



The Synthesis of Some Imine Derivatives *via* Condensation Reactions between Some Aromatic Phenylhydroxylamine Derivatives with Glyoxylic Acid and the Study of their biological activity

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ABSTRACT

This study investigated the conversion of 3,4-dimethylbenzaldehyde and 3,4-dimethoxybenzaldehyde into the corresponding oximes. In this regard, the condensation reaction between *N*-(3,4-dimethoxybenzyl) hydroxylamine with glyoxylic acid was accomplished to obtain the desired carboxylic nitron. The FT-IR, ¹H-NMR, and ¹³C-NMR spectroscopic techniques were used to identify the structure of the produced compound. Finally, the antimicrobial efficacy was assessed in terms of its action against the bacteria (*Escherichia coli*) as Gram-negative bacteria, (*Staphylococcus aureus*) as Gram-positive bacteria and fungus (*Candida albicans*). In conclusion, the activity data proved that this nitron showed a significant antimicrobial activity compared to the standard drugs.

تحضير بعض مشتقات الإيمين من خلال تفاعلات تكثيف بعض مشتقات فينيل هيدروكسيل أمين الأروماتية مع

حامض الكلايوكسيليك ودراسة فعاليتهم البيولوجية

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الملخص

تضمنت هذه الدراسة تحويل 4،3-ثنائي مثيل بنز الديهايد و 4،3-ثنائي ميثوكسي بنز الديهايد إلى الاوكزيمات المقابلة. ثم بعد ذلك، تم إجراء تفاعل التكثيف بين *N*- (4،3)-ثنائي ميثوكسي بنز(هيدروكسيل امين مع حامض الكلايوكسيليك للحصول على كاربوكسيليك نايترون. تم استخدام تقنيات التحليل الطيفي FT-IR و ¹H- NMR و ¹³C-NMR لتحديد بنية المركب الناتج. تم تقييم فعالية مضادات الميكروبات من حيث تأثيرها ضد بكتيريا (*Escherichia coli*) باعتبارها بكتيريا سالبة الجرام، و (*Staphylococcus aureus*) كبكتيريا موجبة الجرام وفطريات (*Candida albicans*) أثبتت بيانات النشاط أن هذا النايترون أظهر نشاطاً كبيراً كمضاد للميكروبات مقارنة بالأدوية القياسية. الكلمات المفتاحية: تفاعلات التكثيف، حامض الكلايوكسيليك، إيمين، الفعالية البيولوجية.

Introduction

Aldehydes hold great significance in organic chemistry as they can be readily converted into various functional groups. They play a crucial role in asymmetric reactions. Similarly, nitrones serve as versatile intermediates and participate in dipole cycloaddition reactions, leading to the formation of heterocyclic derivatives [1][2]. The synthesis of nitrones has been explored using different methods [3][4], which depend on the chemical and physical properties of starting materials, catalysts, solvents, and the chemical structure of the desired nitrones [5]. Among the various synthetic approaches, condensation reactions between *N*-hydroxylamine derivatives and aldehydes are recognized as a simple method for accessing nitrones. However, achieving similar conversions with ketones has traditionally been more challenging [6]. Recently, a work by Beauchemin and co-workers shows the intriguing solvent effect of *t*-BuOH, enabling successful condensation at elevated temperatures [7]. Nitrones have emerged as crucial components in the structure of important drugs due to their diverse biological activities, including anticonvulsant, anti-inflammatory, anti-tuberculosis, and antimicrobial properties [8],[9],[10]. Therefore, this study aims to investigate the condensation reactions between phenylhydroxylamine derivatives and glyoxylic acid to obtain nitrone derivatives and to examine the influence of different starting materials on the synthesis of these compounds.

Experiment

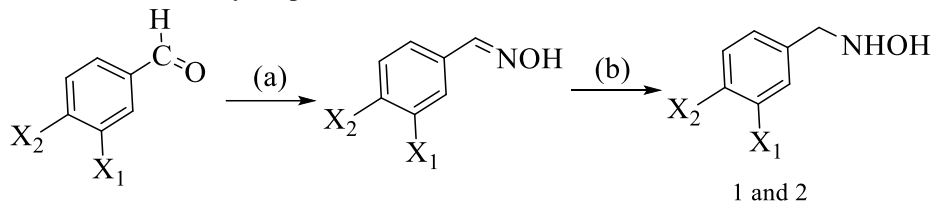
2.1. Materials and Instruments

The materials and instruments utilized in this study consisted of commercially obtained sodium sulfate anhydrous, diethyl ether, petroleum ether (Scharlau), NaOH, KOH (SDFCL), toluene, benzene, dichloromethane, HNO₃, methanol (Romil), and methyl orange (BDH). Hydroxylamine hydrochloride and aldehydes were also acquired from a commercial laboratory. All materials and solvents were used without further purification. The uncorrected melting point of glyoxylic nitrone was determined using a Stuart Scientific melting point apparatus (SMP3). The FT-IR spectra were recorded with a Shimadzu 8300 instrument using the KBr disk at Salahaddin University, Erbil. The ¹H-NMR and ¹³C-NMR spectra were obtained using a 500 MHz and 125 MHz spectrometer, respectively, at the Kurdistan Central Research Center (KCRF) in Iran. TMS was used as an internal reference, and CDCl₃ as a solvent. Chemical shifts were measured in parts per million (ppm) relative to the residual solvent. Coupling constants (*J*) were reported in Hertz.

2.2. The Synthesis of Phenylhydroxylamine Derivatives (1 and 2)[11][12]

General Procedure: (0.034 mol) of NaOH was dissolved in (25 mL) of H₂O, and then (0.015 mol) of one of the substituted benzaldehydes and (0.018 mol) of hydroxylamine hydrochloride were added to the solution. The reaction mixture was stirred for 45 minutes at room temperature. Subsequently, the solution was acidified by adding 10% of aqueous HCl. The resulting mixture was extracted with (3*15) mL of methylene chloride. The organic layer was then dried using anhydrous sodium sulfate sodium (Na₂SO₄) and concentrated by a rotary evaporator.

In the next step, (0.0082 mol) of the obtained material was dissolved in (20 mL) of MeOH and then (0.0064 mol) of NaBH₃CN was added to it. A very small amount of methyl orange was added to the solution as an indicator. The solution was acidified by adding 4N HCl/MeOH until a pink solution was observed. The mixture was stirred for 1.5 hours. Then, MeOH was removed using a rotary evaporator. To the resulting aqueous solution, (20 mL) of H₂O and 4M KOH were added to adjust the pH value to 9. The mixture was extracted with (3*15) mL of CH₂Cl₂. The CH₂Cl₂ layer was dried with Na₂SO₄ and removed via rotary evaporation.



(a)= NaOH, H₂O, NH₂OH.HCl, HCl and CH₂Cl₂.

(b)= NaBH₃CN, MeOH, HCl-MeOH, KOH, H₂O and CH₂Cl₂.

1 = X₁= CH₃ , X₂= CH₃

2 = X₁= OCH₃ , X₂= OCH₃

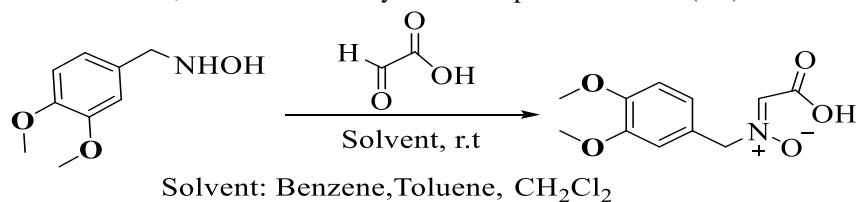
Scheme 1. The Synthesis of Phenylhydroxylamine Derivatives (1and 2)

Table 1- Phenylhydroxylamine Derivatives (1and 2)

Entry	Product No.	Product Name	Yield %
3,4-dimethylbenzaldehyde	1	<i>N</i> -(3,4-dimethylbenzyl)hydroxylamine	93%
3,4-dimethoxybenzaldehyde	2	<i>N</i> -(3,4-dimethoxybenzyl)hydroxylamine	95%

2.3. The Synthesis of Nitron Derivative (3)[11]

In this step, (0.008 mol, 1.46 g) of *N*-(3,4-dimethoxybenzyl)hydroxylamine **2** and (0.012 mol) of glyoxalic acid were dissolved in (20 mL) of a chosen solvent (benzene, toluene, or CH₂Cl₂), each was used separately. The solution was stirred at room temperature overnight, and then the extraction was performed with (2*20 mL) of water. The organic layer was dried using Na₂SO₄, then the solvent was removed via rotary evaporation. The resulting product was subjected to recrystallization from hot ethanol, washed with diethyl ether and petroleum ether (3:1).



Scheme 2. General synthesis of nitron

2.4. The Synthesis of 1-carboxy- *N*-(3,4-Dimethoxybenzyl)methanimine Oxide (3)

Light yellow crystal, m.p= 93-95 °C. IR (KBr/v_{max}/ cm⁻¹): 3002.86 (ArH) stretching, 2960.73 (OH) stretching, 1525.69 (C=N), 1498.69-1446.61 (C=C), 1032.43 (N⁺- O⁻). ¹H-NMR [500 MHz, CDCl₃, δ (ppm)]: 11.55 (s, 1H, H-O-C=O), 7.255 (d, 1H, ArH), 7.15 (d, 1H, ArH), 7.10 (s, 1H, ArH), 4.83 (s, 3H, methoxy H), 4.02 (s, 3H, methoxy H), 2.37 (s, 2H, -CH₂-), 2.39 (s, 1H, H-C-C=O), ¹³C NMR [126 MHz, CDCl₃, δ (ppm)]: 164.56, 139.71, 138.60, 132.08, 130.33, 129.81, 129.33, 128.40, 70.88, 55.07, 21.25.

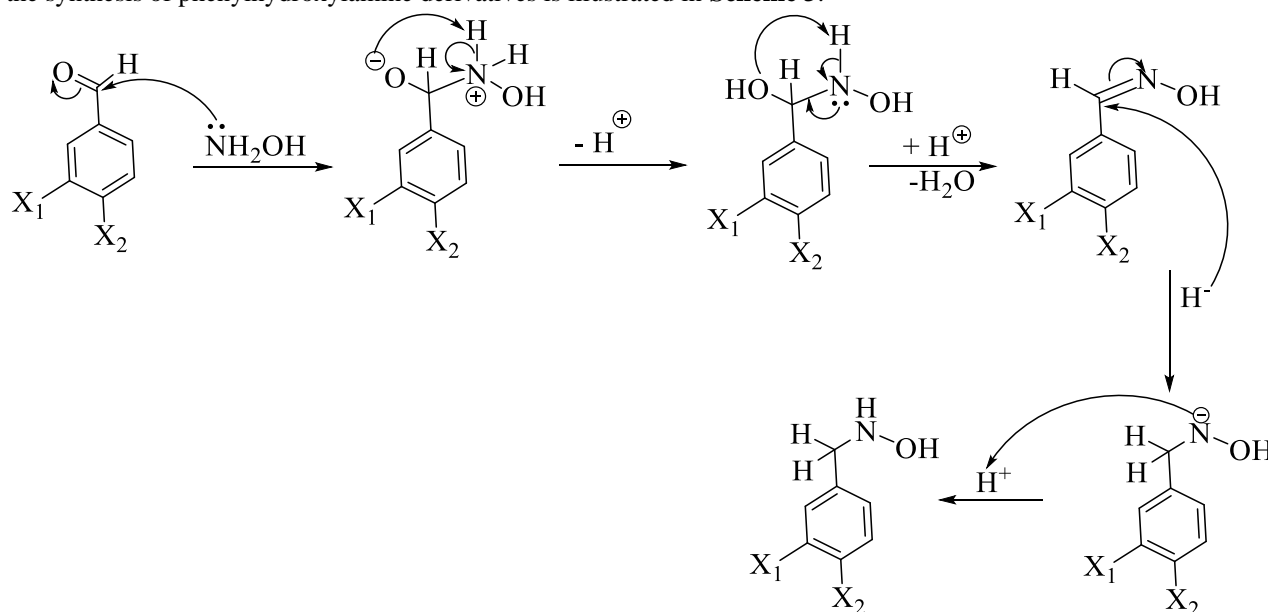
2.5. The Antibacterial and Antifungal Activities of Synthesized Nitron

The synthesized nitron (3) was dissolved in two different concentrations, 800 μg and 400 μg , in 1 mL of dimethyl sulfoxide (DMSO). The antibacterial activity of the nitron at these concentrations was studied towards gram-positive *S. aureus* bacteria and gram-negative *E. coli* bacteria, as well as studying its antifungal activity against *Candida albicans*. The method used for antibacterial activity was agar well diffusion method. First, Muller Hinton Agar (MHA) was heated by autoclave, and then this solution was cooled to 50-55 $^{\circ}\text{C}$ and poured in a regular layer on petri dishes. By using sterilized swabs, *S. aureus* and *E. coli* bacteria were completely streaked on the petri dishes and became solid, and then remained for 30 minutes. After that, in the layers made on agar, four wells with a diameter of 8 mm were cut and (100 μL) of each of two different concentrations of prepared nitron, dimethylsulfoxide and levofloxacin (as a standard drug for comparison with antibacterial activity of nitron) were placed. Petri dishes were incubated at 37 $^{\circ}\text{C}$ for 48 hr, and then the inhibitory zone was measured in mm [13],[14]. The synthesized nitron's antifungal activity was investigated against the *Candida albicans* fungus and its efficacy was compared to that of clotrimazole as a typical drug.

In this experiment, the growth medium was saboraud dextrose. The wells with a diameter of 8 mm were cut and (100 μL) of DMSO. The prepared nitron in two different concentrations (800 and 400 μL), and also the standard drug were placed. The inhibitory zone was measured in mm after a 48-hour incubation period at 37 $^{\circ}\text{C}$ [15].

Results and Discussion

The objective of this research is to create a novel carboxylic nitron compound by employing a conversion of 3,4-dimethoxybenzaldehyde into its corresponding hydroxylamine (see **Scheme 1** and **Table 1**). The general mechanism for the synthesis of phenylhydroxylamine derivatives is illustrated in **Scheme 3**:



Scheme 3. The general mechanism for the synthesis of Phenylhydroxylamine derivatives (1 and 2)

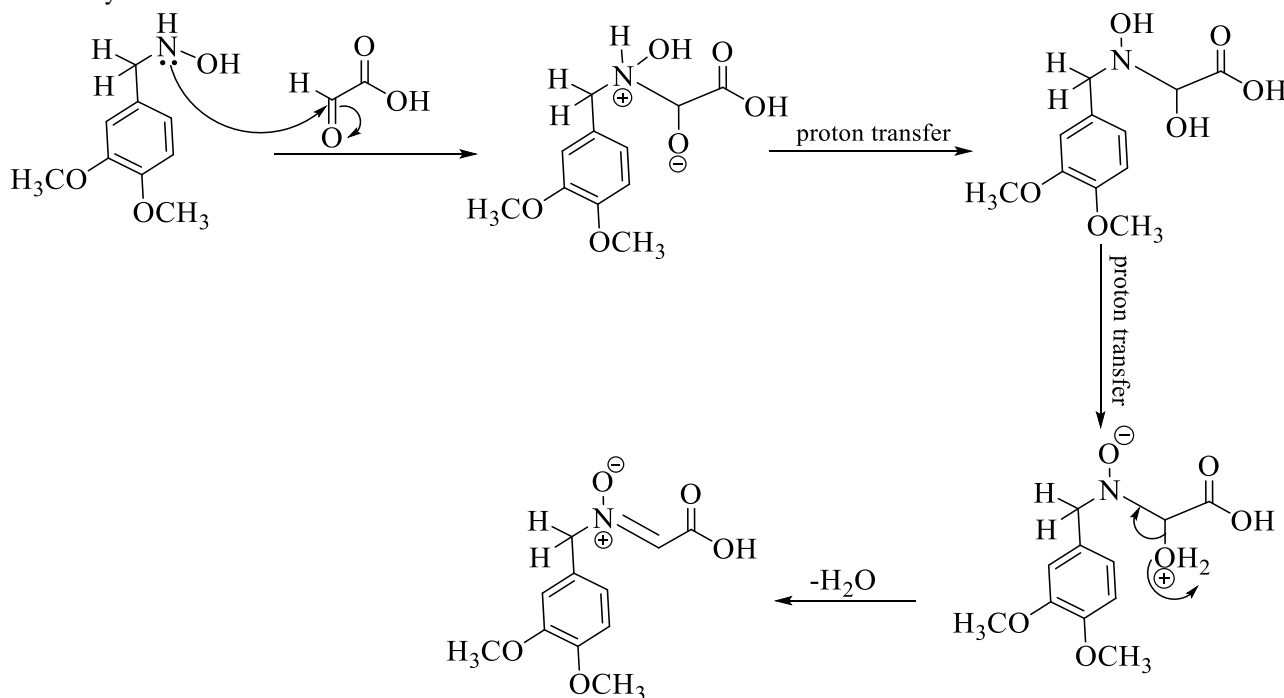
This hydroxylamine will then be utilized in a condensation reaction with glyoxylic acid using various solvents, as illustrated in **Scheme 2** and **Table 2**.

Table 2. benzylhydroxylamine derivatives in condensation reactions with glyoxylic acid

Name	Carbonyl compound	Solvent	Yield%
<i>N</i> -(3,4-dimethoxybenzyl)hydroxylamine	glyoxylic acid	dichloromethane	87
<i>N</i> -(3,4-dimethoxybenzyl)hydroxylamine	glyoxylic acid	benzene	81
<i>N</i> -(3,4-dimethoxybenzyl)hydroxylamine	glyoxylic acid	toluene	83

The requirement for the synthesis of carboxylic nitrones in this method was the condensation reaction between phenylhydroxylamine and aldehyde. According to the research done by John August [11] and shown in (**Scheme 1** &

Scheme 2), the condensation reaction between phenylhydroxylamine and the aldehyde part of glyoxylic acid to prepare the nitrones was necessary. **Scheme 4** provides a demonstration of the general mechanism involved in synthesizing of carboxylic nitron:



Scheme 4. The General mechanism for the synthesis of carboxylic nitron (3)

The FT-IR spectrum provided important absorption bands for the identification of glyoxylic nitron (1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide) (3). Specifically, the absorption bands at 1032.43 cm^{-1} for the ($\text{N}^+\text{-O}^-$) bond, 1525.69 cm^{-1} for the ($\text{C}=\text{N}$) bond and 1737.8 cm^{-1} for ($\text{C}=\text{O}$) group were significant. Furthermore, the disappearance of the secondary amine's signal, and the presence of an absorption band at 3320 cm^{-1} for the (OH) of carboxylic group indicated that the carboxylic part of glyoxylic acid did not undergo any reaction and remained unchanged (Figure 1).

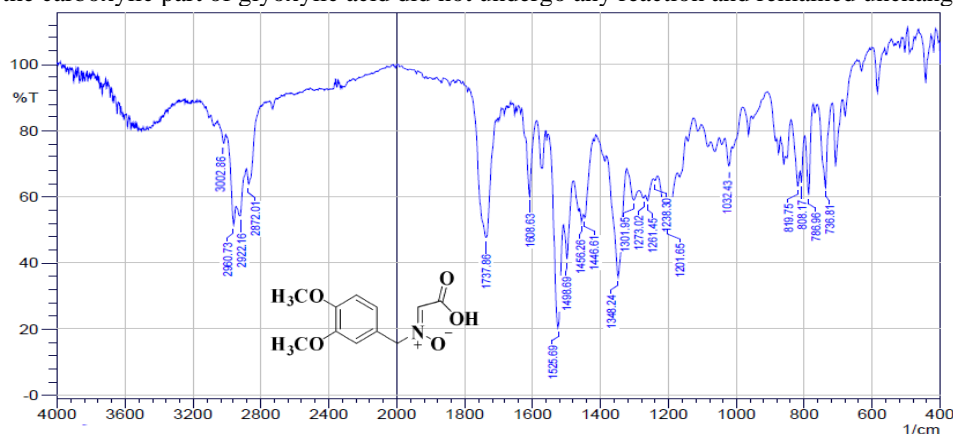


Fig. 1: FT-IR of 1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide (3)

In $^1\text{H-NMR}$, the presence of a singlet signal at (11.5) ppm, which is related to ($\text{H-O-C}=\text{O}$) proton in the carboxylic part of the nitron, showed that the carboxylic moiety of glyoxylic acid remained without any reaction. It also demonstrated the reaction between *N*-(3,4-dimethoxybenzyl)hydroxylamine and the aldehyde moiety of glyoxylic acid. Moreover, the protons of the two methoxy groups (4.84 and 4.02 ppm) displayed two singlet signals. Finally, the protons at ($\text{CH}_2\text{-N}^+\text{-O}^-$) showed two singlet signals at 2.37 and 2.29. With these protons signals, all aromatic and aliphatic proton signals appeared for the synthesized nitron (1-carboxy-*N*-(3,4-dimethoxybenzyl) methanimine oxide) (Figure 2) [2],[11].

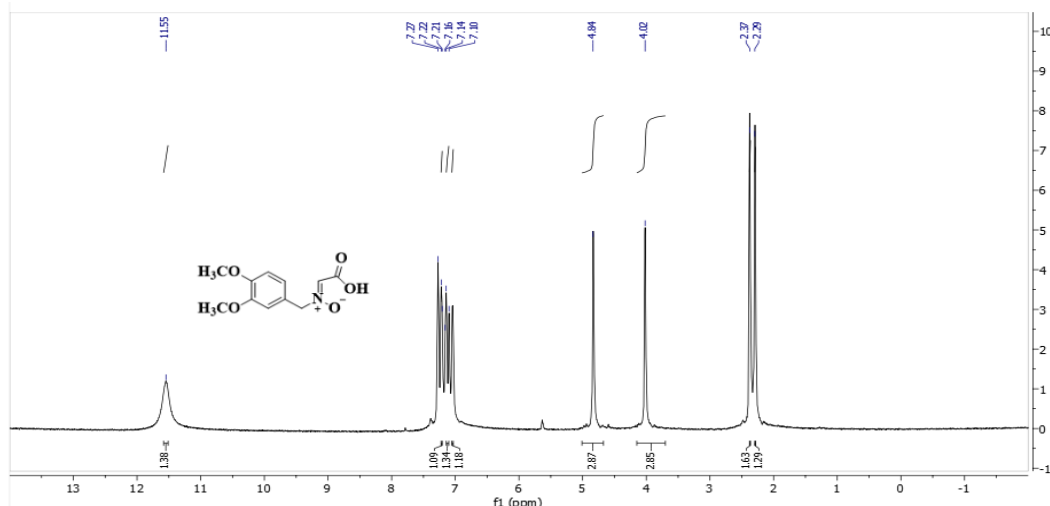


Fig. 2: $^1\text{H-NMR}$ of 1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide (3)

In $^{13}\text{C-NMR}$, the signal corresponding to the carbon in the carboxylic part of the nitron appeared clearly at (164.58) ppm, while the signal corresponding to the carbon in aldehyde in the glyoxylic nitron disappeared. Moreover, the $^{13}\text{C-NMR}$ spectrum of this compound illustrated these results measured by ppm: 139.7 ($\text{CO}_2\text{H-C}$), 138.6 ($\text{H}_3\text{CO-C}$), 132.0 ($\text{H}_3\text{CO-C}$), 55.0 (OCH_3), 134.3 ($\text{N}=\text{CH}_2$), 130.3 (Ar quat.C), 129.8 (ArCH), 129.2 (ArCH), 128.4 (ArCH). Therefore, these results are crucial for the confirmation of the reaction between *N*-(3,4-(dimethoxybenzyl)hydroxylamine and the aldehyde moiety of glyoxylic acid (Figure 3).

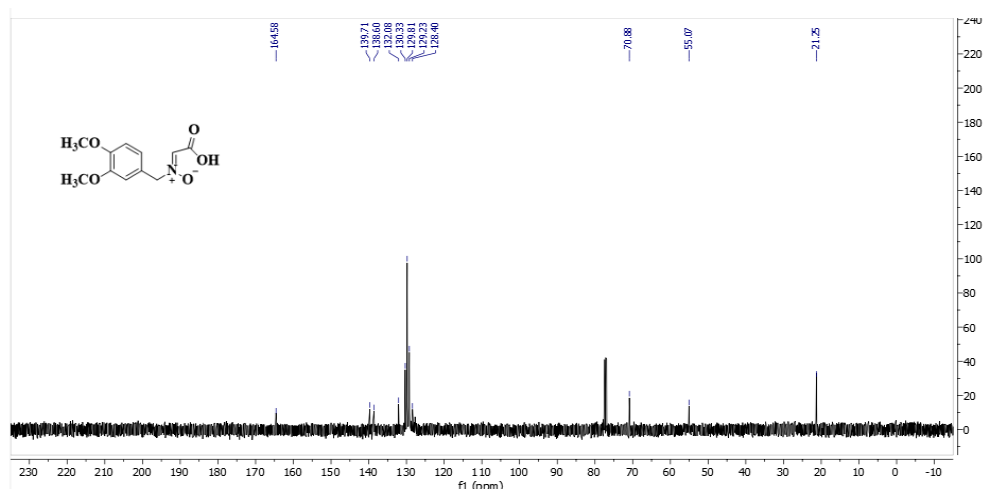


Fig. 3: $^{13}\text{C-NMR}$ of 1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide (3)

The findings of investigating the antibacterial and antifungal activities of synthetic glyoxylic nitron against *Candida albicans* fungus and *E. coli* and *S.aureus* bacteria are presented in Table 3.

Table 3. The antimicrobial activity of 1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide

Microorganism	Inhibition zone (mm) for 800 $\mu\text{g}/ 1 \text{ mL}$ (DMSO)	Inhibition zone (mm) for 400 $\mu\text{g}/ 1 \text{ mL}$ (DMSO)
<i>S.aureus</i> bacteria	9	7
<i>E.coli</i> bacteria	7	7
<i>Candida albicans</i> fungi	24	22
Levofloxacin's 29 mm inhibition zone against <i>S.aureus</i> bacteria		
Levofloxacin's 28 mm inhibition zone against <i>E. coli</i> bacteria		
Clotrimazole's 25 mm inhibition zone against <i>Candida albicans</i> fungi		

The activity of this nitrone toward *E. coli* bacteria was weak in both concentrations and its activity against *S. aureus* bacteria was better in higher concentration when compared to levofloxacin. However, the activity of this nitrone against *Candida albicans* fungus was good when compared to clotrimazole in both concentrations. In general, its activity as antifungal was better than antibacterial (Figure 4).

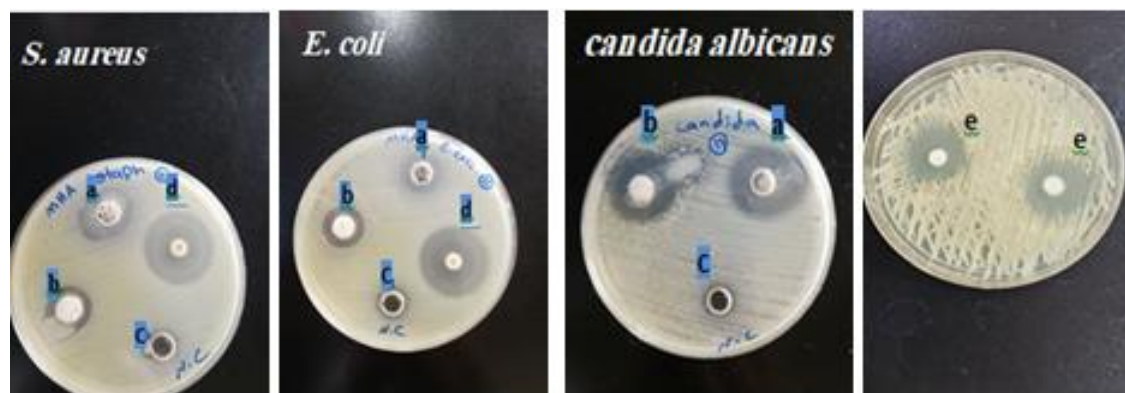


Fig. 4: The antibacterial and antifungal activities of nitrone toward *S.aureus* and *E.coli* bacteria and *Candida albicans* fungi

a: 800 µg nitrone in 1mL DMSO.

b: 400 µg nitrone in 1mL DMSO.

c: DMSO

d: Levofloxacin

e: Clotrimazole.

Conclusion

In conclusion, the experimental data obtained from the FT-IR, ¹H-NMR and ¹³C-NMR spectra confirmed the structure of 1-carboxy-N-(3,4-dimethoxybenzyl)methanimine oxide. Regarding the biological activities, the synthesized carboxylic nitrone demonstrated superior antifungal activity against *Candida albicans* fungus compared to its antibacterial activity against *E.coli* and *S.aureus* bacteria. The biological properties of the synthesized compound can be attributed to the presence of the carboxylic nitrone group, which aligns with the findings reported in literature [11].

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