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Synthesis, Characterization, X-ray Crystallography, Anticholinesterase Inhibition and Antioxidant Activities of Some Novel hydrazone

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Abstract

Alzheimer's disease is the most common form of dementia in persons over the age of 65, and its etiology is linked to oxidative stress. In this work, an easy and efficient access to two new hydrazones Schiff bases as (**2-Cl MHB** and **2-Cl BHB**). These compounds were prepared by condensation of 2-Chloro phenyl hydrazine with benzil.The structure of each of the two compounds was determined by the X-ray diffraction technique performed on single crystals. In a second part, an attempt to evaluate the biological properties is carried out on the two hydrazones such as the antioxidant power (by two methods: DPPH and CUPRAC) and the anti-cholinesterase activity AChE, BChE) against the disease of Alzheimer's. The results obtained must be taken (into account with other studies to improve their inhibitory activity.

Keywords: Hydrazon, DPPH, CUPRAC, AChE, BChE.

1. Introduction

The condensation of hydrazine (or derivate of hydrazine) with carbonyl compounds in acidic medium yields hydrazone (or Schiff bases) compounds. These type of molecules play considerable role in biological processes as antioxidant, antibacterial, antifungal, anti-alzheimer and anti-inflammatory **[1-3]**. They're used in a variety of treatments to treat disorders caused by free radicals **[4]**. Moreover, many researchers have succeeded in discovering the effectiveness of hydrazones against alzheimer's disease **[5]**, the main therapeutic objective in the treatment for AD is the inhibition of AChE and BChE present in forebrain.

Current acetylcholinesterase (AChE) and butyrylcholinesterase inhibitors (BChE) have some adverse side effects and are effective only against the mild type of Alzheimer's disease. As a result, it is very necessary to develop new drugs with better AChE and BChE inhibitors to control this disease neurodegenerative.

In this research work, we have synthesized and characterized Tow new hydrazone derived from the condensation reaction of benzil with 2-Chlro phenylhydrazine (**2-Cl MHB** and **2-Cl BHB**) **Scheme 1**, using FT-IR, UV-vis and X-ray diffraction spectroscopic techniques. In a second part, we report the resultats in vitro antioxidant and anticholinesterase inhibitions of our compounds.

2. Materials and methods

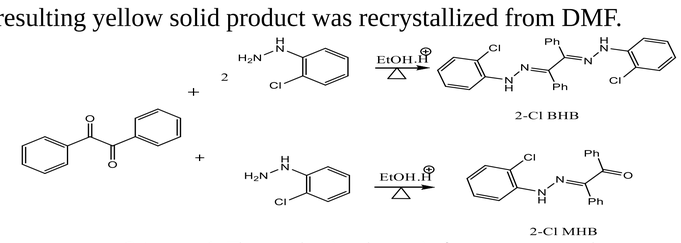
2.1. Synthesis

2.1.1. Synthesis of 2-chloro mono hydrazone benzil **(2-Cl MHB)**

This compound was synthesized by mixing 1,05g (0.005 mol) of benzil and 0,71g (0.005 mol) of 2-Chlorophenyl hydrazine in in 30 mL ethanol and two drops of acetic acid. The mixture was refluxed for 4h and then cooled, filtered and the solid was washed with methanol. The compound was recrystallized using DMF.

2.1.2. Synthesis of 2-chloro bis hydrazone benzil **(2-Cl BHB**)

In this case, we mixture 1,05g (0.005mol) of benzil with 1,42g (0,01 mol) of 2-Chlorophenyl hydrazine in in 50 mL ethanol and 2 drops of acetic acid was added (used as a catalyst), the mixture was refluxed for about 8 h. The reaction was monitored by TLC until completion. The resulting yellow solid product was recrystallized from DMF.



Scheme. 1. The synthesis schematic for our compounds

2.2. Biological evaluation

2.2.1. Antioxidant analysis

DPPH and CUPRAC methods [26] was used for determining the antioxidant potential of the compounds 2-Cl MHB and 2-Cl BHB according to Blois 1958 and Apak 2004 protocols respectively **[6,7]**.

2.2.2. Essay on enzyme inhibition

The well-known Ryan and Elman approach was used to conduct enzyme inhibition investigations **[8]**. Enzyme solutions (AChE/BChE) were mixed with solutions of the synthesized compounds followed by the addition of substrate solution i.e., acetylthiocholine iodide for AChE and butyrylthiocholine chloride for BChE together with DTNB solution and phosphate buffer of pH=8 and mixed properly. The mixture was incubated for 30 min at 37 °C. Substrate hydrolysis was estimated by spectrophotometer by recording absorbance at l=400 nm for AChE and l=412 nm for BChE. Enzyme inhibition rate was measured by the following equation.

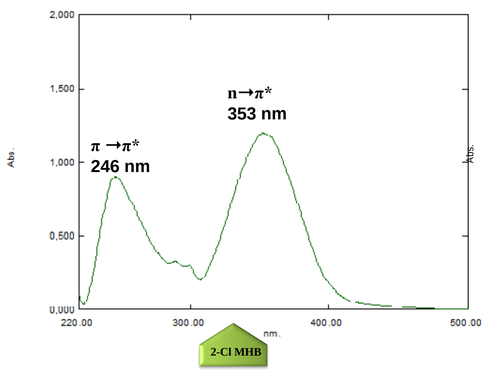
$$\% \text{ Inhibition} = \left(\frac{A_y - A_x}{A_y}\right) \times 100$$

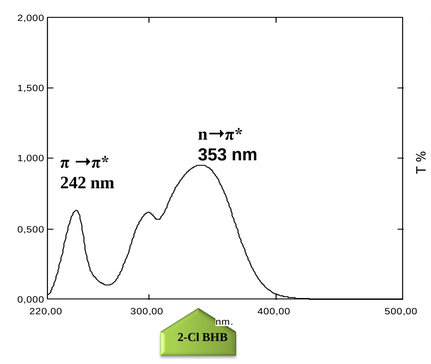
Where A_x Value of Absorption for enzyme including testsample.

A_y Value of Absorption for enzyme devoid of test sample. Every test was performed thrice, and mean was calculated. Galantamine was taken as reference

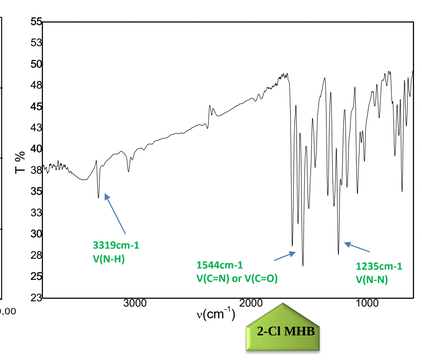
3. Results and Discussion

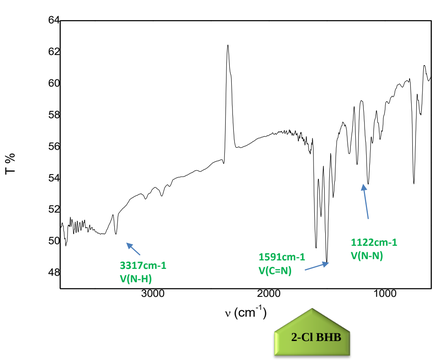
3.1. Caractérisation by UV-vis





3.2. Caractérisation by IR





3.3. Crystallographic studies

The crystals of both compounds were grown through slowly evaporation process in DMF solution. **2-Cl MHB** and **2-Cl BHB** crystallized in a Monoclinic system in `'P 21/c'` space group, with four unit per cell (Z= 4). The ellipsoid plot structures of our compounds are illustrated in **Fig. 1**

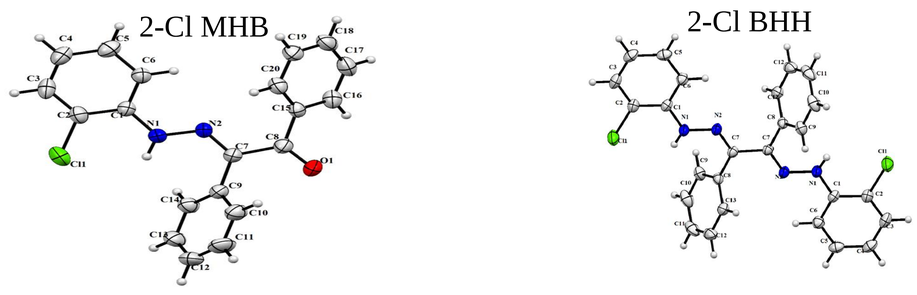


Fig. 1. Molecular structure of 2-Cl MHB and 2-Cl BHB

3.4. Antioxidant activity

The sample exhibited potent anti-oxidant results against DPPH radical and by CUPRAC method as depicted in Table 1, The IC50 of the tested compounds 2-Cl MHB and 2-Cl BHB against DPPH radical is measured as 291,37 and 312,97 µM, respectively, it's very far from IC50 value of standard drug (46,73 Mm). Whereas by CUPRAC method, $A_{0,5}$ of our products was determined to be 7,31 and 12,67 µM, respectively, They are very acceptable values compared to an activity of BHA and BHT drugs.

Table 1. anti-oxidant results of our compound and standards through DPPH and CUPRAC methods

Compounds	DPPH assay IC50 (µM)	CUPRAC assay A0.5 (µM)
2-Cl MHB	291,37±1,88	7,31±0,47
2-Cl BHB	312,97±3,24	12,67±1,98
BHT	46.73±0.41	5.99±0.12
BHA	nt	5.66±0.11

IC50 and A0.5 values are expressed as means±SD of three parallel measurements
BHA= butylated hydroxyanisole, BHT= butylated hydroxytoluene, nt: no tested

3.5. Cholinesterase inhibitory activity

The AChE and BuChE inhibitory activities of prepared hydrazones were determined and compared with Galantamine as standard using modified Ellman approach. This assay reflected that both compounds 2-Cl MHB and 2-Cl BHB have strong tendency to inhibit both AChE and BChE with IC50 values against AChE is measured as 21,80 and 10,38 µM, respectively. Whereas against BChE, it was determined to be 20,95 and 31,21 µM in respective manner and it act as a selective inhibitor of BChE as opposed to Galantamine (IC50= 34,75 µM), **Table 2**. These results are needed to molecular docking studies for its interprétation.

Table 2. In vitro inhibition IC50 values (µM) of our compound and standards for AChE and BuChe.

Compound	Inhibitory values IC50 (µM)	
	AChE	BuChE
2-Cl MHB	21,80 ± 1,10	20.95±1,29
2-Cl BHB	10,38 ± 1,27	31,21±1,50
Galantamine	6.27 ± 1.15	34.75±1.99

4. Conclusions

New hydrazones compounds derivate of benzil weresynthesized in good yield. These molecules were characterized by spectroscopic techniques and were completely elucidated it's structures by X-ray diffraction analysis. Their potential as antioxidant and as anticholinesterases was also evaluated. The 2-Cl MHB and 2-Cl BHB were emerged as the potential candidate as anticholinesterases since having good inhibition potential against both BChE and AChE. This study may be further extended with different hydrazones to find out potential molecules against Alzheimer disease.

5. References

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