*Original Research Article*

**Synthesis, spectrometrical characterization and pharmacological properties of six substituted hydrazones with carbonyl compounds**

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ABSTRACT

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| --- |
| Hydrazones are molecules that prevent the growth of several microbial and parasitic strains by inhibited the replication of DNA sequences due to their chelating properties of metal ions. They are well recognized for Antiparasitic, Antimicrobial, Antibacterial, Antiviral, Antitumoral, Antimalarial and Anticonvulsive activities . The aim of the current study is to synthesized novel substituted hydrazones of ketones and aromatic aldehydes and to study their antiparasitic activities against *Trypanosoma brucei brucei* parasite. Synthesized hydrazone derivatives have been confirmed by elemental analysis and HPLC method, SMHR, 1H and 13C NMR spectroscopy. Three hydrazones derivatives have been synthesized namely phenylhydrazones, 2,4-dinitrophenyl hydrazones and benzohydrazones. The antiparasitic properties of these molecules were evaluated on *Trypanosoma brucei brucei*. The derivatives from phenylhydrazones and benzohydrazones were showing more antiparasitic activity like benzophenone benzohydrazone (D3) and salicylaldehyde phenylhydrazone (B1) compounds, which exhibited moderate activity on *Trypanosoma brucei brucei* (IC50 = 19.49 μM; IC50 = 20.01 μM respectively). Cytotoxicity tests again the human noncancer fibroblast cell line (WI38) were carried out with selected synthesized compounds and it showed that the most compounds were active against *Trypanosoma brucei brucei*. As a result of these findings, hydrazones could be a promising route in the treatment of trypanosomiasis. |

*Keywords: Synthesis, Hydrazones, Characterization, Selectivity, Trypanosoma*

1. INTRODUCTION

Resistance of germs to existing drugs remains and continues to be a very serious concern (Alanis, 2005). It is therefore crucial to develop alternatives for the well-being of humans and animals (Clark, 2013). Among these possibilities, we have synthesis which is a method used in ancient times for the discovery of active ingredients (Oselusi et al., 2024). Thanks to scientific progress, several families of molecules can be developed (Crasta et al., 2021). Among these, we have the substituted hydrazones of carbonyl compounds. Hydrazone is a derivative of aldehyde or ketone formed by a condensation reaction with hydrazine or hydrazine derivatives (Kumar et al., 2016). Biological activities of different hydrazones were reported in the literature. Hydrazone family compounds were known for their antibacterial (Sharma et al., 2020), antimicrobial (Ugochukwu et al., 2023), antitrypanosomal (Shiran et al., 2023), antituberculosis ([Sampiron](https://www.tandfonline.com/author/Sampiron%2C%2BElo%C3%ADsa%2BG) et al., 2019), antimalarial (Rosado-Quiñones et al., 2024) properties. However, some hydrazones and derivatives have not been sufficiently explored to date. Thus, in the present study, the synthesis and characterization of new hydrazones, the evaluation of their antitrypanosomal properties as well as their cytotoxicity will be discussed.

2. material and methods

***2.1. Chemical material***

All substrates, reagents, catalysts and solvents were obtained from chemical societies and used directly for synthesis without any further purification. The substrates, arylketones and arylaldehydes used in this work were : benzaldehyde **A**, salicyladehyde **B**, acetophenone **C** and benzophenone **D** which were obtained from Acros Organics (New Jersey, USA). Phenylhydrazine **1**; 2,4-dinitrophenylhydrazine **2** and benzohydrazide **3** marketed by the Aldrich company were the three reagents used. Their structures are presented in Fig. 1.



**Fig.1: Structures of substrates (A, B, C and D) and reagents (1, 2 and 3) used.**

***2.2. Synthesis of hydrazone compounds***

The hydrazone synthesis was carried out by an acid-catalyzed mechanism method described in our previous work (Atchade et al., 2015; Glinma et al., 2015). For this, the concentrated glacial acetic acid (GAA) marketed by the Prolabo company was used. In a 50 mL flask, 5 mmol of ketone or aldehyde in 05-20 mL ethanol and 1 mL of glacial acetic acid were prepared and stirred for 1 min at r.t. Then, a solution of hydrazine (0.27 g, 2.5 mmol) in 5 mL of ethanol was gradually added. The mixture was stirred at r.t. for 1 min and refluxed for 2 hours. The reaction was carefully monitored by thin layer chromatography (TLC) (hexane: ethyl acetate, 8:2 or 7:3). The crystals formed were filtered, washed with distilled water and dried before being recrystallized to give hydrazone compounds **A2**, **B1**, **C2**, **C3**, **D1** and **D3** with variable yields (70 to 90%).

***2.3. Physicochemical analysis equipment***

The melting points of the synthesized hydrazones were determined with a *fusionometer* of the type *electrothermal* (1A 9000). These values were not corrected.

For checking the purity of synthesized compounds, we used a Thermo Electron Corporation Flash EA 1112 series analyzer equipped with a micro-balance Mettler Toledo  MX5. After sublimation in a furnace at 900°C, the sample passed through a catalytic tube where carbon was converted into CO2, nitrogen into N2, hydrogen into H2O and sulfur into SO2. Then, these passed through a gas chromatographic (GC) system equipped with a flame ionization detector (FID). The detector was connected to software (Eager 300) and provided a chromatogram and the relative percentages of the different elements through a calibration line**.** We used also a Accela type (Thermo Fisher Scientifique, Bremen, Germany) High Performance Liquid Chromatography (HPLC) system equipped with a pump, an auto-sampler and a photodiode PDA detector, with a Phenomenex Licospher type C18 column (4 mm to 250 mm)conditioned with 5μm size particles. A binary solvent system of 1000 μL/mm flow rate was used.

Nuclear Magnetic Resonance (NMR) spectra of the synthesized compounds were recorded with a Bruker-type apparatus (UltraSheild). The mass spectra were acquired in positive mode using an LTQ-Orbitrap XL hybrid (Thermo Fisher Scientifique, Bremen, Germany) equipped with an electrospray ionization ESI and high resolution mass was given in m/z of [MH+]. The 1H proton spectra were analyzed at a frequency of 300 to 400 MHz and that of carbon 13C at 100 MHz in the dimethylsulfoxide (DMSO)-d6 or chloroform CDCl3 solution. Chemical shifts were expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Multiplicity was designated as singlet (s), redoubled doublet (dd) triplet (t), quintuplet (qi) and multiplet (m).

**2.4. Biological material**

It consists of parasite strains of the *Trypanosoma brucei brucei* line 427 (Molteno Institute in Cambridge, UK) (Duszenko et al., 1988) and non-cancerous human fibroblast cell strains WI38 (Coppock et al., 1993) for the determination of selectivity of pure synthesized compounds and larvae of brine shrimp (*Artemia* *salina* Leach) in toxicity tests (Otang et al., 2013).

**2.5. Antitrypanosomal activity test**

***2.5.1. Parasites culture.***

Blood forms of *Trypanosoma brucei brucei* strain 427 were cultured *in* *vitro* in HMI9 medium containing 10% unsupplemented fetal calf serum as described by Kpadonou et al. (2019).

***2.5.2. LILIT, Alamar Blue test.***

The *in vitro* test was performed using the method “LILIT, Alamar Blue” as described by Hoet et al(2004).

**2.6. Toxicity test**

The toxicity test was performed on enkysted eggs of *Artemia* *salina* (10 mg) incubated in 100 mL of seawater, at pH 7- 8 as described in our previous work (Kpadonou-Kpoviessi et al., 2012).

**2.7. Cytotoxicity test**

***2.7.1. Cell culture***

The human non-cancerous fibroblast cell line, WI38 (ATCC N CCL-75 from LGC Standards) was grown *in* *vitro* in DMEM (Gibco) medium containing 4mM of L-glutamine, 1mM sodium pyruvate supplemented with 10% uncomplemented fetal calf serum (Gibco), penicillin-streptomycin (100 IU/mL -100 μg/mL) and fungal (amphotericin D 250 UG/mL). Cells were incubated in a 5% CO2 humidified atmosphere at 37°C.

***2.7.2. MTT test***

Stock solutions were prepared in DMSO at a concentration of 10 mg/mL and kept at 4°C. The cytotoxic activity of pure compounds on WI38 cells was evaluated. The test was performed as described by Kpoviessi et al. (2014) using the tetrazolium salt (MTT) 3-(4,5- dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide (sigma) test which is a colorimetric test based on tetrazolium salt (MTT) reduction in diformazan by living cell dehydrogenases (Kpoviessi et al., 2014).

**2.8. Statistical analysis**

The student t test was used to determine whether the difference between the results for different samples, and between the results for samples and controls are significant. The difference is statistically significant at (P = 0.05) (SAS/STAT, 1990).

3. results and discussion

**3.1. Chemistry**

Tree series of derivatives hydrazones witch were synthesized: Benzaldehyde 2,4-dinitrophenylhydrazone (**A2**), Acetophenone 2,4-dinitrophenylhydrazone (**C2**), Salicylaldehyde phenylhydrazone (**B1**), Benzophenone phenylhydrazone (**D1**), Acetophenone benzohydrazone (**C3**), Benzophenone benzohydrazone (**D3**).

**3.1.1. Yields of synthesis reactions**

The synthesis method of hydrazones used in this study with glacial acetic acid as a catalyst delivered compounds with good yields (70-91%) as resumed in Table 1. These compounds were obtained between 2 to 3 hours approximately against 4-8 hours for other derivatives of hydrazones ([Şener](https://onlinelibrary.wiley.com/authored-by/%C5%9Eener/Ahmet) et al., 2002).

 **Table 1: Physical properties and yields of compounds**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Compounds** | **Crude formula** | **Molecular weight****(g.mol-1)** | **Melting Points (°C)** | **Yield (%)** |
| **A2** | C13H10N4O4 | 286.24 | 181-182 | 79.55 |
| **C2** | C14H12N4O4 | 300.27 | 187-188 | 72.67 |
|  |   |   |   |   |
| **B1** | C13H12N2O | 212.25 | 139-140 | 71.98 |
| **D1** | C19H16N2 | 272.34 | 142-143 | 90.06 |
|  |   |   |   |   |
| **C3** | C16H16N2O | 252.31 | 189-190 | 70.23 |
| **D3** | C20H16N2O | 300.35 | 119-120 | 70.24 |

The analysis of Table 1, showed that the yields of synthesized compounds were higher than 70%. Similar results were obtained by Avaji et al. (2009). This improvement in yields in a reduced time of reaction could be due to the steric and electronic effects of the various carbonyl substituents (Shamsabadia and Chudasama, 2017).

**3.1.2. Results of spectrometric analysis of synthesized products**

High-resolution mass spectroscopy provided the exact mass of the studied compounds, their raw formula and the various fragments used to reconstruct the parent molecular. The values provided by this method of analysis were highly accurate. Table 2 summarizes the crud formula and molecular ion mass [M+H]+, [M+Na]+ and [2M+Na]+ generated during the analysis of our synthesized products.

**Table 2: Crude formulas and molecular ion masses of the synthesized compounds**

|  |  |  |  |
| --- | --- | --- | --- |
| **N°** | **Generated Raw Formula** | **Theoretical exact mass** | **Molecular ion mass M** |
| [M+H]+ | [M+Na]+ | [2M+Na]+ |
| **A2** | C13H10N4O4 | 286.07 | 287.09135 | 309.11203 | - |
| **C2** | C14H12N4O4 | 300.09 | 301.09296 | 323.11043 | - |
|   |   |    |   |   |   |
| **B1** | C13H12N2O | 212.09 | 213.10211 | 235.19210 | - |
| **D1** | C18H16N2 | 272.13 | 273.13815 | 295.11004 | 567.23098 |
|   |   |   |   |   |   |
| **C3** | C15H14N2O | 250.13 | 251.13275 | 273.11474 | 525.23932 |
| **D3** | C20H16N2O | 300.13 | 301.13266 | 323.11472 | 623.23969 |

We find from the analysis in Table 2 that for all synthesized compounds, the masses of the molecular ions (parent peaks) obtained were comparable and almost identical to those of the theoretical molecular ions.

Results of 13C NMR and 1H NMR analysis below confirmed the structures of our compounds.

**Benzaldehyde 2,4-dinitrophenylhydrazone (A2)**

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**13C NMR** (CDCl3, 100 MHz) δ (ppm): 148.61 (CH=N); 144.58 (C-NH); 126.11-139.39 (C- Ar). **1H NMR** (CDCl3, 400 MHz) δ (ppm): 8.3 (s, 1H, CH=N); 9.70 (s, 1H, NH); 11.8 (s, 1H; NH); 7.4-7.6 (m, 5H, H-Ar); 8.4-7.4 (m, 3H, H-Ar). **SM** m/z [MH] +: 287.09135.

**Acetophénone 2,4-dinitrophenylhydrazone (C2)**

 ****

**13C** **NMR** (DMSO-d6, 100 MHz), δ (ppm): 148.6 (C=N); 147.59 (C-NH); 19.01(CH3); 126.11 134.20 (C-Ar). **1H NMR** (DMSO-d6, 400 MHz), δ (ppm): 10.90 (s, 1H, NH); 1.8 (s, 3H, CH3); 7.3-7.8 (m, 5H, H-Ar); 7.9-8.8 (m, 2H, H-Ar). **SM** m/z [MH] +: 301.09296.

**Salicylaldehyde phenylhydrazone (B1)**

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**13C NMR** (CDCl3, 100 MHz), δ (ppm): 148.61 (CH=N); 148.58 (C-NH); 156.47 (C-OH), 134.38 (C-CH=N) 119.31-129.39 (C-Ar). **1H NMR** (CDCl3, 400 MHz): δ (ppm): 8.10 (s, 1H, CH=N); 9.2 (s, 1H, NH); 6,31-7.46 (m, 5H, H-Ar); 7.20-7.90 (m, 4H, H-Ar); 3.8 (s, 1H, OH). **SM** m/z [MH] +: 213.10211**.**

**Benzophenone phenylhydrazone (D1)**

****

**13C NMR** (CDCl3, 100 MHz), δ (ppm):148.61 (C=N); 144.38 (C-NH) ; 126.01-131.18 (C-Ar). **1H NMR** (CDCl3, 400 MHz), δ (ppm): 10.9 (s, 1H, NH); 6.8-7.6 (m, 10H, H-Ar); 7.2-7.9 (m, 5H, H-Ar). **SM** m/z [MH] +: 273.13815.

**Acetophenone benzohydrazone (C3)**

****

**13C** **NMR** (DMSO-d6, 100 MHz), δ (ppm): 154.78 (NH-CO-Ar); 149.11 (C=N); 125.46-143.41 (C-Ar); 21,96 (CH2); 11,04 (CH3). **1H NMR** (DMSO-d6, 400 MHz), δ (ppm): 10.9 (s, 1H, NH); 7.12-7.59 (m, 5H, H-Ar); 7.20 -8.19 (m, 5H, H-Ar); 1.9 (t, 1H, CH3); **SM** m/z [MH] +: 251.13275.

**Benzophenone benzohydrazone (D3)**

****

**13C NMR** (DMSO-d6, 100 MHz), δ (ppm): 158.33 (NH-CO-Ar); 148.48 (C=N); 123.52-144.24 (C-Ar). **1H NMR** (DMSO-d6, 400 MHz), δ (ppm): 10.20 (s, 1H, NH); 7.2-7.70 (m, 5H, H-Ar); 7.45 -8.50 (m, 10H, H-Ar). **SM** m/z [MH] +: 301.13266.

This spectrometric analysis of synthesized compounds : phenylhydrazones, dinitrophenylhydrazone, benzohydrazones and salicylhydrazones showed similar characteristics with some specificities described below.

In 1H NMR, several types of protons were distinguished, two of which were more shielded: the phenolic OH proton and the NH proton. The phenolic proton OH with acidic properties was found at a lower field than the proton NH ([Halim](https://pubmed.ncbi.nlm.nih.gov/?term=Halim+SA&cauthor_id=37264069) and [Abdel-Rahman](https://pubmed.ncbi.nlm.nih.gov/?term=Abdel-Rahman+MA&cauthor_id=37264069), 2023; [Nose](https://pubs.acs.org/action/doSearch?field1=Contrib&text1=Akira++Nose) et al., 2004). Thus, it will be assigned to OH, the highest δ value. Therefore, the latter was a singlet between 11.80 and 11.75 ppm while the NH proton’s singlet was observed between 11.42 and 11.20 ppm. This was done for all compounds synthesized with a hydroxyl group in the ortho position. Aromatic protons appear between 8.36 and 6.90 ppm and confirmed the work of Mohammed and Sultan (2024).

In 1H NMR, the only proton of internal nitrogen NH was a singlet at 10.90 ppm for 2,4-dinitrophenyl hydrazones (**C2**) in DMSO and 9.7 ppm for 2,4-dinitrophenyl hydrazone (**A2**) in CDCl3. The same results were obtained by Çınar and Topal (2022). Thus causing a chemical shift of hydrogen towards the weak fields ([Yao](https://onlinelibrary.wiley.com/authored-by/Yao/Jiandong) et al., 2020). Aromatic protons were observed as a mass between 7.93 and 6.84 ppm. In the 13C NMR spectra, a peak was observed between 153 and 154 ppm which corresponds to the carbon of the group (N-CO-Ar) for the compounds **C3**and **D3**. The carbon of the imine function (C=N) characteristic of the formation of the synthesized compound appears at around 148-149 ppm (Atchade et al., 2015). Other peaks between 158 and 108 ppm correspond to the aromatic nucleus (Okolo et al., 2015); phenolic carbon (aromatic C-OH) showed a high value of 156.47 ppm in the compound **B1** (Atchade et al., 2015).

**3.1.3. Verification of purity of synthesized compounds**

The purity of the synthesized compounds was verified by two methods: the elementary analysis and the high-performance liquid chromatography (HPLC) analysis.

***3.1.3.1. Elementary analysis***

The elemental analysis allowed us to determine the percentage of different atoms of the compound except for the oxygen atom. The difference (in absolute value) between the theoretical and experimental percentages of each atom must be less than 0.5 for the synthesized compound to be considered as pure (Atchade et al., 2015). Table 3 presents the results of the elementary analysis of studied compounds. Analysis of this table shows that these compounds meet the purity criteria for elemental analysis (Aleluia et al., 2023). The same purity of our compounds was verified by the analysis with High-Performance Liquid Chromatography (HPLC) (Atchade et al., 2015).

**Table 3: Elemental analysis of synthesized compounds.**

|  |  |  |
| --- | --- | --- |
| Compound | Atoms | Percentage (%) |
| Weight |  |
| Experimental 1 | Experimental 2 | Average  | Theoretical | |δmex-mth| |
|   |   |   |   |   |   |   |
| **A2** | N | 10.43 | 10.73 | 10.58 | 11.02 | 0.44 |
|   | C | 70.43 | 70.38 | 70.40 | 70.85 | 0.445 |
|   | H | 5.55 | 5.54 | 5.54 | 5.55 | 0.005 |
|   |   |   |   |   |   |   |
| **C2** | N | 8.98 | 8.64 | 8.81 | 9.03 | 0.22 |
|  |  |  |  |  |  |  |
|   | C | 69.26 | 69.16 | 69.21 | 69.66 | 0.45 |
|   | H | 5.82 | 5.83 | 5.82 | 5.85 | 0.025 |
|   |   |   |   |   |   |   |
| **B1** | N | 18.9 | 18.79 | 18.84 | 18.81 | 0.035 |
|   | C | 59.32 | 59.02 | 59.17 | 59.16 | 0.01 |
|   | H | 7.98 | 7.19 | 7.58 | 7.67 | 0.085 |
|   |   |   |   |   |   |   |
| **D1** | N | 11.88 | 11.78 | 11.83 | 11.85 | 0.02  |
|   | C | 81.18 | 81.45 | 81.31 | 81.32 | 0.005 |
|   | H | 6.94 | 6.77 | 6.85 | 5.83 | 0.025 |
|   |   |   |   |   |   |   |
| **C3** | N | 10.43 | 10.73  | 10.58 | 11.02 | 0.45 |
|   | C | 70.43 | 70.38 | 70.40 | 70.85 | 0.444 |
|   | H | 5.55 | 5.54 | 5.54 | 5.55 | 0.005 |
|   |   |   |   |   |   |   |
| **D3** | N | 10.43 | 10.73 | 10.58 | 11.02 | 0.46 |
|   | C | 70.43 | 70.38 | 70.40 | 70.85 | 0.443 |
|   | H | 5.55 | 5.54 | 5.545 | 5.55 | 0.005 |

***3.1.3.2. HPLC analysis***

The analytical method by High Performance Liquid Chromatography (HPLC) was developed to verify the purity of the studied compounds. It allowed to determine the percentage of different compounds in a mixture by calculating the area of the surfaces under the curve of each of them. The percentage of compounds tested for purity by this method must be greater than 95% to be considered pure (Aleluia et al., 2023).

Fig. 2 shows the chromatogram of the compound **D3** with its percentage of purity and proved that this compound was pure at 99.028%.



**Fig. 2: Chromatogram showing the percentage purity of the product D3.**

Table 4 presents the percentage of purity of synthesized compounds. The percentages in Table 4 were higher than 95% and therefore proved that all synthesized compounds were pures (Aleluia et al., 2023).

**Table 4: Percentage of purity of synthesized compounds by HPLC analysis**

|  |  |
| --- | --- |
| Compounds | Percentage of purity |
| **A2** | 97.254 |
| **C2** | 100.021 |
|  |  |
| **B1** | 95.24 |
| **D1** | 97.267 |
|  |  |
| **C3** | 98.349 |
| **D3** | 99.028 |

These two purity verification techniques proved that all our compounds meet the purity requirements for a synthesized compound to be bioassayed.

**3.2. Biological part.**

**3.2.1. Lipinski’s rule.**

Before the biological tests, we conducted a general theoretical analysis of all compounds. This was a theoretical approach that allowed us to estimate certain parameters essential for the pharmacokinetics of molecules. We used the rule established by Lipinski et al. (1997).

Any molecule meeting three of these criteria would have good pharmacokinetics and would be a good candidate drug. Table 5 presents the criteria of the Lipinski rule applied to the synthesized compounds.

**Table 5: Lipinski rule applied to the synthesized compounds**.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  Compounds | Molar mass (g.mol-1) |  Clog P | Number of links H donors | Number of acceptor H-bonds | Number of criteria met |
|   |  |  |  Rules |  |  |
| < 500 | < 5 | ≤ 5 | < 10 | 3 on list |
| **A2** | 286.07 | 3.670 | 1 | 8 | All |
| **C2** | 300.09 | 4.178 | 1 | 8 | All |
|   |   |   |   |   |   |
| **B1** | 212.09 | 3.909 | 2 | 3 | All |
| **D1** | 272.13 | 5.193 | 1 | 2 | All |
|   |   |   |   |   |   |
| **C3** | 250.13 | 4.042 | 1 | 3 | All |
| **D3** | 300.13 | 4.948 | 1 | 3 |  All |

From the analysis of this table, we note that all the synthesized compounds meet at least three of Lipinski’s criteria and could therefore be considered as good drug candidates (Lipinski et al., 1997). These compounds were then tested for their pharmacological properties.

**3.2.2. Toxicity and cytotoxicity of synthesized compounds.**

All synthesized compounds were tested for toxicity against brine shrimp larvae and cytotoxicity against human cells WI38. Results are presented in Table 6. These activities, which provide information on toxicity and cytotoxicity, also allow the evaluation of the selectivity of compounds against parasites (Kpoviessi et al., 2014). Thus, a selective compound would be useful for pest control and a highly toxic compound would be directed to cancer control (Santos et al., 2003; Graminha et al., 2008; Glinma et al., 2014).

**Table 6: Toxic and cytotoxic activity of synthesized compounds**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  Compounds |  \* LC50 (µM) |  Toxic activities |  IC50 (µM) |  Cytotoxic activities  |
| LC50-m | LC50-sd | IC50-m | IC50-sd |
| **A2** | 18.45 | 1.86 | Toxic | >174.68 | - | Not cytotoxic  |
| **C2** | 31.88 | 0.87 | Toxic | 79.55 | 1.91 | Not cytotoxic  |
|  |  |  |   |  |  |   |
| **B1** | 555.52 | 0.78 | Not Toxic | 55.78 | 4.06 | Not cytotoxic  |
| **E1** | 641.87 | 1.73 | Not Toxic | 90.79 | 1.22 | Not cytotoxic  |
|  |  |  |   |  |  |   |
| **C3** | 153.41 | 2.01 | Not Toxic | 179.38 | 1.98 | Not cytotoxic  |
| **D3** | 63.89 | 3.06 | Not Toxic | 85.25 | 10.62 | Not cytotoxic  |

\*LC50: Half-Lethal Concentration, IC50 = Half-Inhibition Concentration, m = mean, sd = standard deviation

For analysis of results in Table 6, camptothecine, the reference compound for these tests was used as a positive control (Kpadonou-Kpoviessi et al., 2014). Its LC50 value was 38.09 μM for larval toxicity, and its IC50 was 1.26 μM for cytotoxicity. All compounds with an LC50 < 38.09 μM and an IC50 < 1.26 μM were respectively toxic against shrimp larvae and cytotoxic against the human cell (Kpadonou-Kpoviessi et al., 2014). The study showed that, except 2,4-dinitrophenyl hydrazine series **A2** and **C2** compounds which showed toxicity against larvae of *Artemia* *salina* leach, all other compounds were not toxic against shrimp larvae and not cytotoxic against non-cancer human cells. Similar results were obtained by Glinma et al. (2014). The 2,4-dinitrophenyl hydrazine series of compounds which showed toxicity against larvae and any cytotoxicity against the non-cancerous fibroblast cell line (WI38) could be a deeper study for applications in cancer cell control.

**3.2.3. Antitrypanosomal activity and selectivity of synthesized compounds**

All the synthesized compounds were tested *in vitro* for their antitrypanosomal activities, particularly on *Trypanosoma brucei brucei* The half-inhibitory concentrations (IC50) were determined and expressed in micromolar (µM) and the selectivity index (SI) of each compound was calculated as summarized in Table 7.

**Table 7: Antitrypanosomal activity, toxicity, cytotoxicity and selectivity of synthesized compounds**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Compounds | IC50 Tbb (µM) | αAntitrypanosomal activities  | CytotoxicityWI38 (µM) | βselectivity index |
|  | IC50-m | IC50-sd |   | IC50-m | IC50-sd |  WI38/*Tbb* |
| **A2** | >87.39 | - | Weak | >34.95 | - | >5 |
| **C2** | >83.31 | - | Weak | >33.32 | 1.91 | <0.96 |
|  |  |  |   |  |  |   |
| **B1** | 20.01 | 0.17 | Moderate | 55.78 | 4.06 | 2.75 |
| **D1** | 29.05 | 0.06 | Weak | 90.79 | 1.22 | 3.13 |
|  |   |   |   |   |   |   |
| **C3** | 245.63 | 68.40 | Weak | 179.38 | 5.59 | 0.73 |
| **D3** | 19.49 | 1.72 | Moderate | 85.25 | 10.62 | 4.37 |
| Camptotecin  |  - | - |  - |  1.26 | 0.34 |  - |
| Suramin | 0.09 | 0 .02 | - | - | - | - |

\*WI38 = human embryonic lung fibroblasts, Tbb = *Trypanosoma brucei brucei*, IC50 = half inhibition concentration, m = mean, sd = standard deviation, βSelecitivity index = IS = IC50 (WI38) / IC50 (Tbb).

To better appreciate the antitrypanosomal power of compounds, we used the activity scale established through the work of Bero et al. (2011). The results in table 7 showed that the antitrypanosomal activities of the studied compounds vary according to the family from which they derive. The most active compounds were in the phenylhydrazones (1) and the benzohydrazones (3) families. Among all synthesized compounds, **D3** and **B1** exhibited moderate activity on *Trypanosoma brucei brucei* with IC50 = 19.49 μM and IC50 = 20.01 μM respectively, according to the activity scale of Bero et al. (2011). The 2,4-dinitrophenyl hydrazine family has low activity (IC50 > 80 μM). The study of the structure-activity relationship at the level of the benzohydrazones family, where the activity was more interesting, has allowed understanding that the replacement of a methyl group in **C3** (IC50 = 245.63 μM) by a phenyl group leading to the **D3** compound with a strong improvement in antitrypanosomal activity (IC50 = 19.49 μM); from low activity, we move to moderate activity (Bero et al., 2011).

|  |  |  |
| --- | --- | --- |
|  |  |  |
| **245,63µM** |  | **19,49 µM** |

**Fig.3 : Improvement of antitrypanosomal activity from C3 to D3 compound.**

The study of the structure-activity relationship revealed that the position of the various substituents at the level of the carbon chain was the basis for the different activities observed at the level of the compounds. Indeed, the replacement of this methyl group by phenyl increased mesomeria at the level of the compound which could justify the biological activity observed at the level of **D3** (Jain et al., 2012).

The selectivity of different molecules was expressed by calculating the selectivity index (SI = IC50-WI38/ IC50 parasite) (Table 7). If the SI value was higher than one, the studied compound is considered to be selective on the parasite. However, if SI was less than one, the concerned compound was more cytotoxic than pest control (Tiuman et al., 2005). The selectivity index was studied only for compounds that showed germ activity (Table 7).

The most active compound **D3** was the most parasite selective with selectivity indices higher than one (SI = 4).

4. Conclusion

Six (6) hydrazones whose purity was proved and whose structure was confirmed by analysis of their SMHR, 1H NMR and 13C NMR spectra were synthesized. The study of the antiparasitic activity of these compounds has shown that the hydrazones of the phenylhydrazines and benzohydrazides families were the most active against *Trypanosoma brucei brucei*. Toxicity and cytotoxicity tests against larvae of *Artemia salina* Leach and WI38 cells respectively, showed that the most active molecules were all non-toxic and non-cytotoxic. These results confirm that hydrazones in general and especially those of the phenylhydrazine family and benzohydrazides could be good drug candidates against sleep sickness.

**Disclaimer (Artificial intelligence)**

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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