Received: 2 May 2013 / Accepted: 30 December 2013
© Springer Science+Business Media Dordrecht 2014

Abstract Thiosemicarbazones have become one of the promising compounds as new clinical candidates due to their wide spectrum of pharmaceutical activities. The wide range of their biological activities depends generally on their related aldehyde or ketone groups. Here, we report the pharmacological activities of some thiosemicarbazones synthesized in this work. Benzophenone and derivatives were used with *N*(4)-phenyl-3-thiosemicarbazide to synthesize corresponding five thiosemicarbazones (1–5). Their structures were characterized by spectrometrical methods analysis IR, NMR ¹H & ¹³C and MS. The compounds were then screened in vitro for their antiparasitic activity and toxicity on *Trypanosoma brucei brucei* and *Artemia salina* Leach respectively. The selectivity index of each compound was also determined. Four thiosemicarbazones such

as 4, 2, 3 and 1 reveal interesting trypanocidal activities with their half inhibitory concentration (IC₅₀) equal to 2.76, 2.83, 3.86 and 8.48 μM respectively, while compound 5 (IC₅₀ = 12.16 μM) showed a moderate anti-trypanosomal activity on parasite. In toxicity test, except compound 1, which showed a half lethal concentration LC₅₀ >281 μM, the others exerted toxic effect on larvae with LC₅₀ of 5.56, 13.62, 14.55 and 42.50 μM respectively for thiosemicarbazones 4, 5, 3 and 2. In agreement to their selectivity index, which is greater than 1 (SI >1), these compounds clearly displayed significant selective pharmaceutical activities on the parasite tested. The thiosemicarbazones 2–5 that displayed significant anti-trypanosomal and cytotoxicity activities are suggested to have anti-neoplastic and anti-cancer activities.

B. Glinma · S. D. S. Kpoviessi · F. A. Gbaguidi · M. Moudachirou · G. C. Accrombessi
Laboratoire de Chimie Organique, Physique et de Synthèse (LaCOPS), Département de Chimie, Faculté des Sciences et Techniques (FAST), Université d'Abomey-Calavi, 01 BP 4521 Cotonou, Benin

C. N. Kapanda · J. Bero · J. Quetin-Leclercq · J. Poupaert
Louvain Drug Research Institute (LDRI), School of Pharmacy, Université catholique de Louvain, B1 7203 Avenue Emmanuel Mounier 72, 1200 Brussels, Belgium

E. W. Gachomo · S. O. Kotchoni (✉)
Department of Biology and Center for Computational and Integrative Biology, Rutgers University, Camden, NJ 08102, USA
e-mail: simeon.kotchoni@rutgers.edu

L. Baba-Moussa
Laboratoire de biologie et de typage moléculaire en microbiologie, département de biochimie et biologie cellulaire, Faculté des sciences et techniques, Université d'Abomey-Calavi, Cotonou, Benin

Keywords Synthesis · *N*(4)-phenyl-3-thiosemicarbazones · Pharmacological activities · Toxicity

Introduction

African trypanosomiasis is a parasitic disease that affects a variety of mammals, including humans, on the sub-Saharan African continent. The main agents of disease are members of the orders Kinetoplastida (*Trypanosoma*, etc.). *Trypanosoma brucei* is the etiological agent responsible for African trypanosomiasis, an infectious pathology which represents a serious problem of public health and economic losses in Sub-Saharan Africa [1]. Animal African trypanosomiasis (AAT), known for its ravages in several parts of agro-ecological zones [2, 3], is one of the greatest constraints of livestock and agricultural production resulting in profound effects on the economy, social structure and quality of life in endemic areas of Africa [4, 5]. Human

African trypanosomiasis (HAT) in association with the animal form (Nagana) caused by *Trypanosoma brucei brucei* (*T. b. brucei*) are responsible for considerable death in Africa. These parasites are one of the major threats to the public, farmers, ranchers, and hunters in sub-Saharan Africa [5–9]. Sleeping sickness caused by this parasite remains the biggest obstacle to rural development in Africa [5]. There is therefore a great need to develop new compounds that could be exploited as new drugs to efficiently treat this recurring disease. It is of critical importance to establish new methods to rapidly design bioactive compounds to fight off the sleeping sickness disease.

In this regard, a class of small molecules, thiosemicarbazones and derivatives, have been studied over the last few years due to their wide pharmacological versatility [10, 11] such as potential activities against microbial and parasitic diseases: antineoplastic, antivirals and anticancer therapeutics [12–15], antimicrobial [16, 17], anticonvulsant [18], antibacterial [19], antioxidant [20], anti-amoebic [21], anti-malarial [22], as well as for their parasiticidal action [4, 23–28]. They represent validated drug leads that kill several species of protozoan parasites through the inhibition of cysteine proteases as well as other novel targets [27]. In our previous research, we synthesized thiosemicarbazones which have revealed potential trypanocidal activity against the parasite, *T. b. brucei*, responsible for AAT [4, 25–27].

In this work, we synthesized *N*(4)-phenyl-3-thiosemicarbazones of benzophenone and its substituted derivatives by the groups amino in ortho, hydroxy in ortho and para and chlorine in meta positions. The compounds were analyzed for their antitrypanosomal activity against *T. b. brucei* and their toxicity on larvae shrimp, *Artemia salina* Leach. The potential of these compounds as anticancer agents is here also highlighted.

Materials and methods

Equipment

All synthesized compounds were characterized by NMR spectra using Bruker Avance 400 UltraShield with

dimethylsulfoxide (DMSO)- d_6 or $CDCl_3$ and then MS using the method of Atmospheric-pressure chemical ionization. The frequencies for 1H and ^{13}C are 400.130 and 100.612 MHz respectively. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane as internal standard. Multiplicity was designated as singlet (s) and multiplet (m). Their melting points were taken on a *fusionometer* of the type *electrothermal 1A 9000* and were not corrected.

Reagents

The 4-phenyl-3-thiosemicarbazide obtained from A ALDRICH^R was used on benzophenone, 2-aminobenzophenone, 2-hydroxybenzophenone, 4-hydroxybenzophenone, glacial acetic acid (GAA) and 2-amino-5-chlorobenzophenone purchased from PROLABO and MERCK-Schuchardt. The hydrochloric acid 37 % and the glacial acetic acid (GAA) used in the reactions was obtained from Riedel-de Haën. All products were used without purifying. Compounds were synthesized via the synthesis route detailed in Fig. 1.

Methods

Synthesis of the compounds

An equimolar mixture (1.67 g, 0.01 mol) of 4-phenyl-3-thiosemicarbazide dissolved in 10 mL ethanol (EtOH 96°) was added slowly to a solution (0.01 mol) of arylketone dissolved in 20–30 mL EtOH in presence of acid (1 N HCl or GAA). The mixture was heated at reflux for 4 h with stirring. After cooling, the precipitate was filtered, washed with cold distilled water until neutrality, dried and then recrystallized in ethanol.

The synthesized compounds were tested for their antitrypanosomal activity on strain 427 of *T. b. brucei* and screened for cytotoxicity on *A. salina* Leach.

Pharmacological property characterization of the compounds

Anti-trypanosomal activity

The activity of the compounds was assessed on bloodstream of *T. b. brucei* strain 427 according to “LILIT

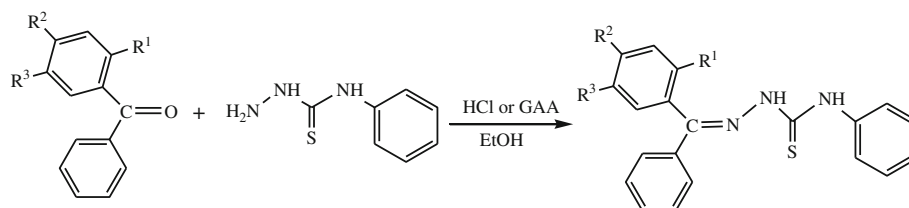


Fig. 1 Synthetic routes of 4-phenyl-3-thiosemicarbazones (scaffold). Compound 1: R¹ = R² = R³ = H; Compound 2: R¹ = NH₂, R² = R³ = H; Compound 3: R¹ = OH, R² = R³ = H; Compound 4: R¹ = R³ = H, R² = OH; Compound 5: R¹ = NH₂, R² = H, R³ = Cl

Alamar Blue™ method [29–31]. Briefly, healthy cell will be monitored through this method using Alamar Blue® cell viability reagent, which functions as healthy cell indicator by using the reducing power of living cells to quantitatively measure the proliferation of various human and animal cell lines. This method will allow the establishment of relative cytotoxicity of agents within various classes of chemical compounds. Resazurin, the active ingredient of Alamar Blue® reagent, is a non-toxic, cell permeable compound that is blue in color and virtually non-fluorescent. Upon entering cells, resazurin is reduced to resorufin, a red and highly fluorescent compound. Viable cells continuously convert resazurin to resorufin, increasing the overall fluorescence and reddish color of the media surrounding cells.

The stock solutions of thiosemicarbazones were prepared from an initial concentration of 10 mg/mL in DMSO. The trypanosomes were grown in a medium containing 10 % of heat-inactivated fetal calf serum and bloodstream form supporting factor. The trypanosome suspensions were adjusted to 5×10^4 tryp/mL. In each well, 50 μ L of different dilutions of the stock solution were added to 50 μ L of suspension of trypanosomes. The plates were then incubated at 37 °C for 72 h in an atmosphere with 5 % CO₂. 10 μ L of “Alamar Blue™” is added to each well and then incubated for 4 h for detecting enzymatic activity. The MIC is represented by the minimal concentration of thiosemicarbazone that inhibits the proliferation of trypanosomes. 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 of 590 nm.

Cytotoxicity screen

The cytotoxicity test was performed on larvae of brine shrimp (*A. salina* Leach) by the method of Michael et al. [32] and modified by Vanhaecke et al. [33] and by Sleet and Brendel [34]. *A. salina* eggs were incubated in seawater until hatching of young larvae (48 h). Then, series of solutions of test substances at varying concentrations were prepared in DMSO/seawater. A defined number of larvae were introduced into each solution and incubated under rocking condition for 24 h. To evaluate the toxicity of the solution, counting of larvae viability was performed under microscope by determining the number of dead larvae in each solution. In the case where there was death in the control medium, the data was corrected by Abbott's formula: % death = [(test – control)/control] \times 100 [35].

Data (dose–response) were transformed by logarithm and the half-lethal concentration LC₅₀ was determined by linear regression [36]. Tests were carried out in triplicates.

All data were expressed as mean \pm standard deviation of triplicate measurements.

Results and discussion

Chemistry characteristics of the compounds

We synthesized five *N*(4)-phenyl-substituted thiosemicarbazones with good yields: benzophenone 4-phenyl-3-thiosemicarbazone (**1**), 2-aminobenzophenone 4-phenyl-3-thiosemicarbazone (**2**), 2-hydroxybenzophenone 4-phenyl-3-thiosemicarbazone (**3**), 4-hydroxybenzophenone 4-phenyl-3-thiosemicarbazone (**4**) and 2-amino-5-chlorobenzophenone 4-phenyl-3-thiosemicarbazone (**5**). Spectrometrical analysis: NMR ¹H & ¹³C were used to characterize the structure of each molecule. The scaffold (Fig. 1) has advantageous properties: low molecular weight, reasonable *ClogP*, good hydrogen bond donating and accepting capabilities (Table 1), easy and economical synthetic routes [37].

Characterization of synthetic compounds

Benzophenone 4-phenyl-3-thiosemicarbazone (**1**)

Synthesis was done using 5 mL of hydrochloric acid (1 N). **Rf** (hexane/ethyl acetate, v/v: 8/2): 0.55. Yield: 82 %; m.p: 153–154 °C; ¹³C NMR (DMSO-_{d6}, δ in ppm): 175.47 (C=S), 149.22 (C=N), 137.11, 135.73, 130.49, 129.65, 129.15, 128.02, 127.75, 125.38 (C-aromatics); ¹H NMR (CDCl₃, δ in ppm): 9.45 (s, 1H, =NNH–), 8.75 (s, 1H, CSNH-Ph), 7.60–7.25 (m, 15H, H-aromatics). **SM** m/z [MH⁺]found: 332.02, [M]theoretical: 331.11. **Molecular formula**: C₂₀H₁₇N₃S.

2-Aminobenzophenone 4-phenyl-3-thiosemicarbazone (**2**)

Synthesis was done using 5 mL of hydrochloric acid (1 N). **Rf** (hexane/ethyl acetate, v/v: 8/2): 0.29. Yield: 73 %; m.p:

Table 1 Synthetic molecules have physical properties compatible with reasonable pharmacokinetics and drug availability

Rule	Molecular weight	<i>ClogP</i>	No. of H bond donors	No. of H bond acceptors	No. of criteria met
	<500	<5	<5	<10	At least 3
1	331	5.400	2	3	3
2	346	4.673	4	4	All
3	347	5.263	3	4	3
4	347	5.263	3	4	3
5	380.5	5.509	4	4	3

136–137 °C; ^{13}C NMR (CDCl_3 , δ in ppm): 176.07 (C=S), 143.74 (C=N), 148.30, 137.91, 135.63, 131.76, 130.51, 129.96, 129.57, 128.86, 127.58, 126.21, 124.33, 119.37, 116.80, 115.49. (C-aromatics); ^1H NMR (CDCl_3 , δ in ppm): 10.30 (s, 1H, =NNH–), 8.87 (s, 1H, CSNH-Ph), 6.87 & 6.70 (s, 2H, NH_2), 7.75–7.17 (m, 14H, H-aromatics). **SM** m/z [MH^+] found: 347.39, [M] theoretical: 346.43. **Molecular formula:** $\text{C}_{20}\text{H}_{18}\text{N}_4\text{S}$.

2-Hydroxybenzophenone 4-phenyl-3-thiosemicarbazone (3)

Synthesis was done using 5 mL of hydrochloric acid (1 N) **Rf** (hexane/ethyl acetate, v/v: 6/4): 0.68. Yield: 57 %; m.p: 186–187 °C ^{13}C NMR (CDCl_3 , δ in ppm): 176.03 (C=S), 148.26 (C=N), 154.56, 138.92, 133.12, 131.67, 129.77, 129.66, 128.53, 128.23, 127.66, 126.10, 125.75, 124.05, 117.69, 116.53 (C- aromatics); ^1H NMR (CDCl_3 , δ in ppm): 10.50 (s, 1H, =NNH–), 10.21 (s, 1H, CSNH-Ph), 8.70 (s, 1H, OH), 7.80–7.00 (m, 14H, H-aromatics). **SM** m/z [MH^+]found: 348.41, [M]theoretical: 347.43. **Molecular formula:** $\text{C}_{20}\text{H}_{17}\text{N}_3\text{OS}$.

4-Hydroxybenzophenone 4-phenyl-3-thiosemicarbazone (4)

Synthesis was done using 2 mL of glacial acetic acid. **Rf** (hexane/ethyl acetate, v/v: 7/3): 0.46. Yield: 65 %; m.p: 172–173 °C; ^{13}C NMR (CDCl_3 , δ in ppm): 175.75 (C=S), 150.33 (C=N), 159.04, 136.72, 131.70, 130.00, 129.89, 129.79, 128.30, 128.25, 127.07, 125.95, 125.60, 121.34, 116.48 (C- aromatics); ^1H NMR (CDCl_3 , δ in ppm): 10.30 (1H, =NNH–), 9.90 (1H, CSNH-Ph), 8.80 (1H, OH), 7.70–6.70 (m, 14H, H-aromatics). **SM** m/z [MH^+]found: 348.41, [M]theoretical: 347.43. **Molecular formula:** $\text{C}_{20}\text{H}_{17}\text{N}_3\text{OS}$.

2-Amino-5-chlorobenzophenone 4-phenyl-3-thiosemicarbazone (5)

Synthesis was done using 5 mL of hydrochloric acid (1 N). **Rf** (hexane/ethyl acetate, v/v: 8/2): 0.29. Yield: 53 %; m.p:

143–144 °C; ^{13}C NMR ($\text{DMSO}-d_6$, δ in ppm): 176.79 (C=S), 150.47 (C=N), 144.20, 136.95, 132.08, 131.51, 131.29, 129.86, 129.56, 128.75, 126.18, 124.38, 118.90, 116.98 (C-aromatics); ^1H NMR (CDCl_3 , δ in ppm): 10.30 (s, 1H, =NNH–), 9.00 (s, 1H, CSNH-Ph), 6.78 & 6.35 (s, 2H, NH_2), 7.70–6.90 (m, 13H, H-aromatics). **SM** m/z [MH^+]found: 381.87, [M]theoretical: 380.89. **Molecular formula:** $\text{C}_{20}\text{H}_{17}\text{ClN}_4\text{S}$.

The spectrometrical analysis data gave: in ^{13}C NMR spectra, peaks of C=S from 176.79 to 175.47 ppm and of C=N between 150.47 and 143.74 ppm in all molecules. All aromatics carbons of the compounds ranged from 159.04 to 115.49 ppm. ^1H NMR spectra gave the characteristic protons in each structure: signals of protons (=NNH–) were identified between 10.50 and 9.45 ppm, protons in (CSNH-Ph) appear from 10.21 to 8.75 ppm in all compounds; in the products **3** and **4** the signal of the typical proton HO was shown respectively at 8.70 and 8.80 ppm; protons in the amino group H_2N in molecules **2** and **5** respectively were identified at 6.87 and 6.70 and 6.78 and 6.35 ppm. Aromatics protons in the compounds were obtained between 7.80 and 6.70 ppm. It is worth mentioning that substituents used here, OH and NH_2 are both electron donating when in the ortho and para positions. Compound **5**, which lies at the end of the physiological scales, has a meta-Cl substituent which is electron withdrawing. The analysis of these spectral data further confirms the structure of each molecule synthesized.

Pharmacology tests

Trypanocidal and cytotoxicity assays of products gave the results summarized in Table 2. We note that compounds present interesting activity. On the parasites, four molecules (**1–4**) revealed a high trypanocidal effect ($\text{IC}_{50} < 10 \mu\text{M}$). Only product **5** ($\text{IC}_{50} = 12.16 \mu\text{M}$) showed a moderate anti-trypanosomal activity. These results are consistent with the scale of trypanocidal activity established in the previous works [4, 24, 26–29]. According to previous studies, thiosemicarbazones are trypanocidal when their IC_{50} values are lower than $10 \mu\text{M}$, and are regarded as moderate anti-trypanosomal agents if these values are between 10 and

Table 2 Pharmacological activities of thiosemicarbazones

No.	Half-inhibitory concentration IC_{50} (μM)	Anti-trypanosomal activities	Half-lethal concentration LC_{50} (μM)	Toxic activities	Selectivity index (SI = $\text{LC}_{50}/\text{IC}_{50}$)
1	8.48 ± 0.89	Trypanocidal	366.76 ± 0.02	Not toxic	43.25
2	2.83 ± 0.17	Trypanocidal	42.50 ± 1.19	Toxic	15.01
3	3.86 ± 0.86	Trypanocidal	14.55 ± 1.32	Toxic	3.76
4	2.76 ± 1.00	Trypanocidal	5.56 ± 0.63	Toxic	2.01
5	12.16 ± 0.44	Moderate	13.62 ± 2.17	Toxic	1.12

100 μM , and have little or no activity when their IC_{50} are higher than 100 μM .

In order to assess the functional characteristics of the chemical compound structures, we performed different substitutions on the chemical core structure of the molecules. We observed that a mono-substitution of H by an amino or hydroxy group in compound structure **1**, resulting in the synthesis of products **2–4** significantly enhanced the trypanocidal activity of these products (**2–4** (MIC = 2.83, 3.86 and 2.76 respectively) compared to product **1** with MIC = 8.48 μM). These IC_{50} values were found to be two to three time lower in the mono-substituted compound products (**2–4**) compared to un-substituted compound **1**. Furthermore, it is evident from this data that the substitution of hydroxy group in the para position was the best trypanocidal compound, i.e., product **4** (MIC = 2.76 μM). The ortho positions (**3**, *o*-hydroxy, and to **2** *o*-amino) were also more actives than un-substituted compound **1** as summarized in Table 2. Comparing the hydroxy-substitution on compound products **3** and **4**, the para-substitution resulted in stronger trypanocidal activity. This could be explained by the steric effect of OH in ortho position as suggested by the different MIC values recorded for these two compounds (LC_{50} values: *p*-OH 2.76 and *o*-OH 3.86 μM).

Normally the cytotoxicity referred value of synthesized thiosemicarbazones on *A. salina* L. known as lethal half-concentration of lapachol (LC_{50}) are reported to be $\text{LC}_{50} = 281 \mu\text{M}$ [38, 39]. Our different LC_{50} values obtained using the synthesized products (**1–5**) exhibited a stronger cytotoxicity activity than lapachol with the exception of compound **1** ($\text{LC}_{50} = 366.76 \mu\text{M}$). Compounds **4**, **5**, **3** and **2** displayed the following $\text{LC}_{50} = 5.56$; 13.62; 14.55 and 42.50 μM on exerted a significant toxic activity on studied (5.56, 13.62, 14.55 and 42.50 μM shrimp larvae respectively (Table 2). Shrimp larvae were selected in this study as biological model. Indeed, there is a correlation between the toxicity of the compounds on shrimp larvae and their cytotoxicity on 9KB and 9PS cells (human carcinoma nasopharygien) [40], and on A-549 cells of lung carcinoma and HT-29 cells of colon carcinoma [41].

Our data showed that compounds **2** and **5** displayed both trypanocidal and cytotoxicity activity opening therefore an avenue to consider these two compounds as potential candidate in the treatment of tumors diseases and could present a potential anti-neoplastic activity [38]. In addition, we noticed that the most strongly trypanocidal compounds were also the most cytotoxic.

Conclusion

We reported in this paper, a series of 4-phenyl-3-thiosemicarbazone synthesized with arylketones like benzophenone

and its derivatives. When subjected to pharmacological study, these compounds revealed interesting anti-trypanosomal activity with low IC_{50} between 2.76 and 12.16 μM . In mass spectrometry, mass of each molecular ion peak (parent peak) obtained is very consistent and comparable to the theoretically estimated mass. Compounds **4**, **5**, **3** and **2** displayed the following $\text{LC}_{50} = 5.56$; 13.62; 14.55 and 42.50 μM and displayed a significant toxic activity on the shrimp larvae respectively (Table 2). Except compound **1**, the other molecules have very toxic effects on *A. salina* larvae ($\text{LC}_{50} < 281 \mu\text{M}$). Their selectivity indexes were greater than a unit ($\text{SI} > 1$). Also, the molecules (**2** and **5**) which were more trypanocidal were also more cytotoxic and could therefore offer a promising avenue in the treatment of tumoral diseases.

Acknowledgments The authors gratefully acknowledged the Belgian National Fund for Scientific Research (FNRS) (T.0190.13) for this work.

References

1. Lanca AS, de Sousa KP, Atouguia J, Prazeres DM, Monteiro GA, Silva MS (2011) *Trypanosoma brucei*: immunisation with plasmid DNA encoding invariant surface glycoprotein gene is able to induce partial protection in experimental African trypanosomiasis. *Exp Parasitol* 127(1):18–24
2. Fajinmi AO, Faleke OO, Magaji AA, Daneji AI, Gweba M (2011) Presence of trypanosome species and determination of *Anaemia* in trade cattle at Sokoto Abattoir, Nigeria. *Res J Parasitol* 6:31–42
3. Dede PM, Omoogun GA, Uzoigwe NR, Njoku CI, Daniel AD, Dadah AJ (2005) Current tsetse and trypanosomiasis situation on the Plateau, Nigeria: epidemiological factors that may enhance disease acquisition and spread. *Rev d'elevage Med Vet Pays Tropicaux* 58:25–32
4. Glinma B, Kpoviessi DSS, Gbaguidi FA, Kapanda CN, Bero J, Quetin-Leclercq J, Moudachirou M, Poupaert J, Accrombessi GC (2012) Synthesis, characterization, trypanosomal activities on *Trypanosoma brucei brucei* and toxicity against *Artemia salina* Leach of *N*(4)-aryl semicarbazones and thiosemicarbazones. *J Chem Pharm Res* 4(2):1016–1021
5. Cattand P (2001) L'épidémiologie de la trypanosomiase humaine africaine: une histoire multifactorielle complexe. *Med Trop* 61:313–322
6. Field MC, Allen CL, Dhir V, Goulding D, Hall BS, Morgan GW, Veazey P, Engstler M (2004) New approaches to the microscopic imaging of *Trypanosoma brucei*. *Microsc Microanal* 10(5):621–636
7. Swallow BM (2000) Impacts of trypanosomosis on African agriculture. PAAT Technical and Scientific Series 2. FAO (Organisation Mondiale pour l'Alimentation et l'Agriculture), Rome, p 52
8. Winkle S (2005) Geißeln der Menschheit. Kulturgeschichte der Seuchen. Artemis & Winkler, Düsseldorf
9. Troeberg L, Chen X, Flaherty TM, Morty RE, Cheng M, Hua H, Springer C, McKerrow JH, Kenyon GL, Lonsdale-Eccles JD, Coetzer TH, Cohen FE (2000) Chalcone, acyl hydrazide, and related amides kill cultured *Trypanosoma brucei brucei*. *Mol Med* 6(8):660–669
10. Tenório RP, Carvalho CS, Pessanha CS, Jde Lima G, de Faria AR, Alves AJ, de Melo EJT, Góes AJS (2005) Synthesis of

- thiosemicarbazone and 4-thiazolidinone derivatives and their in vitro anti-*Toxoplasma gondii* activity. *Bioorg Med Chem Lett* 15:2575–2578
11. Beraldo H, Gambino D (2004) Semicarbazones and thiosemicarbazones: their wide pharmacological profile and clinical applications. *Quim Nova* 27:461–471
 12. Wiecek J, Kovala-Demertzi D, Ciunik Z, Zervou M, Demertzis MA (2010) Diorganotin complexes of a thiosemicarbazone, synthesis: properties, X-ray crystal structure, and antiproliferative activity of diorganotin complexes. *Bioinorg Chem Appl Article*. ID 867195. doi:10.1155/2010/867195
 13. da Silva AP, Martini MV, de Oliveira CMA, Cunha S, de Carvalho IE, Ruiz ALTG, da Silva CC (2010) Antitumor activity of (-)- α -bisabolol-based thiosemicarbazones against human tumor cell lines. *Eur J Med Chem* 45:2987–2993
 14. Richardson DR, Kalinowski DS, Richardson V, Sharpe PC, Lovejoy DB, Islam M, Bernhardt PV (2009) 2-Acetylpyridine thiosemicarbazones are potent iron chelators and antiproliferative agents: redox activity, iron complexation and characterization of their antitumor activity. *J Med Chem* 52(5):1459–1470
 15. Hu W-X, Zhou W, Xia C-N, Wen X (2006) Synthesis and anticancer activity of thiosemicarbazones. *Bioorg Med Chem Lett* 16:2213–2218
 16. Umamatheswari S, Kabilan S (2011) Synthesis and antimicrobial studies of novel 2,4-diaryl-3-azabicyclo[3.3.1]nonan-9-one 4'-phenylthiosemicarbazones. *J Enzyme Inhib Med Chem* 26(3):430–439
 17. Halve AK, Bhashkar B, Sharma V, Bhadauria R, Kankoriya A, Soni A, Tiwari K (2008) Synthesis and in vitro antimicrobial studies of some new 3-[phenyldiazenyl] benzaldehyde *N*-phenyl thiosemicarbazones. *J Enzyme Inhib Med Chem* 23(1):77–81
 18. Aggarwal N, Aggarwal R, Mishra P, Jain JS, Bansal SK, Jha KK (2008) Design and evaluation of semicarbazones and thiosemicarbazones as novel anticonvulsants. *Cent Nerv Syst Agents Med Chem* 8(1):26–28
 19. Salam MA, Affan MA, Saha R, Ahmad FB, Sam N (2012) Synthesis, characterization and in vitro antibacterial studies of organotin(IV) complexes with 2-hydroxyacetophenone-2-methylphenylthiosemicarbazone (H(2)damp). *Bioinorg Chem Appl* 2012:698491
 20. Choudhary A, Sharma R, Nagar M, Mohsin M (2010) Transition metal complexes with N, S donor ligands as synthetic antioxidants: synthesis, characterization and antioxidant activity. *J Enzyme Inhib Med Chem* 26(3):394–403
 21. Bhat AR, Athar F, Van Zyl RL, Chen C-T, Azam A (2008) Synthesis and biological evaluation of novel 4-substituted 1-[[4-(10,15,20-triphenylporphyrin-5-yl)phenyl]methylidene]thiosemicarbazides as new class of potential antiprotozoal agents. *Chem Biodivers* 5(5):764–776. doi:10.1002/cbdv.200890073
 22. Mallari JP, Guiguemde WA, Guy RK (2009) Antimalarial activity of thiosemicarbazones and purine derived nitriles. *Bioorg Med Chem Lett* 19(13):3546–3549. doi:10.1016/j.bmcl.2009.04.142
 23. Fujii N, Mallari JP, Hansell EJ, Mackey Z, Doyle P, Zhou YM, Gut J, Rosenthal PJ, McKerrow JH, Guy RK (2005) Discovery of potent thiosemicarbazones inhibitors of *rhodesain* and *cruzain*. *Bioorg Med Chem* 15(1):121–123
 24. Mallari JP, Shelat A, Kosinski A, Caffrey CR, Michele C, Zhu F, McKerrow JH, Guy RK (2008) Discovery of trypanocidal thiosemicarbazone inhibitors of *rhodesain* and TbcA. *Bioorg Med Chem Lett* 18(9):2883–2885. doi:10.1016/j.bmcl.2008.03.083
 25. Glinma B, Kpoviessi SDS, Fatondji RH, Gbaguidi FA, Kapanda CN, Bero J, Lambert DM, Hannaert V, Quetin-Leclercq J, Moudachirou M, Poupaert J, Accrombessi GC (2011) Synthesis, characterization and anti-trypanosomal activity of R(-) carvone and arylketones-thiosemicarbazones and toxicity against *Artemia salina* Leach. *J pp Pharm Sci* 01(08):65–70
 26. Fatondji HR, Gbaguidi F, Kpoviessi S, Bero J, Hannaert V, Quetin-Leclercq J, Poupaert J, Moudachirou M, Accrombessi GC (2011) Synthesis, characterization and trypanocidal activity of some aromatic thiosemicarbazones and their 1,3,4-thiadiazolines derivatives. *Afr J Pure Appl Chem* 5(1):59–64
 27. Glinma B, Gbaguidi FA, Kpoviessi SDS, Fatondji RH, Poupaert J, Accrombessi GC (2011) Characterization and antiparasitic activity of benzophenone thiosemicarbazones on *Trypanosoma brucei brucei*. *St Cerc St CICBIA* 12(1):33–40
 28. Greenbaum DC, Mache Z, Hansell E, Doyle P, Gut J, Caffrey CR, Lehrman J, Rosenthal PJ, McKerrow JH, Chibale K (2004) Synthesis and structure activity relationships of parasiticidal thiosemicarbazone cysteine protease inhibitors against *P. falciparum*, *T. brucei* and *T. cruzi*. *J Med Chem* 47(12):3212–3219
 29. Baltz T, Baltz D, Giroud C, Crockett J (1985) Cultivation in a semi defined medium of animal infective forms of *Trypanosoma brucei*, *T. equiperdum*, *T. evansi*, *T. rhodesiense* and *T. gambiense*. *EMBO J* 4(5):1273–1277
 30. Hirumi H, Hirumi K (1994) Axenic culture and African *Trypanosome* bloodstream forms. *Parasitol Today* 10(2):80–84
 31. Rüz B, Iten M, Grether-Bühler Y, Kaminsky R, Brun R (1997) The Alamar Blue™ assay to determine drug sensitivity of African trypanosomes (*Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*) in vitro. *Acta Trop* 68:139–147
 32. Michael AS, Thompson CG, Abramovitz M (1956) *Artemia salina* as a test organism for a bioassay. *Science* 123:464
 33. Vanhaecke P, Persoone G, Claus C, Sorgeloos P (1981) Proposal for a short-term toxicity test with *Artemia nauplii*. *Ecotoxicol Environ Safety* 5:382–387
 34. Sleet RB, Brendel K (1983) Improved methods for harvesting and counting synchronous populations of *Artemia nauplii* for use in developmental toxicology. *Ecotoxicol Environ Safety* 7:435–446
 35. Abbott WA (1925) A method of computing the effectiveness of an insecticide. *J Econ Entomol* 18:265
 36. Hafner E, Heiner E, Noack E (1977) Mathematical analysis of concentration-response relationships. *Arzneim-Forsch Drug Res* 27:1871–1873
 37. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ (1997) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv Drug Deliv Rev* 23:3–25
 38. Graminha AE, Batista AA, Louro SRW, Zioli RL, Teixeira LR, Beraldo H (2008) 2-Pyridinoformamide-derived thiosemicarbazones and their iron(III) complexes : potential antineoplastic activity. *Polyhedron* 27:547–551
 39. Santos PLP, Pinto GB, Takahashi JA, Silva LGF, Boaventura MAD (2003) Biological screening of *Annonaceae* Brazilian medicinal plants using *Artemia salina* (Brine shrimp test). *Phytomedicine* 10(2–3):209–212
 40. Pelka M, Danzl C, Distler W, Petschelt A (2000) A new screening test for toxicity testing of dental materials. *J Dent* 28(5):341–345
 41. Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos DC (2002) A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. *BMC Biotechnol* 2:17. doi:10.1186/1472-6750-2-17