Hemi-Synthesized of Trypanocidal Thiosemicarbazones in Three Essential Oils Rich in Carbonyl Compounds

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Abstract

Essential oil of C. citratus, C. schoenantus and E. citriodora were extracted. These oils respectively contain citral (aldehyde: 72.91%) of piperitone (ketone: 68.2%) and citronellal (aldehyde: 67.5%). From these three EO, fourteen thiosemicarbazones were hemi-synthesized in-situ. All these molecules were purified and their structures have been determined by the IR spectrometric analysis, 1H NMR, 13C NMR and MS. From antitrypanosomien testing and toxicity testing on shrimp larvae, the selectivity indices of the molecules were determined. The citral thiosemicarbazone (IS = 51.27), the citral-4-phenyl-3-thiosemicarbazone (IS = 36.07 revealed an impressive selectivity. The selectivity essential oil of Cymbopogon citratus is also interesting (IS = 11 47). This is a job during which new selective molecules of thiosemicarbazones against trypanosome parasites were hemi-synthesized in-situ in essential oils rich in carbonyl compounds.

Keywords: Antitrypanosomal, essential oils, hemi-synthetic, selectivity index, spectroscopic analysis.

Introduction

With a mortality rate of nearly 9 million in 2008, infectious and parasitic diseases account for 26.3% of deaths across the world [1]. Over 90% of human infectious diseases, unfortunately, occur in developing countries where there is great poverty [2-4].

Among these infections, we are trypanosomiasis. This is a group of important human and animal diseases caused by parasitic protozoa of the Trypanosoma genus. This disease is specific to Africa and is deployed in situations of great poverty [5]. Most drugs used against the disease are difficult to use, toxic and were developed several decades ago [6]. In untreated, these diseases can be fatal. The medium and long term prospects for the development of new drugs effective and safe against these diseases must be necessarily considered.

Among active synthetic molecules, thiosemicarbazones caught our attention. They are highly coveted by molecules of pharmaceutical chemistry because of their many interesting biological properties. This is what justifies the importance given to them as part of the fight against bacterial disease [7,8], tumoral disease [9,10], trypanosomal disease [11,12] and viral disease [13] etc.

As thiosemicarbazones molecules result from the condensation of carbonyl compounds with thiosemicarbazides, essential oils rich in carbonyl compounds may serve as substrates in the hemi-synthesis reactions for their syntheses.

This work aims to replace commercial substrates by essential oils rich in carbonyl compounds in the hemi-synthesis reactions leading to the formation of substituted semicarbazones thiosemicarbazones and to determine their selectivity on *Trypanosoma brucei brucei*.
This topic seemed all the more interesting as the Benin flora is extremely rich in highly available aromatic plants, which, in turn, are rich in essential oils.

Material and Methods

The plant material is made from the leaves of *Cymbopogon citratus*, *Cymbopogon schoenantus* and *Eucalyptus citriodora*.

Extraction equipment of essential oils

Hydrodistillation variant used is saturated steam distillation. The extractions are always made from 500 g of plant material. The Clevenger apparatus has enabled us to achieve the extraction of essential oils.

Chemical analysis equipment of essential oil

The analysis is performed on a FOCUS GC with a capillary column CP Wax 52 CB (J & W Scientific from Agilent Technologies Column, No. US1670726A, USA) of dimension 15 x 0.25 mm with 0.25 µm internal diameter. In order to confirm the specificity and selectivity of the GC method, GC/MS analysis were performed on a TRACE GC 2000 series (ThermoQuest, Rodano, Italy), equipped with an AS2000 autosampler (GC System ThermoQuest. coupled to a mass spectrometer type TheroQuest Trace MS) operating in electron impact mode. The compounds are identified by comparing their retention time and mass spectra with those of reference compounds.

Hemi-synthesis

Essential oils extracted were used as substrates in the various hemi-synthesis reactions. During these reactions must respectively reacted *in-situ*, carbonyl compounds such as piperitone, citral (neral and geranial) and citronellal essential oils of *Cymbopogon schoenantus*, *Cymbopogon citratus* and *Eucalyptus citriodora*.
The reagents used are sold by the company Acros Organics and Aldrich. These are: hydrochloride semicarbazide, thiosemicarbazide, 2-methyl thiosemicarbazide, 4-methyl thiosemicarbazide, 4-phenyl semicarbazide, 4-phenyl thiosemicarbazide), triethylamine and hydrochloric acid.

Synthesis and identification of compounds

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 CDCl3 (chloroform-d6) or DMSO-d6 (dimethylsulfoxide-d6) which frequencies for 1H and 13C 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.

Methods of Synthesis

The synthesis methods used to semicarbazones and thiosemicarbazones is similar to those described by Fatondji and Du [14,15].

Citralsemicarbazone 1a

To a stirred mixture of 0.001 mol of EO of *C. citratus* (152 mg) dissolved in 1.5 mL of ethanol at 95° and 0.001 mol of semicarbazide hydrochloride (111.5 mg) dissolved in 1 mL of water, we added two drops of triethylamine (Fatondji et al. 2010). After the appearance of the crystals, stirring is continued for one hour. The precipitate is then filtered, washed with distilled water, dried, weighed and recrystallized in ethanol.

Citralthiosemicarbazone 2a

A mixture of 0.001 mol of EO of *C. citratus* (152 mg) dissolved in 1.5 ml of 95° ethanol is added to 0.001 mol of thiosemicarbazide (91 mg) dissolved in 2 ml of 1N hydrochloric acid. The mixture of the two solutions is stirred at room temperature until crystals thiosemicarbazone formation. Stirring is maintained for one hour. The precipitate is then filtered, washed with distilled water, dried, weighed and recrystallized from ethanol.

Citral-2-methyl-3-thiosemicarbazone 3a; citral-4-methyl-3-thiosemicarbazone 4a; citral-4-phenyl-3-semicarbazone 5a; citral-4-phenyl-3-thiosemicarbazone 6a

0.001 mol of substituted thiosemicarbazide (105 mg of 2-methyl-3-semicarbazide, 105 mg of 4-methyl-3-thiosemicarbazide, 151 mg 4-phenyl-3-semicarbazone or 167 mg 4-phenyl-3-thiosemicarbazide) were dissolved in 2 ml of 1N hydrochloric acid. In each case reaction, the dissolved reagent is added to 0.001 mol of EO of *C. citratus* (152 mg) dissolved in 1.5 ml of 95° ethanol. The stirred mixture sets solid. The mass is gradually dispersed to give crystals. Stirring is continued until the crystals formation. The precipitate obtained in each case was filtered, washed with distilled water, dried, weighed and recrystallized from ethanol.

Pipéritonesemicarbazone 1b

In the solution of 0.002 moles dissolved 1.5 ml of 95° ethanol of EO of *C. schoenantus* (304 mg), we added 0.001 mol of semicarbazide hydrochloride (111.5 mg) previously dissolved in 1 ml water. Two drops of triethylamine are added to the mixture after one minute of agitation. The stirring mixture is heated to a temperature of 50° C for several minutes. It leaves the agitation continued at room temperature. The collected precipitate is filtered, washed with distilled water, dried, weighed and recrystallized in ethanol.

Pipéritonethiosemicarbazone 2b, piperitone-4-phenyl-3-semicarbazone 3b, piperitone-4-phenyl-3-thiosemicarbazone 4b

0.001 mol of substituted or unsubstituted thiosemicarbazide (91 mg of thiosemicarbazide, 151 mg 4-phenyl-3-semicarbazide or 167 mg 4-phenyl-3-thiosemicarbazide) is dissolved in 2 ml of 1N hydrochloric acid. In each case, the dissolved reagent is added to 0.002 mol of *C. schoenantus* EO (304 mg) dissolved in 1.5 ml of 95° ethanol. The stirring mixture is brought to a temperature of 50 ° C. If the mixture is cloudy, add drip ethanol until it is clear. The crystals appear a few minutes later. Stirring is continued at room temperature for 1 hour. The precipitate obtained in each case was filtered, washed with distilled water, dried, weighed and recrystallized in ethanol. The equation of the reaction is summarized in.

Citronellalsemicarbazone 1c

To a stirred mixture of 0.002 mol of *E. citrodora* oil (308 mg) dissolved in 1.5 ml of 95° ethanol and 0.001 mol of semicarbazide hydrochloride (111.5 mg) dissolved in 1 ml of water, add two drops of triethylamine after one minute of agitation. The mixture temperature is brought to 50° C. After the appearance of the crystals after a few minutes, stirring is continued for 1 hour at 50° C. The precipitate is cooled in a refrigerator for 1 hour before being filtered, washed with distilled water, dried, weighed and recrystallized in ethanol.

Citronellalthiosemicarbazone 2c, Citronellal-4-phenyl-3-semicarbazone 3c, Citronellal-4-phenyl-3-thiosemicarbazone 4c

To a mixture of 0.002 mol of *E. citrodora* EO (308 mg) dissolved in 1.5 ml of 95° ethanol, we added 0.001 mol of thiosemicarbazide (91 mg) dissolved in 2 ml of 1N hydrochloric acid. This mixture brought to a temperature of 50 ° C was stirred until thiosemicarbazone crystals formation. The stirring is then continued at room temperature for one hour. The precipitate is cooled, filtered, washed with distilled water, dried, weighed and recrystallized in ethanol.

Biology animal material

The antitrypanosomal tests are performed on 427 strain of *Trypanosoma brucei brucei*. *Artemia salina* (LEACH) is the biological material used in the evaluation of the larval toxicity of the molecules.

Methods of carrying biological tests

The test is performed on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* by the "Lilit Alamar Blue" method [16]. The stock solutions of thiosemicarbazones have been prepared from an initial concentration of 10 mg/ml 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 5.10^{-4} tryp/mL. 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.

Toxicity Test against *Artemia salina*

The test is performed against *Artemia salina* LEACH by the method of Michael resumed by Vanhaecke [17,18]. The eggs of *Arténia salina* are incubated in seawater until hatching of young larvae (48 hours). Then, series of solutions of test substance at varying and progressive concentrations were prepared. A defined number of larvae are introduced into each solution. All solutions and control solution containing no active substance were left stirring for 24 hours. 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)] x 100. Data (dose-response) are transformed by logarithm and the LC_{50} is determined by linear regression.

Results and Discussion

Study of essential oils

After extraction, it appears that plants have a good yield in essential oil. Further analysis reveals a high rate of carbonyl compounds in these oils. This information is favorable for our subsequent reactions. EO of *C. citratus, C. Schoenantus and E. citriodara* are extract with respective yield of 1.48%, 2.5%, 4.3%. They contain respectively 72.91% of citral, 68.20% of piperitone and 67.5% of citronellal.

Pharmacological properties of EO

The antitrypanosomal test revealed that *C. citratus* EO strongly inhibits trypanosome parasites (IC_{50} = 6.80 µg/mL). Antitrypanosomal activities of *C. Schoenantus* and *E. citriodara* EO are rather moderate with respective IC_{50} values of 16.74 µg/mL and 17.96 µg/mL. Given the good antitrypanosomal activity of *C. citratus* EO, we can conclude that this work brings more in the fight against trypanosomiasis disease. The essential oils of *C. citratus C. schoenantus* and *E. citriodora* indicated the respective LC_{50} values of 78 µg/mL, 107 µg/mL and 106 µg/mL during the toxicity assays. All the oils have a LC_{50} value greater than 30µg/mL. According to the literature, they are very little toxic [19]. It would be interesting to note that the oil most active on the parasites is the most toxic. The value of the selectivity index of *C. citratus* EO on trypanosome parasites is the highest (IS = 11.47). *Cymbopogon citratus* EO is the most selective oil. *C. Schoenantus* (IS = 6.39) *E. citriodara* (IS = 5.90) EO are less selective.

Physical properties of semicarbazones and thiosemicarbazones hemi-synthesized in the three oils

Among hemi-synthesized molecules, only the synthesis of compound **2a** and **2c** has been reported in the literature from commercial citral and citronellal [20]. To our knowledge, this was during this work that compounds **3a, 4a, 5a, 6a 1b, 2b, 3b and 4b, 1c, 3c** and **4c** have been synthesized for the first time. Furthermore, the hemi-synthesis method *in-situ* in the essential oils has never been reported in the literature. Citralthiosemicarbazones yields are higher than those of pipéritonethiosemicarbazones which, in turn are higher than those of citronellalthiosemicarbazones (table 1). It is deduced that the reactivity decreases from citral to piperitone, then from piperitone to citronellal. The conjugated bonds in citral and piperitone favor this reactivity.

Determination of structures

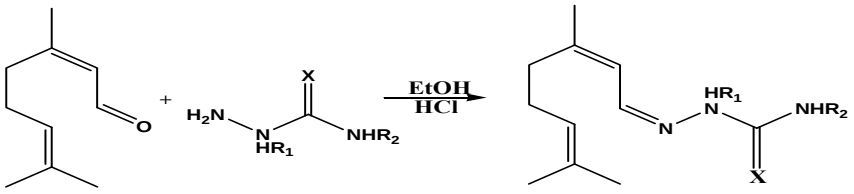
Citralsemicarbazone (1a)

MS: $[MH]^+$ calculated : 210,15 $[MH]^+$ found : 210,04.

IR ν (NaCl, cm^{-1}): 3429 (NH_2); 3310 (NH); 1661 (C=O); 1598 (C=N); 1598 (C=C).

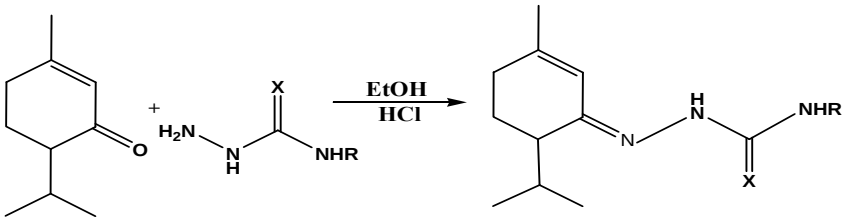
NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 154 (C=O); 147 (C=N); 140, 121 (C=C); 39, 26 (CH_2-CH_2); 23; 24 et 18 (CH_3).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 1,3 (d, 3H, -$CHCH_3$); 1,4 et 1,5 (s, 6H, -$C(CH_3)_2$); 2,1 (m, 4H, -H_2C-CH_2); 4,9 (m, 1H, -CH=$(CH_3)_2$); 5,9 (d, 1H, =C**H**-CH=N-); 7,6 et 7,7 (s, 2H, -NH_2); 7,1 (d, 1H, =CHC**H**=N); 9,8 et 9,9 (s, 1H, =NNH-).



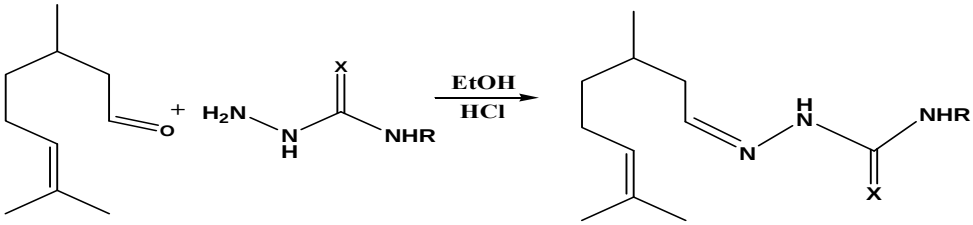
X = O, R_1 = H, R_2 = H: **1a**
X = S, R_1 = H, R_2 = H: **2a**
X = S, R_1 = CH_3, R_2 = H: **3a**
X = S, R_1 = H, R_2 = CH_3: **4a**
X = O, R_1 = H, R_2 = C_6H_5: **5a**
X = S, R_1 = H, R_2 = C_6H_5: **6a**

Figure 1: Global reaction equation of citralthiosemicarbazones



X = O R = H: **1b**
X = S, R = H: **2b**
X = O, R = C_6H_5: **3b**
X = S, R = C_6H_5: **4b**

Figure 2: Global reaction equation of pipéritonethiosemicarbazones



X = O, R = H: **1c**
X = S, R = H: **2c**
X = O, R = C_6H_5: **3c**
X = S, R = C_6H_5: **4c**

Figure 3: Global reaction equation of citronellalthiosemicarbazones.

Citralthiosemicarbazone (2a)

MS : $[MH]^+$ calculated : 226,13 $[MH]^+$ found : 226,10.

IR ν (NaCl, cm^{-1}): 3373 et 3271 (NH_2); 3165 (NH); 1643 (C=N); 1609 (C=C) ; 836 (C=S). **NMR ^{13}C** ($CDCl_3$, 100MHz) δ(ppm): 178 (C=S); 142 (C=N) ; 139 et 121 (C=C) ; 31et 27 (CH_2-CH_2); 25; 22 et 19 (CH_3).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 1,6 (d, 3H, -$CHCH_3$); 1,7, et 1,8 (s, 6H, -$C(CH_3)_2$); 2,2 (m, 4H, -H_2C-CH_2); 5,1 (m, 1H, -CH=$(CH_3)_2$); 5,9 (d, 1H, =CH-CH=N-); 7,1 et 7,2 (s, 2H, -NH_2); 7,9 (d, 1H, =CHCH=N); 10,2 (s, 1H, =NNH-).

Citral-2-méthyl-3-thiosemicarbazone (3a)

MS : $[MH]^+$ calculated : 240,15 $[MH]^+$ found : 240,09.

IR ν (NaCl, cm^{-1}): 3427 et 3251 (NH_2); 1642 (C=N); 1586 (C=C) ; 857 (C=S).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 181(C=S) ; 140 (C=N); 135 et 121 (C=C) ; 40 et 33 (CH_2-CH_2); 26 (NCH_3); 18; 23 et 24 (CH_3).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 1,6 (d, 3H, -$CHCH_3$); 1,7 et 1,8 (s, 6H, -$C(CH_3)_2$); 2,2 (m, 4H, -H_2C-CH_2); 3,6(s, 3H, NCH_3) ; 5 (m, 1H, -CH=$(CH_3)_2$); 5,9 et **6,1** (d, 2H, =CH-C**H**=N-); 7,2 (d, 1H, =CHCH=N); 7,6 (s, 2H, -NH_2).

Citral-4-méthyl-3-thiosemicarbazone (4a)

MS : $[MH]^+$ calculated : 240,15 $[MH]^+$ found : 240,2.

IR ν (NaCl, cm^{-1}): 3424 (NH); 3275 (NH); 1639 (C=N); 1544 (C=C) ; 855 (C=S).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 179 (C=S); 143 (C=N) ; 134 , 122 (C=C) ; 40 ; 32 (CH_2-CH_2) ; 25 (NCH_3); 17, 22, 24 (CH_3).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 1,6 (d, 3H, -$CHCH_3$); 1,7 et 1,8 (s, 6H, -$C(CH_3)_2$); 2,1 (m, 4H, -H_2C-CH_2); 3,1(s, 3H, $NHCH_3$) ; 5 (m, 1H, -CH=$(CH_3)_2$); 5,8, (d, 1H, =C**H**-CH=N-); 7,2 (m, 1H, -$NHCH_3$) ; 7,8 (t, 1H, =CHC**H**=N); 9,9 (s, 1H, =N-NH-).

Citral-4-phényl-3-semicarbazone (5a)

MS : $[MH]^+$ calculated : 286,18 $[MH]^+$ found : 286,19.

IR ν (NaCl, cm^{-1}): 3662 (NHPh); 3443 (NH) ; 1693 (C=O) ; 1594 (C=N); 1539 (C=C).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 153 (C=O); 145 (C=N) ; 131 et 125 (C=C) ; 119, 120, 122 (aromatiques); 38 et 30 (CH_2-CH_2) ; 23 (NCH_3); 17 ; 22 et 23 (CH_3).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 1,7 (d, 3H, -$CHCH_3$); 1.8 et 1.9 (s, 6H, -$C(CH_3)_2$); 2,1 et 2,2 (m, 4H, -H_2C-CH_2) ; 5,1 (m, 1H, -CH=$(CH_3)_2$); 6 (d, 1H, =C**H**-CH=N-); 7 ; 7,3 et 7,5 (aromatiques) 7,8 (d, 1H, =CHC**H**=N); 8 (s, 1H, -NHPh) ; 9,8 (s, 1H, =NNH-).

Citral-4-phényl-3-thiosemicarbazone (6a)

MS : $[MH]^+$ calculated : 302,16 $[MH]^+$ found : 302,10.

IR ν (NaCl, cm^{-1}): 3418 (-NHPh); 3280 (NH) ; 1644 (C=N); 1595 (C=C) ; 855 (C=S).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 181 (C=S); 140 (C=N); 135 ; 121(C=C) ; 121, 122, 124 (aromatiques); 40 ; 35 ; 23 ; 18 (CH_3).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 1,5 (d, 3H, -$CHCH_3$); 1,7, et 1,8 (s, 6H, -$C(CH_3)_2$); 2,1 (m, 4H, -H_2C-CH_2) ; 5,1 (m, 1H, -CH=$(CH_3)_2$); 5,9 (d, 1H, =CH-CH=N-); 7,1 ; 7,2 ; 7,5 (aromatiques) 7,9 (d, 1H, =CHCH=N); 9,1 (s, 1H, -NHPh) ; 10,4 (s, 1H, =NNH-).

Pipéritonesemicarbazone (1b)

MS : $[MH]^+$ calculated : 210,0 $[MH]^+$ found : 210,04.

IR ν (NaCl, cm^{-1}): 3530 et 3440 (NH_2); 3145 (NH); 1576 (C=N); 1671 (C=O); 1471et 1375 (C=C).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 156 (C=O); 145 (C=N); **132** et 128 (-H_2C**C**=CC=N) ; **41** et 36 (-**C**H-CH$(CH_3)_2$); **39** ; 34 (C**C**H_2-CH_2CH); 31 (-CH_3); 19 et 21(C(**C**$H_3)_2$).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 1,2 et 1,3 (d, 6H, -$C(CH_3)_2$); 1.9 (s, 3H, -CH_3); 2,2 et 2,3 (m, 4H, 2CH_2); 2,4 (m, 1H, -CH$(CH_3)_2$); 2,5 (m, 1H,N =CCH-); 5,6 (s, 1H, C=CH); 6,8 et 6,9 (s, 2H, $CSNH_2$); 8,8 (s, 1H, =NNH−).

Pipéritonethiosemicarbazone (2b)

MS : $[MH]^+$ calculated : 226,13 $[MH]^+$ found : 226,04.

IR ν (NaCl, cm^{-1}): 3587et 3523, (NH_2); 3261 (NH); 1606 (C=N); 1495 et 1476 (C=C); 831 (C=S).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 178 (C=S); 145 (C=N); **121** et 118 (-H_2C**C**=CC=N) ; **43** et 29 (-**C**H-$CH(CH_3)_2$); **40** et 28 (C**C**H_2-CH_2CH); 25 (-CH_3); 21et 19(C(**C**$H_3)_2$).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 0,9 et 1,0 (d, 6H, -$C(CH_3)_2$); 1,7 (s, 3H, -CH_3); 1,6 et 1,9 (m, 4H, $2CH_2$); 2,2 (m, 1H, -$CH(CH_3)_2$); 2,3 (m, 1H, -CHC=N); 6,1 (s, 1H, C=CH); 7,1et 7,2 (s, 2H, $CSNH_2$); 8,6 et 8,8 (s, 1H, =NNH−).

Pipéritone-4-phenyl-3-semicarbazone (3b)

MS : $[MH]^+$ calculated : 286,18 $[MH]^+$ found : 286,06.

IR ν (NaCl, cm^{-1}): 3607 (-NHPh); 3198 (NH); 1685 (C=O); 1602 (C=N); 1499 et 1447 (C=C).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 155 (C=O); 142 (C=N); **132** et 122 (-H_2C**C**=CC=N) ; **40** ; 25 (-**C**H-$CH(CH_3)_2$); **32** et 25 (C**C**H_2-CH_2CH); 24 (-CH_3); 20 et 19 (C(**C**$H_3)_2$).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 0,9 et 1,1 (d, 6H, -$C(CH_3)_2$); 1,6 (s, 3H, -CH_3); 2 et 2,1 (m, 4H, $2CH_2$); 2.2 (m, 1H, -$CH(CH_3)_2$); 2,4 (m, 1H, N=CCH); 5.6 (s, 1H, C=CH);: 7,1; 7,3 et 7,5 (H aromatiques) ; 8,3 (s, 1H, CSNH); 9,1; (s, 1H, =NNH−).

Pipéritone-4-phenyl-3-thiosemicarbazone (4b)

MS : $[MH]^+$ calculated : 302,16 $[MH]^+$ found : 302,03.

IR ν (NaCl, cm^{-1}): 3522 (-NHPh); 3323 (NH); 1591 (C=N); 1539 et 1487 (C=C); 853 (C=S).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 182 (C=S); 144 (C=N); **130** et 123 (-H_2C**C**=CC=N) ; **43** et 29 (-**C**H-$CH(CH_3)_2$); **39** et 27 (C**C**H_2-CH_2CH); 22 (-CH_3); 20 et 19 (C(**C**$H_3)_2$).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 0,8 et 1,1 (d, 6H, -$C(CH_3)_2$); 1,6 (s, 3H, -CH_3); 2,1et 2,4 (m, 4H, $2CH_2$); 2,3 (m, 1H, -$CH(CH3)_2$); 2,4 (m, 1H, N=CCH-); 6,1 (s, 1H, CH=C); 7,2; 7,3 et 7,7 (H aromatiques) ; 8,8 (s, 1H, CSNH); 9,3 (s, 1H, =NNH−).

Citronellalsemicarerbazone (1c)

MS : $[MH]^+$ calculated : 212,17 $[MH]^+$ found : 212,17.

IR ν (NaCl, cm^{-1}): 3594 et 3481 (NH_2); 3182 (NH); 1668, (C=O); 1578 (C=N); 1518 et 1449 (C=C).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 158 (C=O); 146 (C=N); 122 et **128** (H_2CC=**C**$(CH_3)_2$) ; **35** et 37 (**C**HCH_2CN); **23** et 34 (HC**C**H_2CH_2C=); 20 (-CH_3); 27 et 28 (=$C(CH_3)_2$).

NMR ^{1}H ($CDCl_3$, 400MHz), δ(ppm): 1,0 (d, 3H, -$CHCH_3$); 1.3, et 1.4 (s, 6H, -$C(CH_3)_2$); 1,6 (m, 2H, -C**H**$_2$CH); 1,9 (m, 2H, -C**H**$_2$CH=); 2,2 (m, 1H, -C**H**CH_2); 2,6 (t, 2H, -C**H**$_2$CH=N); 4,3 (t, 1H, -CH=$C(CH_3)_2$); 6,8 (t, 1H, -CH=N); 7,2 et 7,4 (s, 2H, -NH_2); 8.2 (s, 1H, =NNH-).

Citronellalthiosemicarerbazone (2c)

MS : $[MH]^+$ calculated : 228,15 $[MH]^+$ found : 228,00.

IR ν (NaCl, cm^{-1}): 3534 et 3445 (NH_2); 3177 (NH); 1576 (C=N); 1483 et 1459, (C=C); 979 (C=S).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 178 (C=S); 144 (C=N); 126 et **128** (H_2CC=**C**$(CH_3)_2$) ; **38** et 39 (**C**HCH_2CN); **23** et 38 (HC**C**H_2CH_2C=); 22 (-CH_3); 30 et 31 (=$C(CH_3)_2$).

NMR ^{1}H ($CDCl_3$, 400MHz), 1.6 (d, 3H, -$CHCH_3$); 1.9, et 1.9 (s, 6H, -$C(CH_3)_2$);); 1.6 (m, 2H, -CH_2CH); 2,2 (m, 1H, -$CHCH_2$); 2,5 (m, 2H, -CH_2CH=); 3.5 (t, 2H, -CH_2CH=N); 5.2 (t, 1H, -CH=C); 7.0 et 7,1 (s, 2H, -NH_2); 7.3 (t, 1H, -CH=N); 9.5 (s, 1H=NNH-).

Citronellal-4-phenyl-3-semicarbazone (3c)

MS : $[MH]^+$ calculated : 288,2 $[MH]^+$ found : 288,27.

IR ν (NaCl, cm^{-1}): 3529 (NHPh); 3200 (NH); 1684 (C=O); 1594 (C=N); 1500 et 1446 (C=C).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 158 (C=O); 143 (C=N); 130 et **134** (H_2CC=**C**$(CH_3)_2$) ; **33** et 40 (**C**HCH_2CN); **24** et 26 (HC**C**H_2CH_2C=); 18 (-CH_3); 25 et 26 (=$C(CH_3)_2$) ; les aromatiques : 125 ; 126 ; 128 et 134.

NMR ^{1}H ($CDCl_3$, 400MHz), 1,1 (d, 3H, -$CHCH_3$); 1.6 et 1.8 (s, 6H, -$C(CH_3)_2$); 2 (m, 2H, -CH_2CH); 2,2 (m, 1H, -$CHCH_2$); 2.2 (m, 2H, -CH_2CH=); 2,8 (t, 2H, -CH_2CH=N); 4.2 (t, 1H, -CH=C); 7,2; 7,3 et 7,5 (H aromatiques) 7.7 (s, 1H, -NHPh); 7.9 (t, 1H, -CH=N); 8.6 (s, 1H=NNH-).

Citronellal-4-phenyl-3-thiosemicarerbazone (4c)

MS : $[MH]^+$ calculated : 304,18 $[MH]^+$ found : 304,15.

IR ν (NaCl, cm^{-1}): 3528 (-NHPh); 3181 (NH); 1600 et 1590 (C=N); 1498 et 1458 (C=C); 878 (C=S).

NMR ^{13}C ($CDCl_3$, 100MHz) δ(ppm): 183 (C=S); 142 (C=N); 133; **138** (H_2CC=**C**$(CH_3)_2$) ; **34** et 40 (**C**HCH_2CN); **24** et 27 (HC**C**H_2CH_2C=); 18 (-CH_3); 25 et 26 (=$C(CH_3)_2$) ; les aromatiques : 124 ; 126 ; 128 et 130.

NMR ^{1}H ($CDCl_3$, 400MHz), 1,7 (d, 3H, -$CHCH_3$); 1,8, et 1,8 (d, 6H, -$C(CH_3)_2$); 1,9 (m, 2H, -CH_2CH); 2.3 (m, 1H, -$CHCH_2$); 2,8 (m, 2H, -CH_2CH=); 3,4 (t, 2H, -CH_2CH=N); 4.6 (t, 1H, -CH=C); 7,1; 7,3 et 7,4 (H aromatiques) 7.6 (s, 1H, -NHPh); 7.8 (t, 1H, -CH=N); 9.7 (s, 1H, =NNH-).

Antitrypanosomal activities thiosemicarbazones

The scale used in this work to assess the antitrypanosomal properties of molecules hemi-synthesized, is that adopted by Du and Fujii [21].

Based on this scale described above, it appears that the compounds **2a**, **6a**, **3b** and **4b** are trypanocidal. Among the compounds with moderate activity, there are compounds **3a**, **5a**, **2b**, **3c** and **4c**. The compounds **1a**, **4a**, **1b**, **1c** and **2c** have little effect against *Trypanosoma brucei brucei* (table 2).

Table 1: Physical properties of semicarbazones and thiosemicarbazones hemi-synthesized in the three oils

N°	Names	Empirical formulas	M (g/mol)	Melting Points (°C)	Yield (%)
1a	Citralsemicarbazone	$C_{11}H_{19}ON_3$	209,04	120	79
2a	citralthiosemicarbazone	$C_{11}H_{19}SN_3$	225,10	105	83
3a	Citral-2-methyl-3-thiosemicarbazone	$C_{12}H_{21}SN_3$	239	79	73
4a	Citral-4-methyl-3-thiosemicarbzone	$C_{12}H_{21}SN_3$	239,20	102	80
5a	Citral-4-phenyl-3-semicarbazone	$C_{17}H_{23}ON_3$	285,19	78	81
6a	Citral-4-phenyl-3- thiosemicarbazone	$C_{17}H_{23}SN_3$	301,10	82	91
1b	Piperitonesemicarbazone	$C_{11}H_{19}N_3O$	209,04	196	56
2b	Piperitonethiosemicarbazone	$C_{11}H_{19}N_3S$	225.04	163	67
3b	Piperitone-4-phényl-3-semicarbazone	$C_{17}H_{23}N_3O$	285.06	165	71
4b	Piperitone-4-phényl-3-thiosemicarbazone	$C_{17}H_{23}N_3S$	301.03	120	87
1c	Citronellalsemicarbazone	$C_{11}H_{21}N_3O$	211,17	83	48
2c	citronellalthiosemicarbazone	$C_{11}H_{21}N_3S$	227,0	178	67
3c	Citronellal-4-phényl-3-semicarbazone	$C_{17}H_{25}N_3O$	287,27	120	58
4c	Citronellal-4-phényl-3-thiosemicarbazone	$C_{17}H_{25}N_3S$	303,15	143	77

Table 2: Selectivity index of semicarbazones and thiosemicarbazones

Compounds	LC_{50} (µM)	IC_{50} (µM)	(SI = LC_{50}/IC_{50})
1a	420.39	234.64	1.79
2a	390.22	7.61	**51.27**
4a	275.36	172.84	1.59
5a	101.7	18.10	5.61
6a	70.70	1.96	**36.07**
HE (*C. citratus*)	**78 µg/mL**	**6.80 µg/mL**	**11.47**
1b	373.20	478.47	0.78
2b	86.66	74.58	1.16
3b	85.52	10.90	7.84
4b	32.22	8.63	3.73
HE (*C. schoenantus*)	107 µg/mL	16.74 µg/mL	6.39
1c	462.08	473.93	0.97
2c	121.15	440.53	0.27
3c	45.05	57.26	0.79
4c	96.53	19.63	4.92
HE (*E. citriodora*)	106 µg/mL	17.96 µg/mL	5.90

Toxicity tests on shrimp larvae

When toxicity value is greater than 281 µM, the molecule is not active on the larvae [22]. Among the compounds of the series A, only compounds **5a** and **6a** show activity on shrimp larvae. The others are not active (Table 1). With the exception of the compound **1b**, all compounds of series B are active on the larvae (Table 2). The compound **1c** is not the only asset in the series C (Table 2).

Selectivity index (SI)

Thiosemicarbazones selectivity index are shown in Table 2. All thiosemicarbazones from the *C. citratus* EO are selective on the relevant parasites. The selectivities of compounds **2a** (SI = 51.27) and **6a** (SI = 36.07) are very pronounced and significantly stronger than that of the initial EO (SI = 11.47). In the series of piperitone, selectivities of compounds **2b**, **3b** and **4b** are as remarkable but less significant than in previous cases. Of these, **3b** (SI = 7.84) has a higher selectivity than that of the initial EO (SI = 6.39). One compound from essential oil *E. citriodora* proved selective on *Trypanosoma brucei brucei*. It is the compound **4c**. But oil remains in the last case more selective than compounds. Despite the moderate activity of certain molecules, we note that 10 compounds are still selective about trypanosome parasites.

Structure-activity relationship

Citralthiosemicarbazones are more selective against trypanosome parasite than piperitonethiosemicarbazones, which in their turn, are more selective than citronellalthiosemicarbazones. The substitution of hydrogen with a methyl group decreases the activity. The presence and increasing the mesomeric effect in the molecule increases the trypanocidal activity. This is particularly striking when replacing hydrogen of NH_2 group by an aromatic ring. The reduction of the double C = C also decreases the activity. It is the case when going citral to citronellal. *Lipinski's "Rule of Five"*

The properties concerning the pharmacokinetics should therefore be optimized to result in a medicinal molecule [23]. The compounds hemi-synthesized in this work meet at least three criteria of the "five rule of Lipinski." These molecules could be good drug candidates. The strong trypanocidal activity of N- phenyl substituted is linked to fewer hydrogen donors.

Conclusion

From EO often unstable and aggressive to used, can be hemi-synthesized pure thiosemicarbazones, not aggressive and provided excellent antitrypanosomal activities. This work brings from our local resources more in the field of modern pharmaceutical chemistry. The approach taken in this work has not only allowed us to develop a new line of research in the field of essential oils, but it also led us towards obtaining new active molecules.

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