Studii şi Cercetări Ştiinţifice 2011, **12** (1), pp. 033 – 040
Chimie şi Inginerie Chimică, Biotehnologii, Industrie Alimentară

Scientific Study & Research
Chemistry & Chemical Engineering, Biotechnology, Food Industry *ISSN 1582-540X*

ORIGINAL RESEARCH PAPER

CHARACTERIZATION AND ANTIPARASITIC ACTIVITY OF BENZOPHENONE THIOSEMICARBAZONES ON *Trypanosoma brucei brucei*

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Received October 07, 2010
Accepted: January 10, 2011

Abstract: The structure of four synthesized thiosemicarbazones, substituted or not, of benzophenone has been confirmed by spectrometrical analysis IR, NMR ^{1}H and ^{13}C. Their anti-trypanosomal activities were evaluated on *Trypanosoma brucei brucei*. Among these compounds, benzophenone 4-phenyl-3-thiosemicarbazone **4** has the highest activity with the half-inhibitory concentration (IC_{50}) = 8.48 micromolar (µM). Benzophenone 4-methyl-3-thiosemicarbazone **3** and benzophenone thiosemicarbazone **1** showed moderate anti-trypanosomal activity with IC_{50} values equal to 23.27 µM and 67.17 µM respectively. Benzophenone 2-methyl-3-thiosemicarbazone **2** showed no activity up to IC_{50} = 371.74 µM.

Keywords: *anti-trypanosomal activities, benzophenone, half-inhibitory concentration (IC_{50}), spectrometrical analysis, substituted thiosemicarbazones*

INTRODUCTION

Trypanosomes are pathogens of Chagas disease in Central and South America and sleeping sickness in sub-Saharan Africa [1]. In Africa, the protozoan parasite of the genus *Trypanosoma* causes animal (AAT) and human African trypanosomiasis (HAT) [2]. They are transmitted by tsetse flies (tsetse fly): *Glossina palpalis palpalis* and *Glossina morsitans morsitans* vectors of *Trypanosoma brucei gambiense* and *Trypanosoma brucei rhodesiense* respectively. *Trypanosoma brucei brucei*, responsible of animal trypanosomiasis, can be transmitted both by the *palpalis* group and the *morsitans* group [3]. This type of trypanosome is studied in recent years across Africa (example of Cameroon and Ivory Coast) [4, 5]. 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 [2]. They are lethal if not treated [6]. Therefore, the development of new drugs in the treatment of these diseases is necessary and imperative [1].
The thiosemicarbazones, biologically active molecules, are known in the fight against microbial and parasitic diseases. Through to their multiple pharmacological properties: antiviral [7, 8], antibacterial [9, 10], antiparasitic [11 – 13], antitumor [14 – 16], anticonvulsant [17, 18], they are agents that block the development of microbes and parasites. Moreover, these properties are accentuated when these molecules are N(4)-substituted [19]. The presence of sulfur atom, which cheeks a key role in the antimicrobial activity generally provides a higher activity compared to semicarbazones [20, 21]. Recent studies have shown that thiosemicarbazones exhibit inhibitory effects on parasite including *Trypanosoma brucei* and *Plasmodium falciparum* [12, 22 – 24]. The aim of this work is to synthesize benzophenone thiosemicarbazones substituted or not and to evaluate their anti-trypanosomal activity on *Trypanosoma brucei brucei*.

MATERIALS AND METHODS

Materials

Equipment

The melting points were taken on a fusionometer of the type electrothermal 1A 9000 and have not been corrected. 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 recorded on a Brucker 500 in DMSO-d_6 or $CDCl_3$ and the frequencies for ^{1}H and ^{13}C are 300 – 400 MHz and 100 MHz respectively. Chemical shifts are given in parts per million (ppm) relative to tetramethylsilane as a benchmark.

Reagents

Four thiosemicarbazides: thiosemicarbazide, 2-methyl-3-thiosemicarbazide, 4-methyl-3-thiosemicarbazide and 4-phenyl-3-thiosemicarbazide obtained from ^{A}ALDRICH^{R} (SIGMA-ALDRICH Chemie GmbH, Germany) were used on benzophenone purchased from PROLABO (EMB de 45-Briare, France).

General protocol for the preparation of thiosemicarbazones of benzophenone

We prepared an equimolar mixture (0.001 mol) of benzophenone dissolved in 1.5 mL of ethyl alcohol 95° and thiosemicarbazide dissolved in 1 mL of 1N hydrochloric acid. The mixture is stirred until the appearance of precipitate. We leave the bustle continue 30 minutes to 1 hour depending on the reagent to complete the reaction. The precipitate is filtered, washed with cold distilled water until neutrality, dried and then recrystallized in ethanol 95°.

Pharmacology

Biological material

The material used for the study of pharmacological properties consists of trypanosomes: *Trypanosoma brucei brucei* (Tbb) according to the protocol below. Anti-trypanosomal activity is assessed on the half-inhibitory concentrations IC_{50} expressed in micromolar (μM) to allow comparison with the literature [11, 13].

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

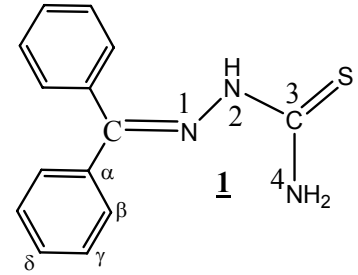
The test is performed on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* by the «LILIT Alamar Blue^{TM}» method [25 – 27]. The stock solutions of thiosemicarbazones have been prepared from an initial concentration of 10 mg·mL^{-1} in DMSO. The trypanosomes are grown in a medium containing 10% of heat-inactivated fetal calf serum and bloodstream form supporting factor. The trypanosome suspensions were adjusted to $5 \cdot 10^{-4}$ tryp·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 CMI 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.

RESULTS AND DISCUSSION

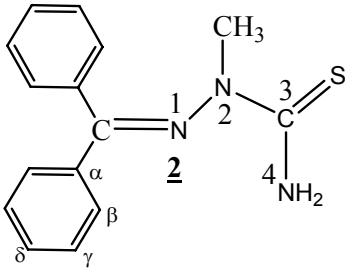
Chemistry

Four thiosemicarbazones (substituted or not) of benzophenone were synthesized by condensation reaction with thiosemicarbazides. Their formulas are given in Figure 1.

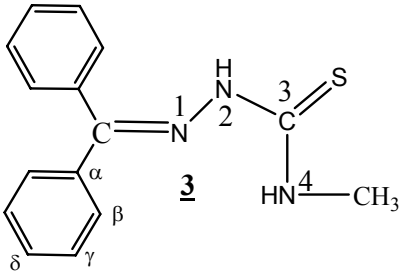
The steric and electronic effects of various substituents of thiosemicarbazides could explain the difference in yield reactions with benzophenone. The 4-phenyl-3-thiosemicarbazide is more reactive, it forms bright yellow crystals. The product **1** is obtained at 50 minutes with 84% (yield) higher performance to 25% (yield) in 30 hours shown in the work of Tarlok *et al*. in 2006 [28]. The physicochemical data are presented in Table 1.



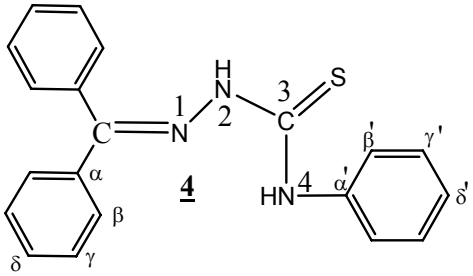
Benzophenone thiosemicarbazone



Benzophenone 2-methyl-3-thiosemicarbazone



Benzophenone 4-methyl-3-thiosemicarbazone



Benzophenone 4-phenyl-3-thiosemicarbazone

Figure 1. *Structures of thiosemicarbazones synthesized*

Table 1. *Physicochemical properties of thiosemicarbazones of benzophenone*

Compound	Raw formulae	Molecular weight [g·mol^{-1}]	Melting point [°C]	Yield [%]
1	$C_{14}H_{13}N_3S$	255	168	84
2	$C_{15}H_{15}N_3S$	269	179	61
3	$C_{15}H_{15}N_3S$	269	164	52
4	$C_{20}H_{17}N_3S$	331	153	82

The structure of these compounds is confirmed by their IR, ^{1}H and ^{13}C NMR. Spectrometrical data are recorded in Tables 2 – 4.

IR spectra

The analysis of the spectra led to vibration frequencies summarized in Table 2.

The terminal NH_2 group of compounds **1** and **2** shows three different frequencies of vibration with two NH (3410; 3248 and 3426; 3292 cm^{-1} respectively for **1** and **2**), which have different chemical environment, and a frequency NH_2 (3151 and 3121 cm^{-1} for **1** and **2**). Compounds **3** and **4** N(4)-substituted did not show the frequency of vibration NH_2 corresponding to this function. Except the product **2**, compound N(2)-substituted, the others show a vibration frequency HN(2) (respectively to 3346; 3315 and 3304 cm^{-1} for **1**, **3** and **4**). For each of the spectra we find three types of vibrations of thioamide group (N-CS-N) [28] (Table 2).

^{1}H NMR spectra

The spectral data, chemical shifts of proton peaks in the structure, of compounds are shown in Table 3.

Table 2. *Frequencies of vibration in IR*

Functions	Frequencies of vibration in cm^{-1}			
	1	**2**	**3**	**4**
NH	3410; 3346; 3248	3426; 3292	3439; 3315	3338; 3304
NH_2	3151	3121	-	-
C=N	1608	1574	1531	1594
(N-CS-N)	1069; 1026; 846	1074; 1030; 884	1073; 1024; 823	1071; 1031; 855

Table 3*. Chemical shifts of protons in ^{1}H RMN*

Functions	Proton chemical shifts in ppm			
	1	**2**	**3**	**4**
H N(4)H	6.42 (s, 2H)	Broad peak from 6.1 to 7.1 (s, 2H)	7.70 (s, 1H)	9.45 (s, 1H)
H N(2)H	8.60 (s, 1H)	-	8.65 (s, 1H)	8.75 (s, 1H)
H Aromatic	From 7.20 to 7.60 (m, 10H)	From 7.25 to7.60 (m, 10H)	From 7.25 to 7.55 (m, 10H)	From 7.25 to 7.60 (m, 15H)
CH_3 (N-CH_3)	-	3.15 N(2) (s, 3H)	3.25 N(4) (s, 3H)	-

s: singlet, m: multiplet

The proton HN(2) of thiosemicarbazone **1** and 4-methyl-3-thiosemicarbazone **3** has a high chemical shift (8.60 and 8.65 ppm for **1** and **3** respectively) due to the effect of electron with drawing groups C=S and C=N. Regarding the 4-phenyl-3-thiosemicarbazone **4**, the proton HN(4) connected to the phenyl group has its chemical shift even higher (9.45 ppm). This value is due to the phenyl group that is relocating the free pair of nitrogen and the effect of the current cycle (diamagnetic anisotropy) both of which deplete their environment by moving downfield (Table 3).

^{13}C NMR spectra

The chemical shifts corresponding to different types of carbons (peaks) present in each of the structures of compounds are given in Table 4.

Table 4. *Chemical shifts of carbon in ^{13}C RMN*

Functions	Carbon chemical shifts in ppm			
	1	**2**	**3**	**4**
C=S	179.05	181.78	178.67	175.15
C=N	150.21	15.27	149.57	149.22
N-CH_3	-	41.62 N(2)	31.27 N(4)	-
C_α	136.50	137.41	136.79	135.73
C_β	128.54	129.30	128.56	129.15
C_γ	127.88	128.39	127.72	128.02
C_δ	131.16	134.97	131.46	130.49
$C_{\alpha'}$	-	-	-	137.11
$C_{\beta'}$	-	-	-	127.75
$C_{\gamma'}$	-	-	-	129.65
$C_{\delta'}$	-	-	-	125.38

The analysis of these results confirms the presence of functions C=S, C=N, aromatic carbons and methyl carbons substituted even in products **2** and **4** (Table 4).

Pharmacology

Our compounds were tested for their anti-trypanosomal activity on *Trypanosoma brucei brucei*. Their IC_{50} values are shown in Table 5. In decreasing order of activity it is the compound **4** (8.48 µM), **3** (23.27 µM), **1** (67.17 µM) and **2** (371.74 µM).
The works of Fujii *et al.* [11] and Du *et al.* [13] showed that the thiosemicarbazones are trypanocidal when their 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 their IC_{50} are higher than 100 µM. This work allows us to classify the compound **4** as trypanocidal, compounds **3** and **1** as moderate anti-trypanosomal agents and compound **2** as having little or no activity on *Trypanosoma brucei brucei* (Table 5).

Table 5. *Anti-trypanosomal activities of benzophenone thiosemicarbazones*

Compound	IC_{50} [µM]	Anti-trypanosomal activity
1	67.17	moderate
2	no effect up to 371.74	little or no
3	23.27	moderate
4	8.48	trypanocidal

It is well known that in most cases, N(4)-alkyl or N(4)-aryl thiosemicarbazones exhibit greater anti-trypanosomal activity than their corresponding unsubstituted, probably due to increased lipophilicity [1, 29]. This is confirmed by our results.
In our case, compound **3** is N(4)-methyl and the compound **4** is N(4)-phenyl thiosemicarbazone with greater activity for **4** (Table 5). Compound **1**, not N(4)-substituted, although active, is more moderated. The compound **2**, though N(2)-substituted, has a little or no effect. The substitution at position N(2) appears to decrease the activity. The literature gives no indication on the anti-trypanosomal activities of our different compounds.

CONCLUSIONS

In total, four thiosemicarbazones have been synthesized, such as: benzophenone thiosemicarbazone **1**, benzophenone 2-methyl-3-thiosemicarbazone **2**, benzophenone 4-methyl-3-thiosemicarbazone **3** and benzophenone 4-phenyl-3-thiosemicarbazone **4**. The yield of product **1** was significantly improved compared to the work of Tarlok *et al.* [28]. The structures were confirmed by spectrometrical analysis IR, ^{1}H and ^{13}C NMR. The evaluation of pest control activities of these compounds on *Trypanosoma brucei brucei*, not yet investigated to our knowledge, reveals that the compound shown anti-trypanosomal activity. Such molecules could open a promising avenue in the fighting against trypanosomiasis.

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