Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(7): 318-326

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis, characterization, purity verification, antiplasmodial activity and toxicity *against Artemia salina* Leach of salicylhydrazones and *p*-tosylhydrazones from S-(+)-carvone and arylketones

BardieuAtchade[1,3], Salomé D. S. Kpoviessi[1,2,3*], Fernand A. Gbaguidi[3], Bienvenu Glinma[1], Léon A. Ahoussi[1], Joachim Gbenou[3], Georges C. Accrombessi[1], R. Frederick[2] and Jacques H. Poupaert[2]

[1]*Laboratory of Physic and Synthesis Organic Chemistry (LaCOPS), Department of Chemistry, Faculty of Sciences and Technologies, University of Abomey-Calavi, Cotonou, Benin*
[2]*Louvain Drug Research Institute (LDRI), School of Pharmacy, Université catholique de Louvain, B1 7203 Avenue Emmanuel Mounier 72, B-1200 Brussels, Belgium*
[3]*Laboratory of Pharmacognosy and Essential Oils(LaPHE) Cotonou, Republic of Benin*

ABSTRACT

*Hydazones are molecules that inhibit the development of several microbes and parasites. They are known for their pharmacological activities: antimicrobial, antiviral, antitumor, antimalarial, Anticonvulsant. In this research, we synthesized with good yields(57-91%), substituted hydazones of 4'-methylacetophenone, 2-acetonaphthone, 7-methoxy-1-tetralone andS (+)-carvone that formed two series: the series of salicylhydrazones(**1a- 4a**) and the series of p-tosylhydrazones of (**1b-4b**). Then we checked purity of molecules by elemental and HPLC analysis. Spectrometric analyzes methods such as HRMS, ^{1}HNMR and ^{13}C were used to confirm their structures. Their antiparasitic properties were evaluated on Plasmodium falciparum and the toxicity against Artemia salina Leach. Molecules of salicylhydrazones series presented moderate activity with good selectivity (7≤ SI ≤ 14). All compounds were found non-toxic to the larvae except **1a**, **3b** and **4b**. In view of the observed activities and those already known, hydrazones could be a promising approach to the treatment of malaria.*

Keywords: Synthesis, hydrazones, aryl ketone, purity, antiplasmodial activity, selectivity

---

INTRODUCTION

Vector-borne diseases account for 17% of infectious diseases and are among the most important diseases in human health, by both the morbidity and the mortality and also by the human poverty they cause[1]. These diseases are mostly prevalent in tropical regions where they are part of the causes and the consequences of under development. Their impact on public health is an obstacle to children's education and economic development of countries [1]. Among these diseases, malaria is for men constant threats to health. The main protozoan disease agents are members of Apicomplexa orders (Plasmodium, Toxoplasma, Eimeria) [2]. This protozoan parasite Plasmodium genus which is endemic by WHO in 2011 caused more than 655,000 deaths and for which 216 million cases were observed mainly in children and in West Africa [3]. Thus, several research groups synthesized new organic molecules for therapeutic purposes[4-7]. The Hydrazone compounds are one of the important classes of molecules in organic chemistry that have been extensively studied [8,9]. They attract more attention because of their biological and pharmaceutical activities as anticancer [10] antianalgesic, antiinflammatory and antipyretic [11-13].These compounds also have antioxidant [14], anti-trypanosome [15], and antimalaria [16]activities.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. The aim of our study was to synthesize hydrazones from well suitable carbonyl compounds and hydrazine derivatives, to assess their purity by elemental and HPLC analysis, to confirm their structures by various

spectrometric analysis methods and to evaluate *in vitro*, their antiplasmodial activity on P*lasmodium falciparum* and their toxicity against Artemia salina L each.

EXPERIMENTAL SECTION

Equipment

Melting points (m.p.) were determined on a *fusionometer* of the type *electrothermal* 1A 9000 and were not corrected. For checking purity of synthesized compounds, we used a Thermo Electron Corporation Flash EA 1112 serie analyzer equipped with a micro-balance Mettler Toledo MX5 and a logiciel : Eager 300. We used also a Accela type (Thermo Fisher Scientifique, Bremen, Germany) HPLC system equipped with a Phenomenextype C18 column.

All synthesized compounds were characterized by Nuclear Magnetic Resonance spectra using Bruker Advance 400 Ultra Sheild with dimethylsufoxide (DMSO)-d_6 or chloroform $CDCl_3$ and the Mass spectra were acquired in positive mode using an LTQ-Orbitrap XL hybride (Thermo Fisher Scientifique, Bremen, Germany) equipped with an electrospray ionization ESI and high resolution mass was 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), redoubled doublet (dd) triplet (t), quintuplet (qi) and multiplet (m).

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 were : S-(+)-carvone, 4'-methylacetophenone, 7-methoxy-1-tetralone, 2-acetylnaphthalene; glacial acetic acid, Technical ethanol (EtOH) andsalicylhydrazide. The para-toluenesulfonylhydrazide (*p*-tolylhydrazide) is prepared from the hydrazine monohydrate with the *p*-toluenesulfonyl chloride following the method described in the literature [17].

Chemistry

Synthesis of p-tolylhydrazide (*p*-toluenesolfonylhydrazide)

Into a1 L round-bottomed three-necked flask fitted with a thermometer, a mechanical stirrer, and a dropping funnel are placed200 g (1.05 moles) of *p*-toluenesulfonyl chlorideand350 mL of tetrahydrofuran. The stirred mixture is cooled in a nice 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 Celiteto remove suspended particles and foreign matter (if any). The Celite is washed with a little tetrahydrofurane to remove any absorbed to sylhydrazide. 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.

Synthesis of 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 from technic ethanol.

Synthesis of 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.

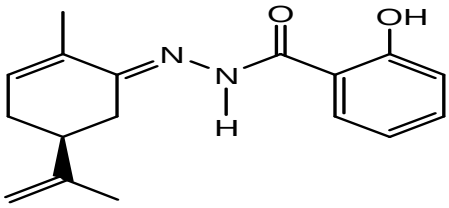
The reactions are followed in Thin Layer Chromatography (TLC), the product is dissolved in chloroform and the eluent is composed of a mixture of hexane / ethyl acetate (Hex / EtOAc v / v: 7/3; 8/2). All compounds after synthesis, purification and purity checking have been submitted to the *in vitro* antiplasmodial and toxicity tests.

Purity checking by elementary and HPLC analysis

The elementary analysis was used to check purity of the synthesized products. This method determines the percentage of the different atoms of the compound except oxygen. The difference between theoretical and experimental percentages (δmex-mth) of pure synthesized compound must be less than0.5[18,19]. HPLC method was also used. It gives the percentage of each compound in a mixture. With this method, the percentage of the pure synthesized compound must be at least equal to 95% [18,19].

Characterization of synthesized compounds

S-(+)-carvone salicylhydrazone (1a)

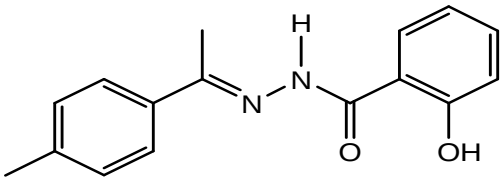


Yield : 78% ; m.p. : 189-190°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.57 ; NMR ^{13}C (DMSO-d_6, δ in ppm) : 161,78 (N-CO-Ar); 147.53 (C=N); 156.36 (C–OH phenolic); 133.87, 130.45, 119.59, 117.95, 116.77 (other C-Ar); 153.99, 133.16, 132.28, 110.32, 40.10, 29.57, 29.45, 20.44, 17.85 (C-carvone).

NMR ^{1}H (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=CH_2); 2.75 (qi, 1H, CH_2-C**H**-CH_2); 2.45 (t, 2H, C=CH-C**H_2**-CH); 2.25 (d, 2H, HC-C**H_2**-C=N); 1.90 (s, 3H, CH_3); 1.78 (s, 3H, CH_3).

MS m/z [MH^+]found : 285.37 ; [M]theoretical : 284.35 ; Molecular formula : $C_{17}H_{20}N_2O_2$

4'-methylacetophenone salicylhydrazone (2a)

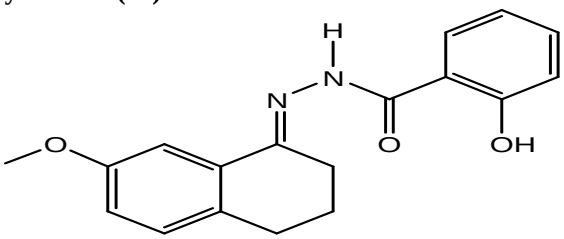


Yield : 79% ; m.p. : 237-238°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.63 ; NMR ^{13}C (DMSO-d_6, δ in ppm): 163.14 (N-CO-Ar); 153.51 (C=N); 157.67 (C–OH phenolic); 140.18, 136.27, 134.47, 131.69, 130.12, 127.52, 120.82, 119.02, 118.01 (other C-Ar); 21.99 (H_3C-Ar); 14.93 (CH_3).

NMR ^{1}H (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, H_3C-Ar), 2.31 (s, 3H, CH_3).

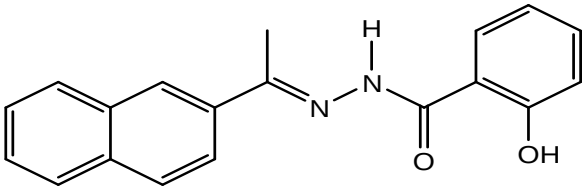
MS m/z [MH^+]found : 269.27 ; [M]theoretical : 268.31 ; Molecular formula : $C_{16}H_{16}N_2O_2$

7-methoxy-1-tetralone salicylhydrazone (3a)



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 (C_{Ar}–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"–CH_2-"). NMR ^{1}H (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, 3CH_2). MS m/z [MH^+]found : 311.33 ; [M]theoretical : 310.34 ; Molecular formula : $C_{18}H_{18}N_2O_3$

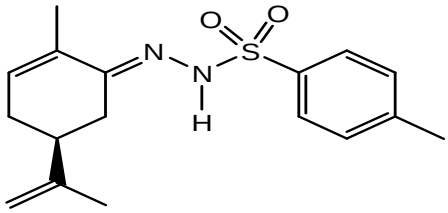
2-acetynaphthalene salicylhydrazone (4a)



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).

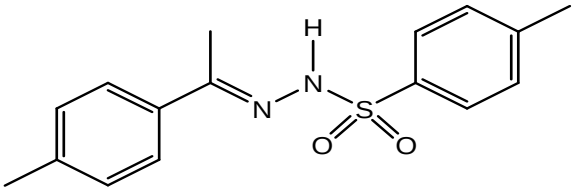
NMR ^{1}H(DMSO-d_6, δ in ppm): 11.80 (s, 1H, OH); 11.42 (s, 1H, NH); 8.36-7.05 (m, 11H, H-Ar); 2.33 (s, 3H, CH_3). MS m/z [MH^+]found : 305.32 ; [M]theoretical : 304.34 ; Molecular formula : $C_{19}H_{16}N_2O_2$.

S-(+)-carvone*p*-tosylhydrazone (1b)



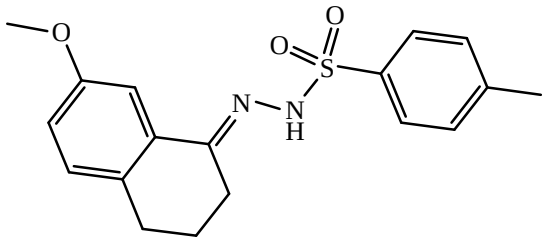
Yield : 57% ; m.p. : 167-168°C ; R_f (Hex/AcOEt, v/v, 8/2) : 0.46 ; NMR ^{13}C ($CDCl_3$, δ in ppm) : 154.80 (C=N); 144.02, 132.46, 129.38, 128.26 (C-Ar); 147.11, 135.17, 133.59, 110.40, 40.36, 29.96, 29.09, 21.64, 17.65 (C-carvone); 20.65 (H_3C-Ar). NMR ^{1}H ($CDCl_3$, δ in ppm) : 7.90 (s, 1H, NH); 7.65 & 7.30 (2s, 4H, H-Ar); 6.05 (t, 1H, C=CH–); 4.75 (dd, 2H, C=C**H$_2$**); 2.60 (m, 1H, CH_2-C**H**-CH_2); 2.45 (t, 2H, C=CH-C**H$_2$**-CH); 2.25 (d, 2H, HC-C**H$_2$**-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 : $C_{17}H_{22}N_2O_2S$

4'-methylacetophenone *p*-tosylhydrazone (2b)



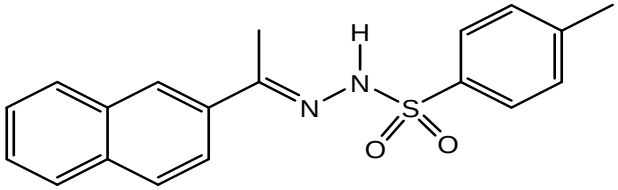
Yield : 81% ; m.p. : 190-191°C ; **R_f** (Hex/AcOEt, v/v, 7/3) : 0.51 ; NMR ^{13}C(DMSO-d_6, δ in ppm) : 153.17 (C=N); 143.26, 138.98, 136.21, 134.62, 129.40, 128.90, 127.57, 125.86 (C-Ar); 20.97, 20.75 (H_3C-Ar); 14.17 (CH_3). NMR ^{1}H (DMSO-d_6, δ in ppm) : 10.45 (s, 1H, NH); 7.82-7.15 (m, 8H, H-Ar); 2.37 (s, 3H, H_3C-Ar); 2.30 (s, 3H, H_3C-Ar); 2.17 (s, 3H, CH_3). MS m/z [MH^+]found : 303.37 ; [M]theoretical : 302.39 ; Molecular formula : $C_{16}H_{18}N_2O_2S$

7-methoxy-1-tetralone *p*-tosylhydrazone (3b)



Yield : 90% ; m.p. : 200-201°C ; R_f (Hex/AcOEt, v/v, 8/2) : 0.17 ; NMR ^{13}C($CDCl_3$, δ in ppm) : 152.57 (C=N); 157.99 (C_{Ar}–OCH_3); 144.20, 135.42, 132.42, 132.41, 129.57, 129.42, 128.23, 117.08, 108.21 (other C-Ar); 55.30 (O-CH_3); 28.43, 25.31, 21.64 ($3CH_2$& $1CH_3$). NMR ^{1}H($CDCl_3$, δ in ppm) : 8.10 (s, 1H, NH); 7.91-6.84 (m, 7H, H-Ar); 3.85 (s, 3H, H_3C-O); 2.63, 2.45, 1.83 (m, 6H, $3CH_2$); 2.34 (s, 3H, H_3C-Ar). MS m/z [MH^+]found : 345.41 ; [M]theoretical : 344.43 ; Molecular formula : $C_{18}H_{20}N_2O_3S$

2-acetylnaphthalene *p*-tosylhydrazone (4b)



Yield : 74% ; m.p. : 181-182°C ; R_f (Hex/AcOEt, v/v, 7/3) : 0.23 ; NMR $^{13}C(CDCl_3$, 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 (H_3C-Ar); 13.29 (CH_3). NMR $^1H(CDCl_3$, δ in ppm) : 8.10 (s, 1H, NH); 7.93-7.25 (m, 11H, H-Ar); 2.39 (s, 3H, H_3C-Ar); 2.25 (s, 3H, CH_3). MS m/z [MH^+] found : 339.38 ; [M]theoretical : 338.42 ; Molecular formula : $C_{19}H_{18}N_2O_2S$.

Pharmacology

Parasites and media

Plasmodium falciparum chloroquine-sensitive strain 3D7 (from Prof. Grellier of Museum d'Histoire Naturelle, Paris-France) asexual erythrocytic stages were cultivated continuously *in vitro* according to the procedure described by Trager and Jensen (1976) at 37 °C and under an atmosphere of 5% CO_2, 5% O_2 and 90% N_2. The host cells were human red blood cells (A or O Rh+). The culture medium was RPMI 1640 (Gibco) containing 32 mM $NaHCO_3$, 25 mM HEPES and 2.05 mM L-glutamine. The medium was supplemented with 1.76 g/L glucose (Sigma–Aldrich), 44 mg/mL hypoxanthin (Sigma–Aldrich), 100 mg/L gentamycin (Gibco) and 10% human pooled serum (A or O Rh+). Parasites were subcultured every 3–4 days with initial conditions of 0.5% parasitaemia and 1% haematocrit.

The eggs of *Artemia salina* L each were obtained from JBL society (JBL Gmbh&Co.KG, Germany).

Antiplasmodial test

Parasite viability was measured using parasite lactate dehydrogenase (pLDH) activity according to the method described by Makler et al. [20]. The *in vitro* test was performed as described by Murebwayire et al. [21]. Chloroquine (Sigma) or artemisinin (Sigma) were used as positive controls in all experiments with an initial concentration of 100 ng/mL. First stock solutions of essential oils and pure compounds were prepared in DMSO at 20 mg/mL. The solutions were further diluted in medium to give 2 mg/mL stock solutions. The highest concentration of solvent to which the parasites were exposed was 1%, which was shown to have no measurable effect on parasite viability. Essential oils were tested in eight serial threefold dilutions (final concentration rang: 200-0.09 µg/mL, two wells/concentration) in 96-well microtiter plates. The parasitaemia and the haematocrit were 2% and 1%, respectively. All tests were performed in triplicate.

Toxicity test

The toxicity test was performed on larvae of brine shrimp (*Artemia salina* Leach) by the method of [22] *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)] x 100 [23] with = number of dead larvae.

Camptothecin(Sigma) was used as positive reference compound.

Data (dose-response) were transformed by logarithm and the half-lethal concentration LC_{50} was determined by linear regression [24]. Tests were carried out in triplicates. All data were expressed as mean ± standard deviation of triplicate measurements.

RESULTS AND DISCUSSION

Chemistry

We have synthesized two series of derivatives hydrazones in goods yields (57-91%). There are: S-(+)-carvone salicylhydrazone (**1a**), 4'-methylacetophenonesalicylhydrazone (**2a**), 7-methoxy-1-tetralone salicylhydrazone (**3a**) and 2-acetylnaphthalenesalicylhydrazone (**4a**), S-(+)-carvone*p*-tosylhydrazone (**1b**), 4'-methylacetophenone *p*-tosylhydrazone (**2b**), 7-methoxy-1-tetralone *p*-tosylhydrazone (**3b**) and 2-acetylnaphthalene *p*-tosylhydrazone (**4b**).

The hydrazine or hydrazide having the same radical $H_2N—NH—R$ 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 [25]; the synthesis using benzaldehyde salicylic acid hydrazide and 4-dimethylaminobenzaldehyde or 4-nitrobenzaldehyde for 4 hours [26]. To enhance the reaction, we used during our work in the synthesis of the salicylhydrazones (**a**) and *p*-tosylhydrazones (**b**) the glacial acetic acidic 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 (**1a**) 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.

Their elementary analysis(table1) showed that the compounds**2a**, **3a**,**4b** and**3b** meet the purity criteria for this analysis with the variation of percentage (δ)of each atom less than0.5 [18-19].

Table 1: Elementary Analyses of the synthesized products

Compounds	Atom	Experience N° 1	Experience N°2	Average	theoretic	δmex-mth
1a	N	09.64	09.99	09.815	09.85	-0.035
	C	71.02	70.76	70.89	71.81	-0.92
	H	07.09	07.09	07.09	07.09	0.00
2a	N	10.61	09.9	10.255	10.44	-0.44
	C	71	71.15	71.075	71.62	-0.445
	H	05.96	05.98	05.97	06.01	-0.005
3a	N	08.98	08.64	08.81	09.03	-0.22
	C	69.26	69.16	69.21	69.62	-0.45
	H	05.82	05.83	05.825	05.85	-0.025
4a	N	9.21	08.62	08.915	09.20	-0.285
	C	74.34	74.34	74.34	74.98	-0.64
	H	05.28	05.27	05.275	05.30	-0.025
1b	N	8.71	8.69	8.7	8.8	-0.1
	C	64.55	63.88	64.215	64.12	0.095
	H	6.84	6.88	6.86	6.96	-0.1
	S	9.56	8.96	9.26	10.07	-0.81
2b	N	9.01	9.09	9.05	9.26	-0.21
	C	62.58	62.85	62.715	63.55	-0.835
	H	5.94	5.78	5.86	6	-0.14
	S	10.16	10.96	10.56	10.6	-0.04
3b	N	08.02	08.09	08.05	08.13	-0.08
	C	62.55	62.55	62.69	62.77	-0.08
	H	05.84	05.88	05.86	05.85	-0.01
	S	09.16	08.96	09.06	09.31	-0.25
4b	N	08.10	08.13	08.115	08.28	-0.165
	C	67.71	67.13	67.65	67.13	0.32
	H	05.41	05.42	05.415	05.36	0.055
	S	09.52	09.48	09.50	09.47	0.03

The other compounds(**1a**, **4a**, **1b** and **2b**) were then purified again and subjected to HPLC analysis for purification checking. These compounds (**1a, 4a**, **1b** and **2b)** fulfilled the purity criteria for HPLC analysis with percentages of purity higher than95 % (figure 1; Table 2) [18, 19].

Table 2: Purity checking by HPLC method

Compound	Percentage of purity
1a	99.028
2a	-
3a	-
4a	100
1b	96.432
2b	97.254
3b	-
4b	-

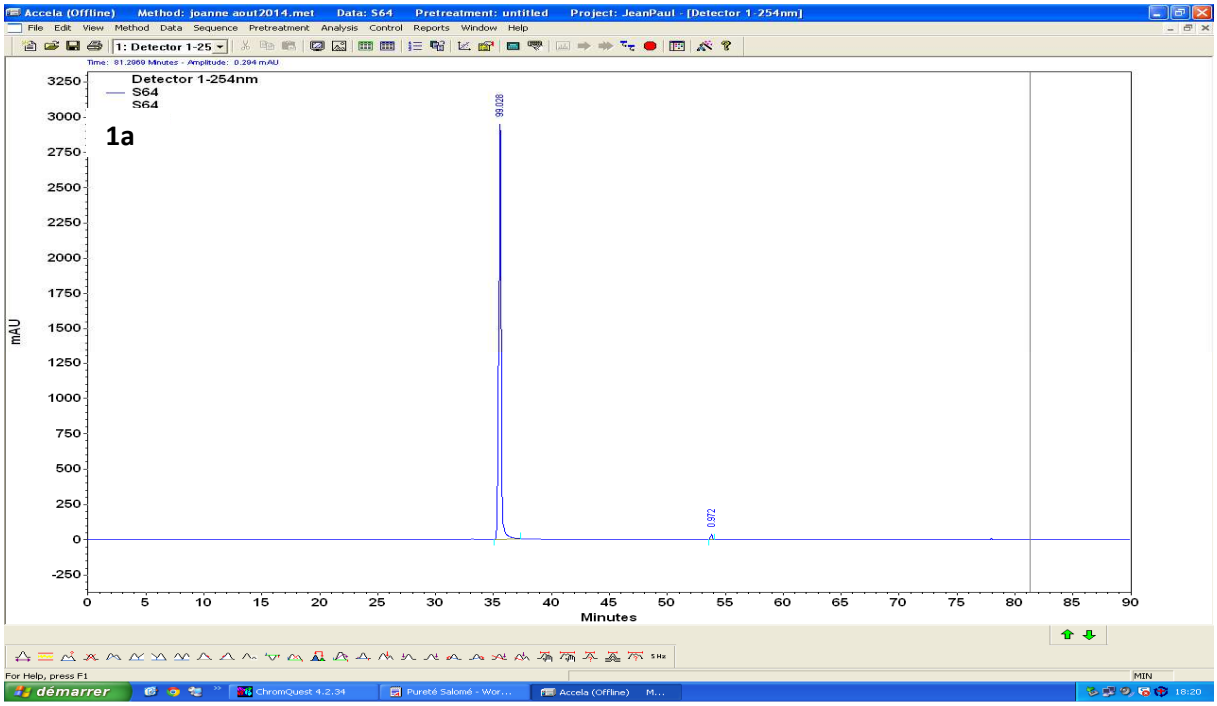


Figure 1 : **Chromatogram with percentage of purity of compound 1a**

In total, the two purity verification methods showed the purity of all synthesized products. These products can therefore be used for biological analysis. Their structures were at first characterized with the TLC frontal rapport (R_f), spectrometrical analysis HRMS, and NMR ^1H &^{13}C.Molecules of each series showed similar spectrometric data.

The analysis of the ^{13}C NMR spectra of molecules from *salicylhydrazones* series (**1a - 4a**)showed 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 [27, 28]. 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.

In the series of *p-tosylhydrazones* compounds(**1b - 4b**),^{13}C NMR spectral analysis 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 (**2b**) in DMSO-d_6 and 8.10 ppm (**3b** & **4b**) and 7.90 ppm (**1b**) ppm in $CDCl_3$. This difference is due to the effect of DMSO solvent which generates a strong hydrogen bond between oxygen and the NH proton [29] 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.

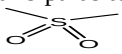
The scaffold has advantageous properties: low molecular weight, reasonable *C*log *P*, good hydrogen bond donating and accepting capabilities (table 3), easy, and economical synthetic routes [24].

Table 3: Properties Compatible with Reasonable Pharmacokinetics and Drug Availability, Rules of Lipinski [30] **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**
1a	284.35	3.093	2	4	all
2a	268.31	2.436	2	4	all
3a	310.34	2.358	2	5	all
4a	304.34	3.111	2	4	all
1b	318.43	4.772	1	4	all
2b	302.39	4.115	1	4	all
3b	344.43	4.199	1	5	all
4b	338.42	4.790	1	4	all

Pharmacology

Antiparasitic activity of compounds was evaluated *in vitro* on the chloroquine-sensitive strain 3D7 of *Plasmodium falciparum* and their toxicity on larvae of *Artemia salina* L. The half-inhibitory concentration (IC_{50}) and half-lethal concentration (LC_{50}) were respectively determined and expressed in micromolar (µM) to be compared with the scales of antiplasmodial and toxic activities. Then, the selectivity index (SI) of each compound was calculated as showed in table 4.

The antiplasmodial test reveals that molecules from *p*-tosylhydrazide series **b** were less active onthis parasites than those from salicylhydrazide ones. This could be explained by the presence of the sulfone group () in *p*-tosylhydrazide compounds. The replacement of this group by the carbonyl group () in the passage of series **b** compounds to series **a** ones seemed to enhance antiplasmodial activity of these compounds. S-(+)-carvonesalicylhydrazone (**1a**) (IC_{50} = 18.65±2.99µM) and 2-acetylnaphthalenesalicylhydrazone (**4a**) (IC_{50} = 13.05±4.84 µM) were the most actives compounds followed by 4'-methylacetophenonesalicylhydrazone (**2a**), and 7-methoxy-1-tetralone salicylhydrazone (**3a**). Among the synthesized arylketone compounds, the increasing of conjugation with C=N groups seemed to enhance antiplasmodial activity from **3a** to **4a**. **1a** and **4a** showed moderate activity with IC_{50} values between 2 and 20 µM and the others compounds, lower activities(IC_{50}>20 µM)(table 4) according tothe scale of Beroand *al.*[31].All synthesized compounds were none toxic against larvae of *Artemia salina* L. except **4b** which exhibited an IC_{50} value lower than camptothecin one (IC_{50} = 38.09±0.06 µM) that was the positive reference used for this test (table 4).

From the analyses of their selectivity index, the synthesized products with interesting activity (**1a**-**4a**) also showed good selectivity on the parasite (SI>1) (table 4). These results were in perfect agreement with the work of Tiuman*et al.*[32] in which if the SI value is higher than unity, the test compound is considered to be selective on the parasite and if SI value is lower than unity, the test compound is more cytotoxic than antiparasitic.

Table 4: Antiplasmodial activity, toxicity and selectivity index of synthesized compounds

Compounds	IC_{50} (µM)	Activity[γ]	LC_{50} (µM)	Toxicity[µ]	Selectivity Index (SI = LC50 / IC50)
1a	18.65± 2.99	moderate	219.44 ± 2.17	no toxic	9.80
2a	20.83±2.82	lower	352.57 ± 1.32	no toxic	11.62
3a	95.25±32.95	lower	731.13 ± 3.27	no toxic	7.74
4a	13.05± 4.84	moderate	310.50 ± 1.93	no toxic	13.97
1b	>314.33	lower or no	536.38 ± 2.35	no toxic	< 1.70
2b	>331.01	lower or no	368.06 ± 1.87	no toxic	< 1.11
3b	>290.60	lower or no	137.32 ± 0.77	no toxic	< 0.47
4b	>198.10	lower or no	35.45 ± 1.41	toxic	< 0.17

[γ]Activity classified by using the scale of Beroand al.[22].[µ]Toxicity by using camptothecin value (IC_{50} = 38.09±0.06 µM) as reference.

CONCLUSION

In this study, eight hydrazone derivatives were synthesized, purified and characterized. Their biological activities were evaluated and products showed interesting antiplasmodial activity on the studded parasite with good selectivity. To our knowledge, this is the first time that the antiparasitic activities of these molecules are evaluated on *Plasmodium falciparum* and then they could open an interesting opportunity to the treatment of malaria.

Acknowledgments

We thank Professors J. Quetin-Leclercq and D. Lambert and all the staff of LDRI at the Université Catholique de Louvain, Brussels, Belgium.

REFERENCES

[1] APoinsignon, *Thèse de Doctorat en Parasitologie*., Université de Montpellier I, **2008**.
[2] MC Field;CL Allen;Dhir V; Goulding D; BS Hall; GW Morgan;P Veazey; M Engstler, *Microsc. Microanal*., **2004,** 10(5), 621–636.
[3] WHO,*World Health OrganisationMalaria*; *Fact sheet* N°94, **2011**.
[4] M Shakir;AAbbasi, *J. Chem. Pharm*. Res., **2015**, 7(5), 375-382.
[5] DD Desai; GC Desai,*J. Chem. Pharm*. Res., **2014**, 6(7), 1704-1708.
[6] AB Samel; NR Pai, *J. Chem. Pharm. Res*., **2010**,2(4), 60-67.
[7] SAM Shedid; HM Hassan; FA Kora; RM El- Eisawy, *J. Chem. Pharm. Res*., **2011**, 3(1), 388-394.
[8] N Kumar; LS Chauhan,*J.Chem. Pharm. Res.*,**2014**, 6(12), 916-934.
[9] A John Maria Xavier; M Thakur; J Margaret,*J. Chem. Pharm. Res.*, **2012**, 4(2), 986-990.
[10] SM Sondhi;M Dinodia;A Kumar,*Bioorg. Med. Chem*.,**2006**. 14, 4657-4663. doi: 10.1016/j.bmc.2006.02.014.
[11] WWWardakhan; NN Eid El-Sayed;RM Mohareb,*Acta Pharm*., **2013**, 63, 45-57.
[12] YL Xia;F Chuan-Dong;BXZhao;J Zhao;DS Shin; JYMiaom. *Eur. J.Med. Chem.*, **2008**, 43, 2347-2353. doi: 10.1016/j.ejmech.,2008.01.021.
[13] SG Küçükgüzel; AMazi;F Sahin;S Öztürk;JP Stables,*Eur. J. Med. Chem*., **2003**, 38(11), 1005-1013. doi: 10.1016/j.ejmech.2003.08.004
[14] Y Wang;N Serradell;J Bolos; E Rosa,*Drugs Fut.*, **2007**, 32, 118-122.
[15] B Glinma; FA Gbaguidi; UC Kassehin; SDS Kpoviessi; A Houngbeme; HD Houngue; GC Accrombessi; JH Poupaert,*J. App. Pharm. Sci.*, **2015**, 5(06), 001-007. DOI: 10.7324/JAPS.2015.50601.
[16] Melnyk P;Leroux V;Sergheraert C;Grellier P, *Library. Bioorg. Med. Chem*. Lett., **2006**, 16(1), 31-35. doi:10.1016/j.bmcl.2005.09.058.
[17] L Friedman; RL Litle; WR Reichle,*Org Syntheses.*, **1960**; 40-93.doi: 10.15227/orgsyn.040.0093
[18] R Urich;R Grimaldi;T Luksch; AF Julie;R Brenk;GW Paul.*J. Med. Chem*., **2014**, 57 (18), 7536–7549. DOI: 10.1021/jm500239b
[19] T Xue;S Ding;B Guo; Y Zhou; P Sun; H Wang;W Chu; G Gong;Y Wang;X Chen;YJ Yang,*J. Med. Chem*., **2014**, 57 (18), pp 7770–7791
[20] MT Makler;JM Ries; JA Williams; JE Bancroft; RC Piper;BL Gibbins;DJ Hinrichs,*Am J Trop Med Hyg*,**1993**, 48: 739–741.
[21] S Murebwayire;M Frederich;V Hannaert; MC Jonville;P Duez,*Phytomedicine*.,**2008**,15, 728-733.
[22] RB Sleet;K Brendel,*Ecotoxicol Environ Safety.*,**1983**; 7, 435-446.
[23] AJW Abbott,*J.Econ. Entomol*.,**1925**, 18, 265.
[24] E Hafner,E Heiner,E Noack,*Mathematical analysis of concentration- response relationships. Arzneim.-Forsch./ Drug. Res*., **19**77, 27, 1871-1873.
[25] M Ashraf,*Can J Chem.*, **1976**; 54: 448-454.
[26] OIH Al-Ajrawy; Al-Anbar,*J Vet Sci.*, **2011**, 4(2), 129-135.
[27] R Baligar; V Revankar,*J Serb ChemSoc*,**2006**,71(12), 1301–1310.
[28] T Govindasami; A Pandey; N Palanivelu; A Pandey,Int*J Org Chem*, **2011,** 1, 71-77.
[29] RM Silverstein, FX Webster, D Kiemle,Spectrometric identification of organic compounds. De Boeck, 2nd Edition,**2007**.
[30] CA Lipinski; Lombardo F;BW Dominy;PJ Feeney,*Adv Drug Delivery Rev.*, **1997**, 23, 3-25.
[31] J Bero, M-F Hérent, GSchmeda-Hirschmann, MFrédérich, J Quetin-Leclercq,*J. Ethnopharmacol***.**, **2014**,149, 76–183.
[32]. TS Tiuman; T Ueda-Nakamura; DA Garcia Cortez; BP Dias Filho; JA Morgado-Diaz; W de Souza; CV Nakamura,*Antimicrob. Agents chemother*.,**2005**, 492, 176-182.