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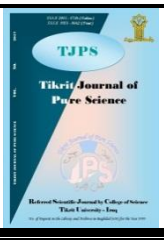

The Synthesis of Some Imine Derivatives *via* Condensation Reactions between Some Aromatic Phenylhydroxylamine Derivatives with Glyoxylic Acid and the Study of their biological activity

Jihad Haji Mohammed[1], Akram Noori Mohammed Qaddo[2], Nabaz Abdulmajeed Muhammad Salih Perxdry[1*], Faeza Burhan Omar[3]

[1]Department of Chemistry, Faculty of Science, Soran University, Kurdistan Region, Iraq
[2]Department of Chemistry, Education College for Girls, University of Mosul, Iraq
[3]Department of Biology, Faculty of Science, Soran University, Kurdistan Region, Iraq

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Corresponding Author*:
Nabaz Abdulmajeed Muhammad
nabaz.mohammad@soran.edu.iq

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 glyoxalic acid was accomplished to obtain the desired carboxylic nitrone. The FT-IR, ^{1}H-NMR, and ^{13}C-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 nitrone showed a significant antimicrobial activity compared to the standard drugs.

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تحضير بعض مشتقات الإيمين من خلال تفاعلات تكثيف بعض مشتقات فينيل هيدروكسيل أمين الأروماتية مع حامض الكلايوكسيليك ودراسة فعاليتهم البايولوجية

جهاد حاجي محمد[1]، اكرم نوري محمد قدو[2]، نەبەز عبدالمجيد محمد صالح پيرخدري[1]*، فائزة برهان عمر[3]

[1]قسم الكيمياء ، كلية العلوم، جامعة سوران، إقليم كردستان العراق

[2]قسم الكيمياء ، كلية التربية للبنات، جامعة الموصل، العراق

[3]قسم علوم الحياة، كلية العلوم، جامعة سوران ، إقليم كردستان العراق

الملخص

تضمنت هذه الدراسة تحويل 4،3-ثنائي مثيل بنزالديهايد و 4،3-ثنائي ميثوكسي بنزالديهايد إلى الاوكزيمات المقابلة. ثم بعد ذلك، تم إجراء تفاعل التكثيف بين *N*-(4،3-ثنائي ميثوكسي بنزيل)هيدروكسيل امين مع حامض الكلايوكسيليك للحصول على كاربوكسيليك نايترون. تم استخدام تقنيات التحليل الطيفي FT-IR و ^{1}H-NMR و ^{13}C-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 نشاطًا كبيرًا كمضاد للميكروبات مقارنة بالأدوية القياسية.

الكلمات المفتاحية: تفاعلات التكثيف، حامض الكلايوكسيليك، إيمين، الفعالية البايولوجية.

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

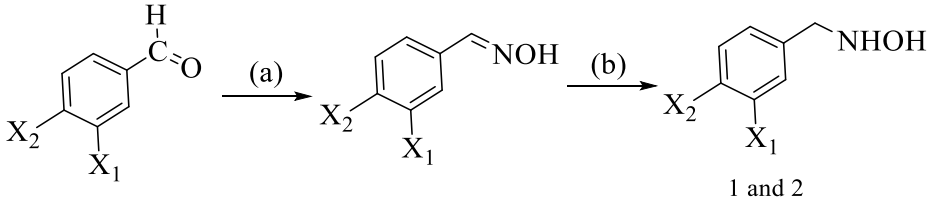
2.1. Materials and Instruments

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, HNO_3, 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 ^{1}H-NMR and ^{13}C-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 $CDCl_3$ 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 H_2O, 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 (Na_2SO_4) 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 $NaBH_3CN$ 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 H_2O and 4M KOH were added to adjust the pH value to 9. The mixture was extracted with (3*15) mL of CH_2Cl_2. The CH_2Cl_2 layer was dried with Na_2SO_4 and removed via rotary evaporation.



1 and 2

(a)= NaOH, H_2O, $NH_2OH.HCl$, HCl and CH_2Cl_2.
(b)= $NaBH_3CN$, MeOH, HCl-MeOH, KOH, H_2O and CH_2Cl_2.
1 = X_1= CH_3 , X_2= CH_3
2 = X_1= OCH_3 , X_2= OCH_3

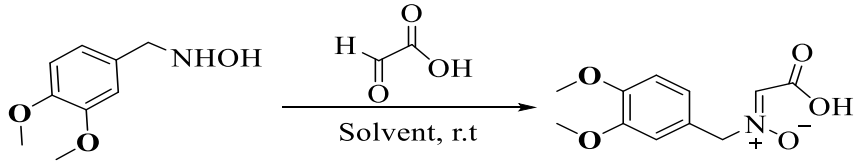
Scheme 1. The Synthesis of Phenylhydroxylamine Derivatives (1and 2)

Table 1- Phenylhydroxylamine Derivatives (1and 2)

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

2.3. The Synthesis of Nitrone 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 CH_2Cl_2), 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 Na_2SO_4, 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).



Solvent: Benzene,Toluene, CH_2Cl_2

Scheme 2. General synthesis of nitrone

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

Light yellow crystal, m.p= 93-95 ^oC. 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^-). ^{1}H-NMR [500 MHz, $CDCl_3$, 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, -CH_2-), 2.39 (s, 1H, H-C-C=O), ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)]: 164.56, 139.71, 138.60, 132.08, 130.33, 129.81, 129.33, 128.40, 70.88, 55.07, 21.25.

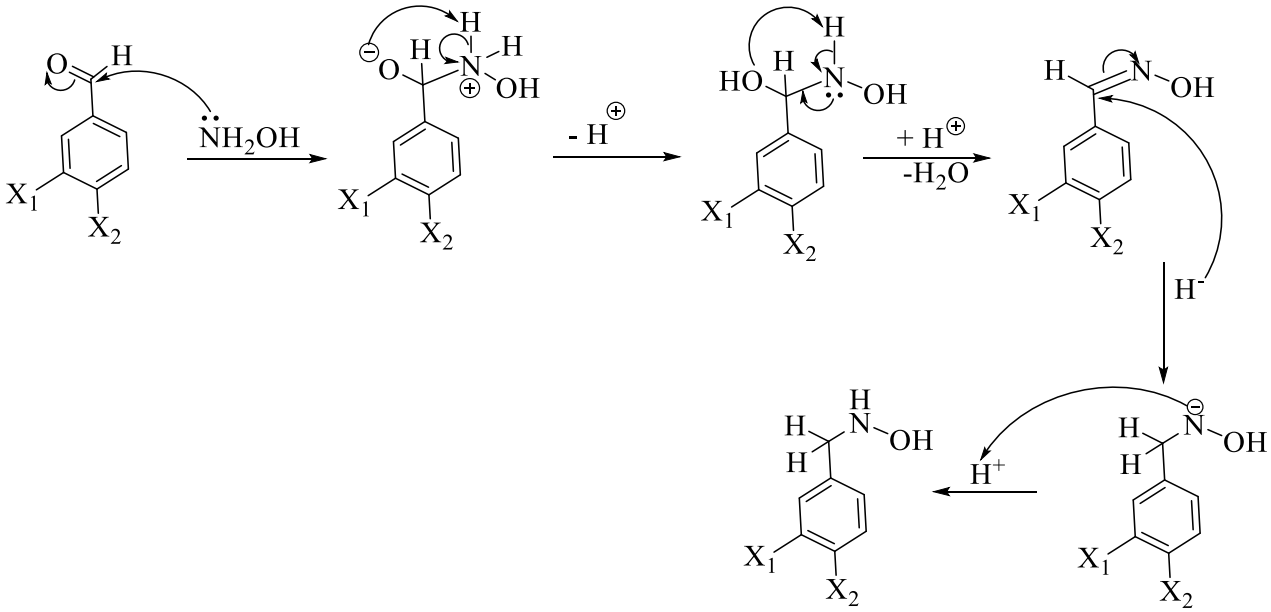
2.5. The Antibacterial and Antifungal Activities of Synthesized Nitrone

The synthesized nitrone (3) was dissolved in two different concentrations, 800 µg and 400 µg, in 1 mL of dimethyl sulfoxide (DMSO). The antibacterial activity of the nitrone 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 °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 nitrone, dimethylsulfoxide and levofloxacin (as a standard drug for comparison with antibacterial activity of nitrone) were placed. Petri dishes were incubated at 37°C for 48 hr, and then the inhibitory zone was measured in mm [13],[14]. The synthesized nitrone'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 nitrone 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°C [15].

Results and Discussion

The objective of this research is to create a novel carboxylic nitrone 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**:



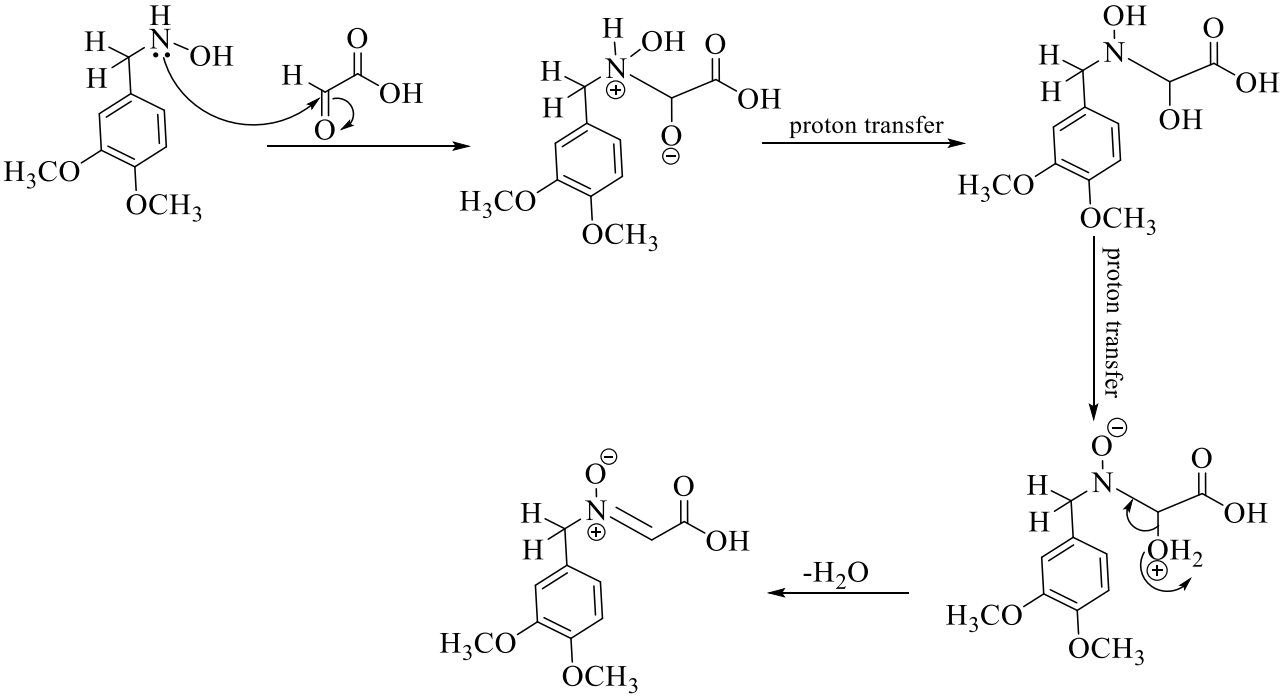
Scheme 3. The general mechanism for the synthesis of Phenylhydroxylamine derivatives (1 and 2)

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%
N-(3,4-dimethoxybenzyl)hydroxylamine	glyoxylic acid	dichloromethane	87
N-(3,4-dimethoxybenzyl)hydroxylamine	glyoxylic acid	benzene	81
N-(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 nitrone:



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

The FT-IR spectrum provided important absorption bands for the identification of glyoxylic nitrone (1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide) (**3**). Specifically, the absorption bands at 1032.43 cm^{-1} for the (N^{+}-O^{-}) bond, 1525.69 cm^{-1} for the (C=N) bond and 1737.8 cm^{-1} for (C=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).

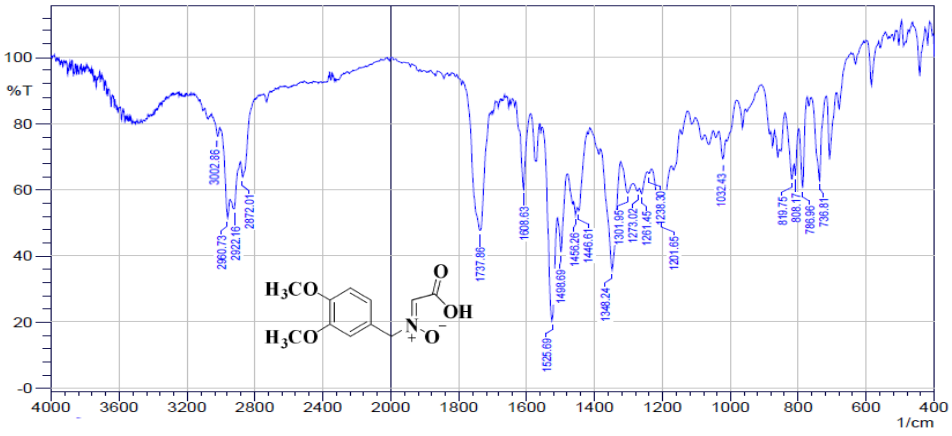


Fig. 1: FT-IR of 1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide (3)

In ^{1}H-NMR, the presence of a singlet signal at (11.5) ppm, which is related to (H-O-C=O) proton in the carboxylic part of the nitrone, 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 (CH_2-N^{+}-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 nitrone (1-carboxy-*N*-(3,4-dimethoxybenzyl) methanimine oxide) (Figure 2) [2],[11].

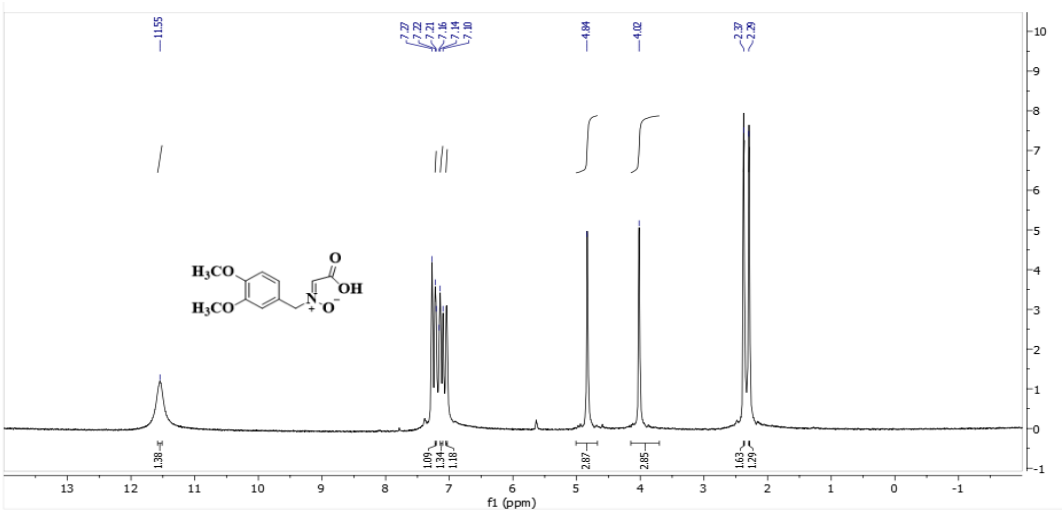


Fig. 2: ^{1}H-NMR of 1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide (3)

In ^{13}C-NMR, the signal corresponding to the carbon in the carboxylic part of the nitrone appeared clearly at (164.58) ppm, while the signal corresponding to the carbon in aldehyde in the glyoxylic nitrone disappeared. Moreover, the ^{13}C-NMR spectrum of this compound illustrated these results measured by ppm: 139.7 (CO_2H-*C*), 138.6 (H_3CO-*C*), 132.0 (H_3CO-*C*), 55.0 (OCH_3), 134.3 (N=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).

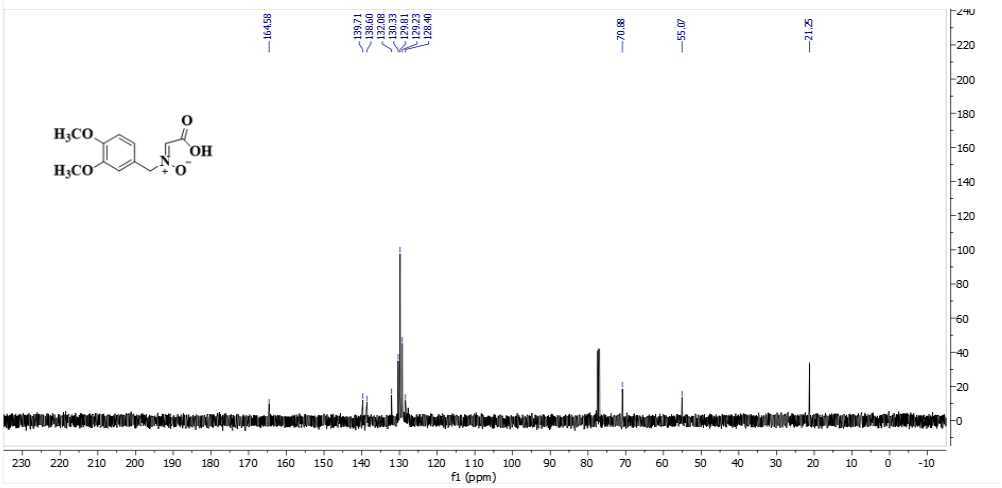


Fig. 3: ^{13}C-NMR of 1-carboxy-*N*-(3,4-dimethoxybenzyl)methanimine oxide (3)

The findings of investigating the antibacterial and antifungal activities of synthetic glyoxylic nitrone 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 µg/ 1 mL (DMSO)	Inhibition zone (mm) for 400 µg/ 1 mL (DMSO)
S.aureus bacteria	9	7
E.coli bacteria	7	7
Candida albicans fungi	24	22
Levofloxacine's 29 mm inhibition zone against *S.aureus* bacteria Levofloxacine's 28 mm inhibition zone against *E. coli* bacteria Clotrimazole's 25 mm inhibition zone against *Cadida albicans 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).

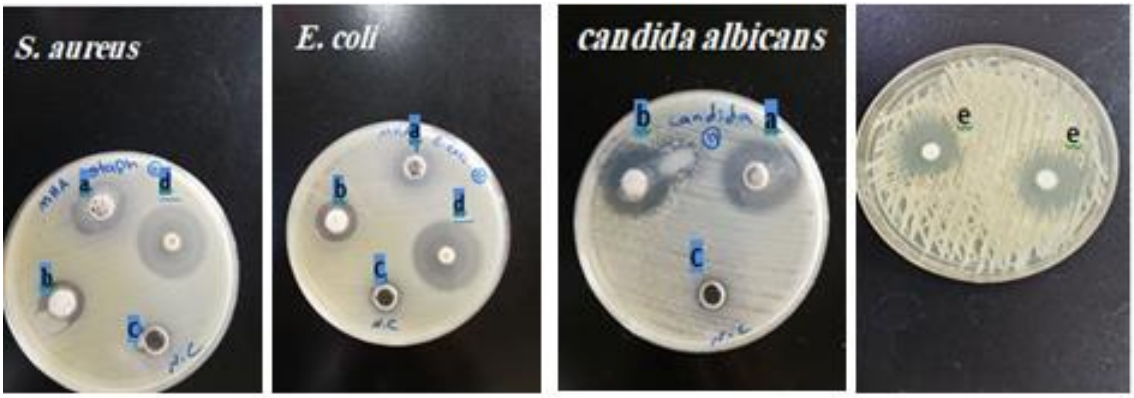


Fig. 4: The antibacterial and antifungal activities of nitrone toward *S.aureus* and *E.coli* bacteria and *Candida albicans* fungi

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, ^{1}H-NMR and ^{13}C-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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