

Synthesis and trypanocidal activity of salicylhydrazones and *p*-tosylhydrazones of S-(+)-carvone and arylketones on African trypanosomiasis

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ABSTRACT

Hydrazones are nowadays considered to be good candidates for various pharmaceutical applications. Here, we have synthesized two series of hydrazones: salicylhydrazones (GS1-4) and *p*-tosylhydrazones (GT1-4) from S-(+)-carvone and three arylketones with good yields (57-91%). Molecules were characterized by elemental analyses; TLC, NMR ¹H, NMR ¹³C and MS. Submitted, *in vitro*, to their antiparasitic testing on *Trypanosoma brucei brucei*, and toxicity on *Artemia salina* Leach, all compounds except GT2 showed significant antitrypanosomal activity IC₅₀ ranging from 1 to 34 micromolar (μM). Among them, 2-acetylnaphthalene salicylhydrazone GS4 (IC₅₀ = 1.97 ± 0.42 μM) and 7-methoxy-1-tetralone *p*-tosylhydrazone GT3 (IC₅₀ = 7.98 ± 1.65 μM) exhibited good trypanocidal activity and the other are moderates on parasite; when the compounds GS1, GT3 and GT4 presented toxic activity on larvae. In agreement to their selectivity index, which is greater than 1 (SI > 1), products turn out quite selective on the parasite: a series of salicylhydrazones revealed more selective (SI ≥ 11), especially GS4 (SI = 157) than the series of *p*-tosylhydrazones showed 1 ≤ SI ≤ 22. The synthesized compounds clearly displayed significant selective pharmaceutical activities on the parasite tested. Compounds developing could open promising route to news drug-candidates.

INTRODUCTION

Hydrazides and hydrazones are nowadays an important class of biologically active compounds (Ali *et al.*, 2012; Narasimhan *et al.*, 2010). They exhibit a wide range of interesting pharmaceutical derivatives and are also important intermediates in organic synthesis (Rollas *et al.*, 2007). In addition, these compounds have shown anti-cancer properties (Wandakhan *et al.*, 2013; El-Sabbagh and Rady, 2009; Zhang *et al.*, 2004), anti-HIV (Vicini *et al.*, 2009) and are potential inhibitors for many enzymes (Xu *et al.*, 2008; Hassanien *et al.*, 2008; Singh et Kumar, 2006), DNA synthesis and cell growth

(Sreeja *et al.*, 2004) and therefore they acquired an important place in medicinal chemistry.

Some hydrazide-hydrazones derivatives have been commercialized: the nifurtimox is used for the treatment of Chagas' disease (Melnyk *et al.*, 2006) and nifuroxazide (D) as intestinal antiseptic (Küçükgülzel *et al.*, 2003). Hydrazones can act as polydentate ligands, depending on the nature of the substituent attached to the hydrazone unit. We also note that their complexing properties give them the opportunity to be interesting candidates for drugs based on transition metals.

In general, the ligands act synergistically with metals for their biological activity (Badiger *et al.*, 2012). Hydrazides-hydrazones are also used as analytical reagents, a polymer coating of ink, pigments and fluorescent materials (El-Tabl *et al.*, 2008).

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These molecules are known possessing several interesting biological properties, among others, antibacterial (Özkay *et al.*, 2010; Bedia *et al.*, 2006), anticonvulsant (Kaushik *et al.*, 2010), antitrypanosomal (Troberg *et al.*, 2000), anti-fungal (Cui *et al.*, 2011, Loncle *et al.*, 2004), antipyretic (Cocco *et al.*, 2006), malaria (Xia *et al.*, 2008).

Based on higher bio-activity, the biological importance of the hydrazone group and its derivatives, it appears important to design, synthesize new derivatives with the pharmacophore hydrazone group and evaluate their pharmacological activities.

In this study we have synthesized two derivatives of hydrazones: the salicylhydrazones (series GS) and the *p*-tosylhydrazones (series GT) respectively with *S*-(+)-carvone (GS1, GT1), 4'-methylacetophenone (GS2, GT2), 7-methoxy-1-tetralone (GS3, GT3) and 2-acetylnaphthalene (GS4, GT4) and then evaluate their parasitic activity on *Trypanosoma brucei brucei*. The selectivity of each compound was also determined.

MATERIALS AND METHODS

Equipment

All synthesized compounds were characterized by Nuclear Magnetic Resonance spectra using Bruker Avance 400 UltraShield with dimethylsulfoxide (DMSO)- d_6 or chloroform $CDCl_3$ and then Mass Spectrophotometer spectra obtained using the method of Atmospheric-pressure chemical ionization and mass is given in m/z of $[MH^+]$.

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), doublet (dd), triplet (t), quintuplet (qi) and multiplet (m). Melting points (m.p.) were determined on a fusionometer of the type *electrothermal* 1A 9000 and were not corrected.

Reagents

All reagents were obtained from chemical societies: Sigma-Aldrich, Acros Organic, Janssen Chimica, Prolabo and Riedel-de Haen. Substrates, reagents, catalysts and solvents were used directly for syntheses without any further purification. There are: *S*-(+)-carvone, 4'-methylacetophenone, 7-methoxy-1-tetralone, 2-acetylnaphthalene; glacial acetic acid (AAG), Technical ethanol (EtOH), tetrahydrofuran (THF); salicylhydrazine and the *p*-toluenesulfonylhydrazide (*p*-tolylhydrazide) is prepared from the hydrazine monohydrate with the *p*-toluenesulfonyl chloride following the method described in the previous literature (Goldman *et al.*, 1960).

Chemistry

General methods of synthesis

p-tolylhydrazide (*p*-toluenesulfonylhydrazide)

Into a 1 L round-bottomed three-necked flask fitted with a thermometer, a mechanical stirrer, and a dropping funnel are placed 200 g (1.05 moles) of *p*-toluenesulfonyl chloride and 350 mL of tetrahydrofuran. The stirred mixture is cooled in an ice bath

to 10–15°C; then a solution of hydrazine in water (135 mL of 85% hydrazine hydrate, 2.22 moles) is added at such a rate that the temperature is maintained between 10° and 20°C. Stirring is continued for 15 minutes after the addition is complete. The reaction mixture is transferred to a separatory funnel. The lower layer is drawn off, and discarded. The upper tetrahydrofuran layer is filtered by suction through a bed of Celite to remove suspended particles and foreign matter (if any). The Celite is washed with a little tetrahydrofuran to remove any absorbed tosylhydrazide. The clear, colorless filtrates are stirred vigorously during the slow addition of two volumes of distilled water. *p*-toluenesulfonylhydrazide separates as fluffy white crystalline needles. The product is filtered through a Büchner funnel; washed several times with distilled water, and air-dried.

p-tosylhydrazones

In a 100 mL flask, we prepare a solution of 0.01 mole of ketone in 10-40 mL of ethanol and 2 mL of glacial acetic acid (GAA) and then we add gradually a solution of *p*-tosylhydrazide (1.76 g) dissolved in 10 mL ethanol. The mixture is maintained at reflux for 2 hours and the reaction is followed by Thin Layer Chromatography TLC (Hex / AcOEt: 8/2 or 7/3). The crystals formed are filtered, washed with distilled water and dried before being recrystallized with ethanol.

Salicylhydrazones

We prepare in a 100 mL flask a salicylhydrazine solution (1.52 g in 10 mL of ethanol) that we gradually add to a solution of ketone (0.01 mole) dissolved in 10-40 ml of ethanol and 2 mL of glacial acetic acid. The mixture is brought to reflux for 2 hours and the reaction is followed by TLC (Hex / AcOEt: 8/2 or 7/3). After cooling, the precipitate is filtered off, washed with distilled water and dried and then is recrystallized from technical ethanol.

The reactions are followed in Thin Layer chromatography (TLC), the product is dissolved in chloroform and the eluent is composed of a mixture of hexane / ethyl acetate (Hex / EtOAc v / v: 7/3 ; 8/2).

All compounds after synthesis have been submitted to the *in vitro* anti-trypanosomal testing activity on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* and were evaluated for their *in vitro* cytotoxicity on *Artemia salina* Leach following standard biological methods.

Pharmacology

Anti-trypanosomal test

The assessment is performed on the bloodstream form of the strain 427 of *T. b. brucei* by the «LILIT Alamar Blue™» method (Baltz *et al.*, 1985 ; Ráz *et al.*, 1997). The stock solutions of each hydrazone have been prepared from an initial concentration of 10 mg/mL in dimethylsulfoxide (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 μ L 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 BlueTM" is added to each well and then incubated for 4 hours. The dye "Alamar BlueTM" 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 half-inhibitory concentration is the concentration of unstained wells in which there is the lowest amount of hydrazones. 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. We carried out the test in triplicate for each compound. All data were expressed as means \pm standard deviation of triplicate measurements.

Cytotoxicity screen

The cytotoxicity test was performed on larvae of brine shrimp (*Artemia salina* Leach) by the method of Sleet and Brendel (1983). *A. salina* eggs were incubated in seawater until hatching of young larvae (48 hours). Then, series of solutions of test compound 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 = [(nd test - nd control) / nd control] 9 100 (Abott, 1925).
nd : number of dead larvae.

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

RESULTS AND DISCUSSION

We have synthesized two series of derivatives hydrazones in goods yields (57-91%). There are: S-(+)-carvone salicylhydrazone (**GS1**), 4'-methylacetophenone salicylhydrazone (**GS2**), 7-methoxy-1-tetralone salicylhydrazone (**GS3**) and 2-acetylnaphthalene salicylhydrazone (**GS4**); S-(+)-carvone *p*-tosylhydrazone (**GT1**), 4'-methylacetophenone *p*-tosylhydrazone (**GT2**), 7-methoxy-1-tetralone *p*-tosylhydrazone (**GT3**) and 2-acetylnaphthalene *p*-tosylhydrazone (**GT4**).

The hydrazine or hydrazide having the same radical $\text{H}_2\text{N}-\text{NH}-\text{R}$ reacts with a carbonyl compound according to the same mechanism. Steric and electronic effects of the various substituents of the carbonyl are responsible for the difference in reactivity and yields when the same hydrazine derivative is reacted with various substrates and vice versa.

In the literature, it was described in 1976 the synthesis of acetophenone *p*-tosylhydrazone without catalyst for 5.5 hours with 68% yield (Ashraf, 1976); the synthesis using benzaldehyde

salicylic acid hydrazide and 4-dimethylaminobenzaldehyde or 4-nitrobenzaldehyde for 4 hours (Al-Ajrawy, 2011). To enhance the reaction, we used during our work in the synthesis of the salicylhydrazones (**GSn**) and *p*-tosylhydrazones (**GTn**) the glacial acetic acid and technical ethanol. The mixture is heated to reflux for 2 hours and the reaction followed by TLC with yields ranging from 57 to 90%. The low yield 57% obtained of S-(+)-carvone *p*-tosylhydrazone (**GT1**) is due to the nature of this α - β unsaturated ketone. We note well the presence of adduct 1-4 minority (Michael addition) which was removed after purification.

The scaffold has advantageous properties : low molecular weight, reasonable $\text{Clog } P$, good hydrogen bond donating and accepting capabilities (table 1), easy, and economical synthetic routes (Lipinski *et al.*, 1997)..

The structures of synthesized compounds were characterized with the TLC frontal rapport (R_f), spectrometrical analysis MS, especially with NMR ^1H & ^{13}C .

Table 1 : Properties Compatible with Reasonable Pharmacokinetics and Drug Availability, Rules of Lipinski *et al.*, (1997) applied to hydrazones.

Compounds	Molecular weight (g.mol-1)	C logP	No. of H bond donors	No. of H bond acceptors	No. of criteria met
Rules	< 500	< 5	≤ 5	< 10	At least 3
GS1	284.35	3.093	2	4	all
GS2	268.31	2.436	2	4	all
GS3	310.34	2.358	2	5	all
GS4	304.34	3.111	2	4	all
GT1	318.43	4.772	1	4	all
GT2	302.39	4.115	1	4	all
GT3	344.43	4.199	1	5	all
GT4	338.42	4.790	1	4	all

Characterization of synthesized compounds

S-(+)-carvone salicylhydrazone (**GS1**) (Figure 1)

Yield : 78% ; m.p. : 189-190°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.57 ; NMR ^{13}C (DMSO- d_6 , δ in ppm) : 161.78 (N-CO-Ar); 147.53 (C=N); 156.36 (C-OH phenolic); 133.87, 130.45, 119.59, 117.95, 116.77 (other C-Ar); 153.99, 133.16, 132.28, 110.32, 40.10, 29.57, 29.45, 20.44, 17.85 (C-carvone).

NMR ^1H (DMSO- d_6 , δ in ppm) : 11.75 (s, 1H, OH); 11.20 (s, 1H, NH); 7.97-6.95 (m, 4H, H-Ar); 6.25 (t, 1H, C=CH-); 4.84 (d, 2H, C=CH₂); 2.75 (qi, 1H, CH₂-CH-CH₂); 2.45 (t, 2H, C=CH-CH₂-CH); 2.25 (d, 2H, HC-CH₂-C=N); 1.90 (s, 3H, CH₃); 1.78 (s, 3H, CH₃).

MS m/z [MH^+]found : 285.37 ; [M]theoretical : 284.35 ;
Molecular formula : $\text{C}_{17}\text{H}_{20}\text{N}_2\text{O}_2$

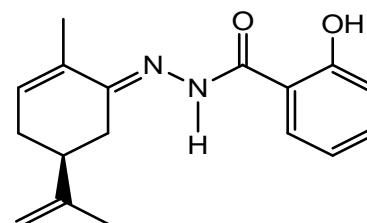


Fig. 1: S-(+)-carvone salicylhydrazone (**GS1**).

4'-methylacetophenone salicylhydrazone (GS2) (Figure 2)

Yield : 79% ; m.p. : 237-238°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.63 ; NMR ^{13}C (DMSO- d_6 , δ in ppm): 163.14 (N-CO-Ar); 153.51 (C=N); 157.67 (C-OH phenolic); 140.18, 136.27, 134.47, 131.69, 130.12, 127.52, 120.82, 119.02, 118.01 (other C-Ar); 21.99 ($\text{H}_3\text{C-Ar}$); 14.93 (CH_3).

NMR ^1H (DMSO- d_6 , δ in ppm): 11.80 (s, 1H, OH); 11.30 (s, 1H, NH); 8.00-6.97 (m, 8H, H-Ar); 2.45 (s, 3H, $\text{H}_3\text{C-Ar}$), 2.31 (s, 3H, CH_3).

MS m/z [MH^+]found : 269.27 ; [M]theoretical : 268.31 ; Molecular formula : $\text{C}_{16}\text{H}_{16}\text{N}_2\text{O}_2$

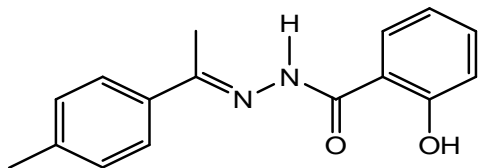


Fig. 2: 4'-methylacetophenone salicylhydrazone (GS2)

7-methoxy-1-tetralone salicylhydrazone (GS3) (Figure 3)

Yield : 85% ; m.p. : 223-224°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.47 ; NMR ^{13}C (DMSO- d_6 , δ in ppm): 161.82 (N-CO-Ar); 151.75 (C=N); 157.64 (C-OH phenolic); 156.42 ($\text{C}_{\text{Ar}}-\text{OCH}_3$); 133.31, 133.04, 132.56, 130.57, 129.62, 119.69, 117.90, 116.83, 116.35, 108.18 (other C-Ar); 55.10 (O- CH_3); 27.92, 25.55, 21.53 (3s, 6H, 3 $^{\text{''}}$ - CH_2 -).

NMR ^1H (DMSO- d_6 , δ in ppm): 11.80 (s, 1H, OH); 11.33 (s, 1H, NH); 8.00-6.90 (m, 7H, H-Ar); 3.80 (s, 3H, O- CH_3); 3.35, 2.69, 1.89 (s, 6H, 3 CH_2).

MS m/z [MH^+]found : 311.33 ; [M]theoretical : 310.34 ; Molecular formula : $\text{C}_{18}\text{H}_{18}\text{N}_2\text{O}_3$

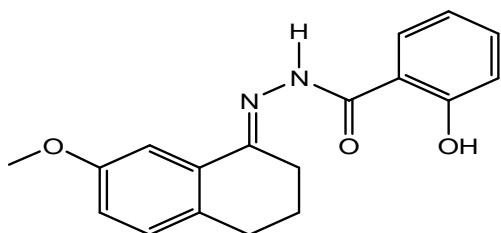


Fig. 3: 7-methoxy-1-tetralone salicylhydrazone (GS3)

2-acetylnaphthalene salicylhydrazone (GS4) (Figure 4)

Yield : 91% ; m.p. : 241-242°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.33 ; NMR ^{13}C (DMSO- d_6 , δ in ppm) : 162.01 (N-CO-Ar); 151.83 (C=N); 156.47(C-OH phenolic); 135.26, 133.39, 133.28, 133.05, 132.74, 130.66, 128.54, 127.73, 127.48, 126.89, 123.63, 119.72, 117.92, 116.87 (other C-Ar); 13.60 (CH_3).

NMR ^1H (DMSO- d_6 , δ in ppm): 11.80 (s, 1H, OH); 11.42 (s, 1H, NH); 8.36-7.05 (m, 11H, H-Ar); 2.33 (s, 3H, CH_3).

MS m/z [MH^+]found : 305.32 ; [M]theoretical : 304.34 ; Molecular formula : $\text{C}_{19}\text{H}_{16}\text{N}_2\text{O}_2$.

Analyses of the ^{13}C NMR spectra of this series of hydrazones show the peak of the amide carbon function (N-CO-

Ar) between 163 and 161 ppm. The carbon of the imine's function, characteristic group obtaining during the reaction (C=N) appears around 153-147 ppm. Other peaks between 158 and 108 ppm correspond to the aromatic ring; phenolic carbon (aromatic C-OH) has a high value between 157 and 156 ppm. In ^1H NMR, there are two types of protons: the phenolic OH proton and that of NH. The phenolic OH proton having acidic properties and may establish a hydrogen bond with the carbonyl in ortho is to a lower field than the NH proton (Baligar and Revankar, 2006; Govindasami *et al.*, 2011). So it will be assigned to OH, the higher δ value. Therefore, it is a singlet between 11.80 and 11.75 ppm whereas the NH proton singlet occurs at 11.42 to 11.20 ppm. The aromatic protons appear between 8.36 and 6.90 ppm.

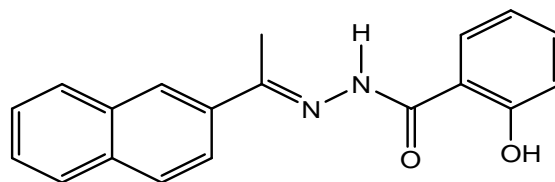


Fig. 4: 2-acetylnaphthalene salicylhydrazone (GS4)

S-(+)-carvone p-tosylhydrazone (GT1) (Figure 5)

Yield : 57% ; m.p. : 167-168°C ; R_f (Hex/AcOEt, v/v, 8/2) : 0.46 ; NMR ^{13}C (CDCl_3 , δ in ppm) : 154.80 (C=N); 144.02, 132.46, 129.38, 128.26 (C-Ar); 147.11, 135.17, 133.59, 110.40, 40.36, 29.96, 29.09, 21.64, 17.65 (C-carvone); 20.65 ($\text{H}_3\text{C-Ar}$).

NMR ^1H (CDCl_3 , δ in ppm) : 7.90 (s, 1H, NH); 7.65 & 7.30 (2s, 4H, H-Ar); 6.05 (t, 1H, C=CH-); 4.75 (dd, 2H, C=CH $_2$); 2.60 (m, 1H, $\text{CH}_2\text{-CH-CH}_2$); 2.45 (t, 2H, C=CH- $\text{CH}_2\text{-CH}$); 2.25 (d, 2H, $\text{HC-CH}_2\text{-C=N}$); 1.95 (m, 3H, CH_3); 1.77 (s, 3H, CH_3); 1.70 (s, 3H, CH_3).

MS m/z [MH^+]found : 319.33 ; [M]theoretical : 318.43 ; Molecular formula : $\text{C}_{17}\text{H}_{22}\text{N}_2\text{O}_2\text{S}$

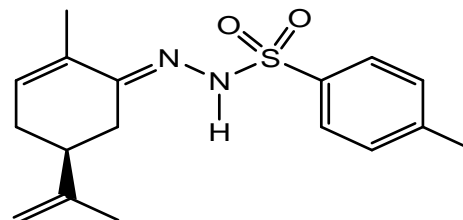


Fig. 5: S-(+)-carvone p-tosylhydrazone (GT1)

4'-methylacetophenone p-tosylhydrazone (GT2) (Figure 6)

Yield : 81% ; m.p. : 190-191°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.51 ; NMR ^{13}C (DMSO- d_6 , δ in ppm) : 153.17 (C=N); 143.26, 138.98, 136.21, 134.62, 129.40, 128.90, 127.57, 125.86 (C-Ar); 20.97, 20.75 ($\text{H}_3\text{C-Ar}$); 14.17 (CH_3).

NMR ^1H (DMSO- d_6 , δ in ppm) : 10.45 (s, 1H, NH); 7.82-7.15 (m, 8H, H-Ar); 2.37 (s, 3H, $\text{H}_3\text{C-Ar}$); 2.30 (s, 3H, $\text{H}_3\text{C-Ar}$); 2.17 (s, 3H, CH_3).

MS m/z [MH^+]found : 303.37 ; [M]theoretical : 302.39 ; Molecular formula : $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_2\text{S}$

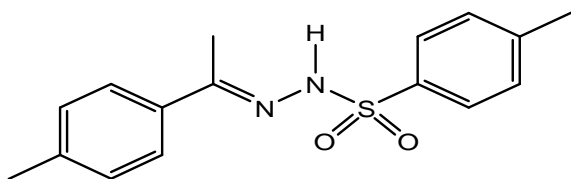


Fig. 6 : 4'-methylacetophenone *p*-tosylhydrazone (GT2)

7-methoxy-1-tetralone *p*-tosylhydrazone (GT3) (Figure 7)

Yield : 90% ; m.p. : 200-201°C ; R_f (Hex/AcOEt, v/v, 8/2) : 0.17 ; NMR ¹³C (CDCl₃, δ in ppm) : 152.57 (C=N); 157.99 (C_{Ar}-OCH₃); 144.20, 135.42, 132.42, 132.41, 129.57, 129.42, 128.23, 117.08, 108.21 (other C-Ar); 55.30 (O-CH₃); 28.43, 25.31, 21.64 (3CH₂ & 1CH₃).

NMR ¹H (CDCl₃, δ in ppm) : 8.10 (s, 1H, NH); 7.91-6.84 (m, 7H, H-Ar); 3.85 (s, 3H, H₃C-O); 2.63, 2.45, 1.83 (m, 6H, 3CH₂); 2.34 (s, 3H, H₃C-Ar).

MS m/z [MH⁺]found : 345.41 ; [M]theoretical : 344.43 ; Molecular formula : C₁₈H₂₀N₂O₃S

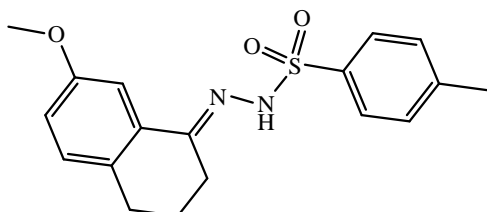


Fig. 7: 7-methoxy-1-tetralone *p*-tosylhydrazone (GT3)

2-acetylnaphthalene *p*-tosylhydrazone (GT4) (Figure 8)

Yield : 74% ; m.p. : 181-182°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.23 ; NMR ¹³C (CDCl₃, δ in ppm) : 152.37 (C=N); 144.26, 135.42, 134.67, 133.85, 132.88, 129.67, 128.58, 128.19, 128.04, 127.64, 126.92, 126.42, 126.29, 123.56 (C-Ar); 21.64 (H₃C-Ar); 13.29 (CH₃).

NMR ¹H (CDCl₃, δ in ppm) : 8.10 (s, 1H, NH); 7.93-7.25 (m, 11H, H-Ar); 2.39 (s, 3H, H₃C-Ar); 2.25 (s, 3H, CH₃).

MS m/z [MH⁺]found : 339.38 ; [M]theoretical : 338.42 ; Molecular formula : C₁₉H₁₈N₂O₂S

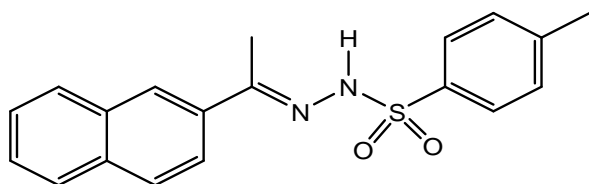


Fig. 8: 2-acetylnaphthalene *p*-tosylhydrazone (GT4).

In this series of compounds, ¹³C NMR spectral analyses presented the group C=N characteristic of the formation of products between 154 and 152 ppm. Aromatic carbons are in the region 144-108 ppm, depending on the structure of the test compound. We remark in ¹H NMR, the single proton of the internal nitrogen NH is in the form of a singlet at 10.45 ppm for the 4'-methylacetophenone *p*-tosylhydrazone (GT2) in DMSO-d₆ and 8.10 ppm (GT3 & GT4) and 7.90 ppm (GT1) ppm in CDCl₃. This difference is due to the effect of DMSO solvent which generates a strong hydrogen bond between oxygen and the NH proton (Silverstein *et al.*, 2007) causing a chemical shift of hydrogen downfield. Aromatic protons are observed as bedding from 7.93 to 6.84 ppm. The molar mass of each synthesized molecule given by mass spectrometry is consistent with theoretical mass found. Various spectrometrical analyses done on each compound have really confirmed the presence of functional groups and different types of protons and carbons in each structure. The spectrometric data are in conformity with the structures suggested for the products.

Pharmacology

Anti-parasitic activity of compounds was evaluated *in vitro* on the bloodstream form of the strain 427 of *Trypanosoma brucei brucei* and their toxicity on larvae of *Artemia salina* L. The half-inhibitory concentration (IC₅₀) and half-lethal concentration ((LC₅₀) were respectively determined and expressed in microlar (μM) to be compared with the scales of trypanocidal and toxic activities. And then, the selectivity index (SI) of each compound was calculated. The results are reported in table 2.

According to the works of Du *et al.*, 2002 and Fujii *et al.*, 2005 studying thiosemicarbazones, compounds that have IC₅₀ values below 10 μM can be considered as trypanocidal molecules, when IC₅₀ values are between 10 and 100 μM, they are considered as moderate antitrypanosomal. The compounds which have their IC₅₀ higher than 100 μM showed little or no activity.

In our work, we note that compounds 2-acetylnaphthalene salicylhydrazones GS4 and 7-methoxy-1-tetralone *p*-tosylhydrazone GT3 exhibited a trypanocidal activity (IC₅₀ = 1.97 ± 0.42 and 7.98 ± 1.65 μM respectively) when other except GT2 (IC₅₀ > 100 μM, low activity) showed moderate trypanocidal activity (11 < IC₅₀ < 34 μM) (table 2).

Indeed, Du *et al.* found that macrophage cells are generally sensitive to concentrations above 10 μM of thiosemicarbazones.

Table 2: Trypanocidal, toxic activity and selectivity index of synthesized hydrazones.

Compounds	IC50 (μM)	Activity	LC50 (μM)	Toxicity	Selectivity index (SI = LC50 / IC50)
GS1	18.42 ± 0.80	Moderate	219.44 ± 2.17	toxic	11.91
GS2	20.53 ± 0.74	Moderate	352.57 ± 1.32	no toxic	17.17
GS3	11.11 ± 1.77	Moderate	731.13 ± 3.27	no toxic	65.80
GS4	1.97 ± 0.42	Trypanocidal	310.50 ± 1.93	no toxic	157.61
GT1	23.99 ± 5.11	Moderate	536.38 ± 2.35	no toxic	22.35
GT2	IC50 > 100	Little or no	368.06 ± 1.87	no toxic	< 3.68
GT3	7.98 ± 1.65	Trypanocidal	137.32 ± 0.77	toxic	17.20
GT4	33.62 ± 7.29	Moderate	35.45 ± 1.41	toxic	1.05

SI > 1
All compounds were selective

Thus, in addition to inhibiting property of the parasite, they would preserve the human macrophage host cell (Du *et al.*, 2002 ; Fujii *et al.*, 2005). On this basis, hydrazones **GS4** ($1.97 \pm 0.42 \mu\text{M}$) and **GT3** ($7.98 \pm 1.65 \mu\text{M}$) were selected as good trypanocidal compounds. It remarks in this study that salicylhydrazones synthesized **GS1-4**, IC_{50} ranging from 1 to 21 μM , were more trypanocidal than their analogues *p*-tosylhydrazones **GT1-4** ($\text{IC}_{50} = 7$ to $> 100 \mu\text{M}$).

The toxicity study showed that only compounds **GS1**, **GT3** and **GT4** ($\text{LC}_{50} = 219.44$, 137.32 and $35.45 \mu\text{M}$ respectively) exerted a toxic activity whereas the other presented no effect ($\text{LC}_{50} > 281 \mu\text{M}$) on larvae of *Artemia salina*. To assess the toxicity with the LC_{50} values of compounds (table 2), we have referred to the LC_{50} value of lapachol ($\text{LC}_{50} = 281 \mu\text{M}$) which is known as reference compound (Graminha *et al.*, 2008; Santos *et al.*, 2003). These tests that are a summary assessment of the toxicity of products reflects the sensitivity of shrimp larvae to the synthesized compounds 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). Thus, the three toxic products would exert activity on the cells.

From the analyses of their selectivity index ($1 < \text{SI} < 140$), we note that all products turn out quite selective on the parasite *Trypanosoma brucei brucei*. These results are in perfect agreement with the work of Tiunan *et al.*, (2005) in which if the SI value obtained is greater than unity, the test compound is considered to be selective on the parasite and if SI value is less than unity, the test compound is more cytotoxic than anti-parasitic. Especially, some of the group: the salicylhydrazones of 2-acetylnaphthalene **GS4**, 7-methoxy-1-tetralone **GS3** ($\text{SI} = 157$, 65 respectively), *S*-(+)-carvone *p*-tosylhydrazone **GT1** ($\text{SI} = 22$) were the most selective. Based on the different tests values, it appears that the *S*-(+)-carvone *p*-tosylhydrazone **GT1** and the salicylhydrazones obtained from 4'-methylacetophenone **GS2**, 7-methoxy-1-tetralone **GS3** and 2-acetylnaphthalene **GS4** exhibited significant trypanocidal activity without effect on *Artemia salina* L. and selective would be able to be used at higher doses for trypanosomiasis treatment.

CONCLUSION

In this study, eight hydrazones derivatives were synthesized and characterized. Their biological activities were evaluated and products showed interesting trypanocidal activity on the parasite study and were selective. To our knowledge, this is the first time these molecules are synthesized and their antiparasitic evaluated on *Trypanosoma brucei brucei* and then they could open an interesting opportunity to the treatment of trypanosome.

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