Available online at sjuoz.uoz.edu.krd

Science Journal of University of Zakho
Vol. 11, No. 2, pp. 160 – 169, April-June 2023

journals.uoz.edu.krd

p-ISSN: 2663-628X
e-ISSN: 2663-6298

SYNTHESIS AND CHARACTERIZATION OF SOME NEW NITRONES DERIVATIVES AND SCREENING THEIR BIOLOGICAL ACTIVITIES

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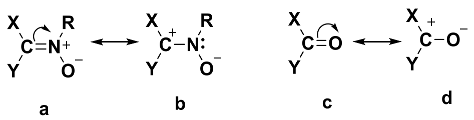
Received:5 Mar..,2023 /Accepted: 15 Mar.,2023/Published:8 Apr.,2023 https://doi.org/10.25271/sjuoz.2023.11.2.1149

ABSTRACT:
Synthetic approached towards the synthesis of some novel nitrones derivatives have been started with reduction of nitrobenzene derivatives as starting material bearing electron withdrawing and electron donating groups to corresponding phenylhydroxylamine in presence of zinc dust as reducing agent in aqueous solution of ammonium chloride (NH4Cl). The prepared phenylhydroxylamine derivatives were reacted with different substituted benzaldehydes to give the target derivatives of nitrone. The structures of the synthesized nitrones were characterized by spectroscopic methods FT-IR, 1H-NMR and 13C NMR. Finally, the newly synthesized compounds were screened for their microorganism activities at different concentration, and inhibited growth of *Escherichia coli* (*E. coli*) Gram negative, *Staphylococcus aureus* (*S. aureus*) Gram positive, and fungi (*candida albicans*).
KEYWORDS: Phenylhydroxylamines, Nitro Compound Derivatives; Electron Withdrawing Groups; Electron Donating Groups Biological active compounds;

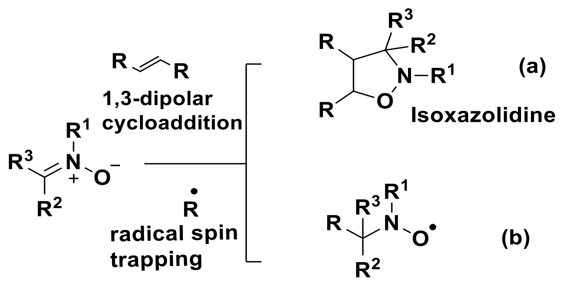
INTRODUCTION

The name of nitrone, which is abbreviation for nitrogen ketone, was proposed by P. Fieffer in 1916 to emphasize the similarity of these compounds to ketones. The reason for these similarities was the mesomeric effect, which was similar in both nitrones and ketones. The polarization of nitrones depends on the substitutions in their structures. The presence of different types of R, X or Y substitutions can change their polarity (Delpierre & Lamchen, 1965; Ferraz *et al*., 2017) (scheme 1).



Scheme 1- General structure of nitrones (a and b) and ketones (c and d)

They are important substances that are widely used in organic synthesis. These substances are also important synthetic intermediates. Several nitrones have been found as essential components in the structure of important drugs (Cai *et al*., 2021; Salman & Majeed, 2013; Thakur *et al*., 2021). These compounds have an important role in trapping free radicals in the body (Besson *et al*., 2019; Deletraz *et al*., 2020; Floyd *et al*., 2002; Janzen & Blackburn, 1968; Jung *et al*., 2021). In addition, they have been used in intermolecular cycloadditions and 1,3-dipolar cycloadditions that were converted to isoxazolidines by reaction with alkenes (scheme 2) (Mutlaq *et al*., 2021).



Scheme 2- Nitrone reactions in 1,3-dipolar cycloaddition and radical spin trapping

Their activities against bacteria and fungi are interesting. They also have used as anticonvulsant and anti-tuberculosis (Al Adhreai *et al*., 2022; Ibrahim *et al*., 2012; Salman, 2019).
Various methods have been used for the synthesis of nitrone derivatives (Murahashi & Imada, 2019), but their preparation by condensation reaction between derivatives of N-monosubstituted hydroxyamines and different substituted aldehydes or ketones is the most common method (Mahieddine *et al*., 2016; West & Davis, 1989).
The aim of the present study includes the synthesis of some new biologically active nitrone derivatives, because, the development of novel antimicrobial drugs is still in demand as there is increasing resistance of microorganisms to currently available antimicrobial drug.

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MATERIALS AND METHODS

The substances and solvents used in this study as well as the characterization were as follows:

NH_4Cl, sodium sulphate anhydrous and zinc dust (SDFCL), diethyl ether (Scharlau), ethyl acetate (Licrosolv), ethanol (Hongwell), and all aldehyde and nitro compounds were obtained from commercial lab in China. All materials and solvents were utilized without purification.

The progress of the reactions and purity of the synthesized nitrones were monitored by thin layer chromatography (TLC) plate (60F-254-Buchs, Switzerland) in which the aluminum plate was pre-coated with silica gel. Ethyl acetate and toluene (3:1) were used as the developing solvent, and the results were observed by UV light.

^{1}H-NMR and ^{13}C NMR characterization were done by 500 and 126 MHz (Ascend) respectively in Kurd Central Research Facilities (KCRF) in Iran. FT-IR spectrometer was determined by Shimadzu, KBr disk in Salahaddin University-Erbil. Melting points were also measured in Salahaddin University-Erbil by Stuart Scientific melting point apparatus (SMP3).

2.1. Synthesis of phenylhydroxylamine derivatives

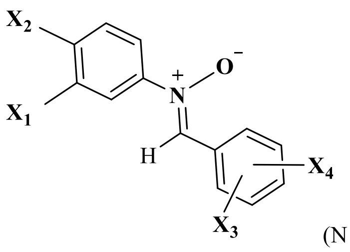
All procedures were modified and derived from (Mahieddine *et al*., 2016; West & Davis, 1989).

In a (500 mL) beaker was added (0.033 mol) of substituted nitrobenzene to (240 mL) distilled water, then added (0.087 mol) of Zn dust and stirring for (20) minutes. The temperature was in the ranges of (60-65) °C. At the end of 20 minutes, the solution was stirred for another 15 minutes. The mixture was filtered and washed with hot distilled water. The filtrate was supersaturated with NaCl and kept in an ice container for 2 hr in a dark place, then extracted with (3*70 mL) of diethyl ether.

Diethyl ether part dried with sodium sulphate anhydrous, and then evaporated to obtain the desired corresponding phyneylhydroxylamine. The product was unstable and was put directly into the next reaction.

2.2. Procedure A: Synthesis of Nitrone (N1-N8)

In a (50 mL) round bottom flask, a mixture of (0.0046 mol) of phenylhydroxyamine derivatives and (0.0046 mol) of one of the substituted benzaldehydes in (15 mL) of the pure ethanol was refluxed for 2-3 hr in the range of (50-55) °C. The progress of the reaction was monitored by TLC plate. Then the mixture was stirred at r.t in the dark for 18 hr. The desired product (figure 1), was filtered, recrystallized with ethanol, and then washed with diethyl ether.



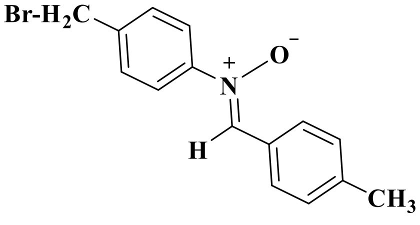
(N1-N8)

N1 : X_1= Cl, X_2=Cl, X_3= H, X_4= H
N2 : X_1 = Cl, X_2= Cl, X_3= p-CH_3, X_4= H
N3 : X_1 = Cl, X_2= Cl, X_3= o-NO_2, X_4= H
N4 : X_1 **= Cl,** X_2= Cl, X_3 = m-(OCH_3), X_4= p-(OCH_3)
N5 : X_1= CH_3, X_2=CH_3, X_3= H, X_4= H
N6 : X_1 = CH_3, X_2= CH_3, X_3= p-CH_3, X_4= H
N7 : X_1 = CH_3, X_2= CH_3, X_3= o-NO_2, X_4= H
N8 : X_1 **=** CH_3**,** X_2= CH_3, X_3 = m-(OCH_3), X_4= p-(OCH_3)

Figure 1: Structure of synthesized nitrones (N1-N8)

2.3. Procedure B: Synthesis of nitrone (N9)

In a (100 mL) conical flask, (0.0046 mol) of 4-nitrobenzyl bromide with (35 mL) of ethanol 85% and (0.0046 mol) of NH_4Cl were mixed. The temperature was set in the range of (7-10) °C, and then (0.0184 mol) of zinc dust was added gradually over 2 hr with stirring. After the addition was complete, the mixture was filtered and 3-methylbenzaldehyde was added to the filtrate and then refluxed in the range of (50-55) °C. The progress of the reaction was monitored by TLC plate, and after 2 hr the mixture was stirred overnight in the dark at r.t. The desired nitrone (figure 2) was filtered, and then recrystallized with ethanol and washed with diethyl ether.



N9

Figure 2Structure of N-(4-(bromomethyl) phenyl)-1-(p-tolyl) methanimine oxide

2.4. -(3,4-dichlorophenyl)-1-phenylmethanimine oxide (N1)

White crystal, m.p= 140-141 ^{0}C, yield= 65%, R_f= 0.64

IR (KBr/ v_{max}/ cm^{-1}): 3099.61 (Ar-H) stretching, 1577.77 (C=N), 1463.97-1546.91 (C=C), 1068.56 (N^+-O^-), 761.88 (Ar-H) out-of-plane/ bending. ^{1}H-NMR [500 MHz, $CDCl_3$, δ (ppm)]: 8.3701(s, 1H, H-C_{12}), 7.9306 (s, 1H, H-C_5), 7.9266 (d, 1H, H-C_2), 7.8962 (d, 1H, H-C_1), 7.4748-7.6387 (m, 5H, H-C_{16}, H-C_{15} and H-C_{17}, H-C_{14} and H-C_{18}) H-C_{15} and H-C_{17} have a same chemical shifting, ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)]: 147.79, 134.82, 134.17, 133.32, 131.51, 130.77, 130.17, 129.25, 128.77, 123.93, 120.81.

2.5. N

2.6. N-(3,4-dichlorophenyl)-1-(p-tolyl)methanimine (N2)

White, m.p = 165-167 ^{0}C, yield=56.0 % , R_f = 0.61

IR (KBr/ v_{max}/ cm^{-1}): 3099.61 (Ar-H) stretching, 2835.66 aliphatic (C-H), 1575.84 (C=N), 1544.98-1415.75, (C=C), 1070.49 (N^+-O^-), 725.23 (Ar-H) out-of-plane/ bending. ^{1}H-NMR [500 MHz, $CDCl_3$, δ (ppm)]: 8.2898 (s, 1H, H-C_{12}), 8.0305-7.7869 (m, 4H, H-C_{14}, H-C_{15}, H-C_{17}, H-C_{18}), 7.7548-

7.3017 (m, 3H, H-C_1, H-C_2, H-C_5), 2.4286 (s, 3H, H-C_{19}). ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)]: 163.05, 134.87, 134.01, 133.28, 130.75, 130.72, 129.59, 129.35, 127.55, 123.91, 120.86, 21.93.

2.7. **N-(3,4-dichlorophenyl)-1-(2-nitrophenyl) methanimine oxide (N3)**

Yellow powder, m.p= 117-119 ^0C, yield= 74%, Rf= 0.71

IR (KBr/ v_{max}/ cm^{-1}): 3097.68 (Ar-H) stretching, 1568.13 (C=N), 1568.13-1419.61(C=C), 1078.43 (N^+-O^-), 731.02 (Ar-H) out-of-plane/ bending. ^1H-NMR [500 MHz, $CDCl_3$, δ (ppm)]: 8.5897 (d, 1H, H-C_{18}), 8.5781 (s,1H, H-C_{12}), 8.4090 (d, 1H, H-C_1), 8.4067 (d, 1H, H-C_{15}), 8.1339 (t, 1H, H-C_{16}), 7.9961 (t,1H, H-C_{17}), 7.5387 (d, 1H, H-C_2), 7.5299 (s, 1H, H-C_5). ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)]: 147.85, 147.52, 135.06, 133.66, 133.65, 130.98, 130.92, 129.52, 128.80, 125.19, 124.13, 124.06, 120.81.

2.8. **N-(3,4-dichlorophenyl)-1-(3,4-dimethoxyphenyl)methanimine oxide (N4)**

yellow powder, m.p=78-80 ^0C, yield= 49%, R_f= 0.57

IR (KBr/ v_{max}/ cm^{-1}): 3080.32 (Ar-H) stretching, 2935.66 and 2833.43 aliphatic (C-H), 1587.42 (C=N), 1575.84-1456.26 (C=C), 1070.64 (N^+-O^-), 740.67 (Ar-H) out-of-plane/ bending. ^1H-NMR [500 MHz, $CDCl_3$, δ (ppm)]: 8.4517 (s,1H, H-C_{12}), 7.9204 (s,1H, H-C_5), 7.8333 (s, 1H, H-C_{18}), 7.6338 (d, 1H, H-C_1), 7.5028 (d, 1H, H-C_2), 6.9198 (d, 2H, H_{14}, H-C_{15}), 3.9409 (s, 3H, H-C_{20} in methoxy), 3.9532 (s, 3H, H-C_{20} in methoxy). ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)]: 151.68, 148.54, 147.52, 134.85, 133.84, 133.26, 130.73, 124.52, 123.66, 123.41, 120.57, 111.16, 110.73, 55.99.

So far, in practical work, nitrobenzene containing electron withdrawing groups (two chlorine groups) have been studied, and from this section to 2.11 the nitrobenzene with electron donating groups (two methyl groups) have been considered.

2.9. **N-(3,4-dimethylphenyl)-1-phenylmethanimine oxide (N5)**

Pale yellow powder, m.p=66-68 ^0C, yield= 69.3%, R_f= 0.76.

IR (KBr/ v_{max}/ cm^{-1}): 3064.89 (Ar-H) stretching, 2839.22 Aliphatic (C-H), 1581.63 (C=N), 1517.98-1456.26 (C=C), 1078.14 (N^+-O^-), 775.38 (Ar-H) out-of-plane/ bending. ^1H-NMR [500 MHz, $CDCl_3$, δ (ppm)]: 8.3888 (d,1H, H-C_1), 7.8911 (s,1H, H-C_{12}), 7.8525 (s, 1H, H-C_5), 7.5719-7.4603 (m, 5H, H-C_{14}, H-C_{15}, H-C_{16}, H-C_{17}, H-C_{18}), 7.2052 (d, 1H, H-C_2), 2.3301 (s, 3H, H-C_8), 2.3108 (s, 3H, H-C_9). ^{13}C NMR [300 MHz, $CDCl_3$, δ (ppm)]: 147.02, 138.83, 137.76, 134.21, 130.81, 130.461, 130.03, 129.02, 128.61, 122.73, 118.79, 19.91, 19.53.

2.10. **N-(3,4-dimethylphenyl)-1-(p-tolyl)methanimine oxide (N6)**

Yellow crystal, m.p=101-102 ^0C, yield= 61.9%, R_f= 0.73.

IR (KBr/ v_{max}/ cm^{-1}): 3022.45 (Ar-H) stretching, 2976.16-2918.30 Aliphatic (C-H), 1598.99 (C=N), 1564.27-1417.68 (C=C), 1070.49 (N^+-O^-), 768.32 (Ar-H) out-of-plane/ bending. ^1H-NMR [500 MHz, $CDCl_3$, δ (ppm)]: 8.2928 (d,2H, H-C_{14}, H-C_{18}) 7.8429 (s, 1H, H-C_{12}), 7.5725 (s, 1H, H-C_5), 7.4646 (d,1H, H-C_1) 7.2889 (d, 2H, H-C_{15}, H-C_{17}), 7.1872 (d, 1H, H-C_2), 2.4035 (s, 3H, H-C_{19}), 2.3196 (s, 3H, H-C_8), 2.2989 (s, 3H, H-C9). ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)]: 147.03, 141.25, 138.58, 137.67, 134.04, 129.97, 129.57, 129.31, 129.06, 128.26, 122.69, 118.72, 21.76, 19.90, 19.51.

2.11. **N-(3,4-dimethylphenyl)-1-**

(2-nitrophenyl)methanimine oxide (N7) yellow powder, m.p=116-118 ^0C, yield= 81%, R_f= 0.72.

IR (KBr/ v_{max}/ cm^{-1}): 3032.10 (Ar-H) stretching, 2935.66-2914.44 Aliphatic (C-H), 1564.27 (C=N), 1516.05 (C=C), 1072.42 (N^+-O^-), 790.81(Ar-H) out-of-plane/ bending. ^1H-NMR [500 MHz, $CDCl_3$, δ (ppm)]: 8.5507 (s,1H, H-C_{12}), 8.0700 (d,1H, H-C_1), 7.7466 (t, 1H, H-C_{17}), 7.5529 (d, 1H, H-C_{15}), 7.4973 (d, 1H, H-C_{18}), 7.2402 (t,1H, H-C_{16}), 7.2322 (d, 1H, H-C_2), 7.1063 (s,1H, H-C_5),2.3455 (s,3H, H-C_9), 2.3276 (s, 3H, H-C_8). ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)]: 154.57, 147.51, 147.22, 139.66, 137.97, 130.24, 129.42, 127.79, 124.97, 124.75, 122.70, 118.89, 106.83, 19.93, 19.60.

2.12. **1-(3,4-dimethoxyphenyl)-N-(3,4-dimethylphenyl)methanimine (N8)**

Light-yellow powder, m.p=108-110 ^0C, yield= 58% , R_f= 0.78.

IR (KBr/ v_{max}/ cm^{-1}): 3020.53 (Ar-H) stretching, 2964.59-2918.30 Aliphatic (C-H), 1577.77 (C=N), 1508.33 (C=C), 1076.27 (N^+-O^-), 759.96 (Ar-H) out-of-plane/ bending. ^1H-NMR [500 MHz, $CDCl_3$, δ (ppm)]: 8.3551(s,1H, H-C_{12}), 8.0723 (s, 1H, H-C_5), 8.0099 (d, 1H, H-C_1), 7.9977 (s, 1H, H-C_{14}), 7.2410 (d, 1H, H-C_2), 7.1565 (d, 1H, H-C_{17}), 6.9371 (d, 1H, H-C_{18}), 4.0098 (s, 3H, H-C_{19}), 3.9598 (s, 3H, H-C_{20}), 2.3667 (s, 3H, H-C_9), 2.3364 (s, 3H, H-C_8). ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)]: 159.04, 140.55, 137.27, 136.84, 130.31, 129.71, 126.85, 123.08, 122.99, 122.24, 119.61, 118.10, 110.47, 56.04, 19.90, 19.72.

2.13. **N-(4-(bromomethyl) phenyl)-1-(p-tolyl) methanimine oxide (N9)**

Yellow powder, m.p=176-178 ^0C, yield= 38.5%, R_f= 0.72.

IR (KBr/ v_{max}/ cm^{-1}): 3010.88 (Ar-H) stretching, 2956.87-2866.22 Aliphatic (C-H), 1597.06 (C=N), 1419.81-1577.77 (C=C), 1076.14 (N^+-O^-), 759.95(Ar-H) out-of-plane/ bending. ^1H-NMR [300 MHz, $CDCl_3$, δ (ppm)]: 8.5353(s,1H, H-C_{10}), 8.3316-7.9014 (m, 4H, H-C_1, H-C_2, H-C_4, H-C_5), 7.8721- 7.6776 (m, 4H, H-C_{12}, H-C_{13}, H-C_{15}, H-C_{16}), 3.0569 (s, 2H, H-C18), 2.4448 (s, 3H, H-C17). ^{13}C NMR [126 MHz, $CDCl_3$, δ (ppm)] 136.84, 130.96, 130.39, 126.84, 124.51, 123.08, 122.98, 122.53, 119.61, 57.18, 19.90.

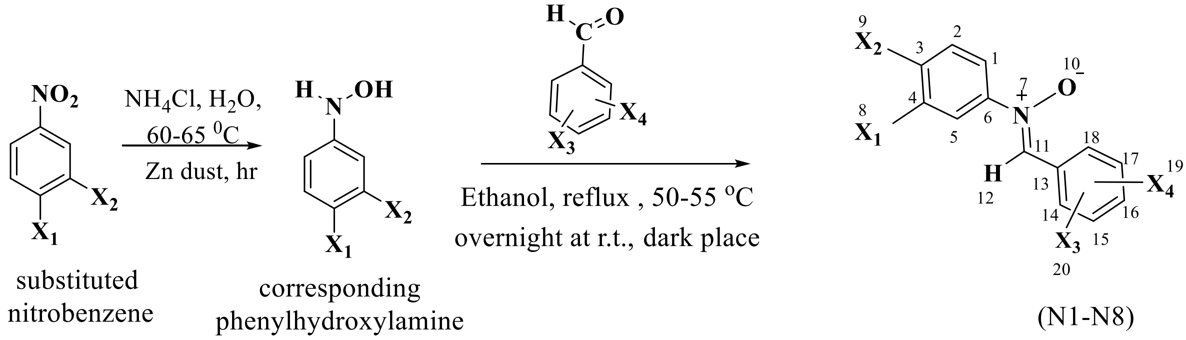
2.14. **Antibacterial and antifungal activity of nitrone derivatives**

Activities of nitrones (N1-N9) were studied with two concentrations (1000 and 500) µg/ mL in dimethyl sulfoxide (DMSO) as antibacterial against *E. coli* as Gram-negative bacteria and *S.aureus* as Gram-positive bacteria and also as an antifungal *Candida albicans* fungi. Agar well diffusion method was used for the antibacterial activity of synthesized nitrones. After autoclaving of Mueller-Hinton Agar (MHA) was cooled to 55°C and poured onto petri dishes. Then, with sterilized swabs, *E. coli* and *S.aureus* bacteria were completely streaked on them until they solidified and left for half an hour. After that, four wells of 8 mm were made on agar and (100 µL) of dimethyl sulfoxide, two different concentrations of synthesized nitrones and levofloxacin as a standard drug were placed. After incubation at 37°C for 28 hr, the zones of inhibition were determined in mm (Lino & Deogracious, 2006).

Antifungal activities of synthesized nitrones were compared with clotrimazole as a standard drug against *Candida albicans*. Sabouraud dextrose was used as the growth medium. 8 mm wells were cut and (100 µL) of two different concentrations of synthesized nitrone, DMSO and standard drug were placed. After incubation at 37°C for 28 hr, the zones of inhibition were determined in mm (Salman & Majeed, 2013) .

RESULTS AND DISCUSSION

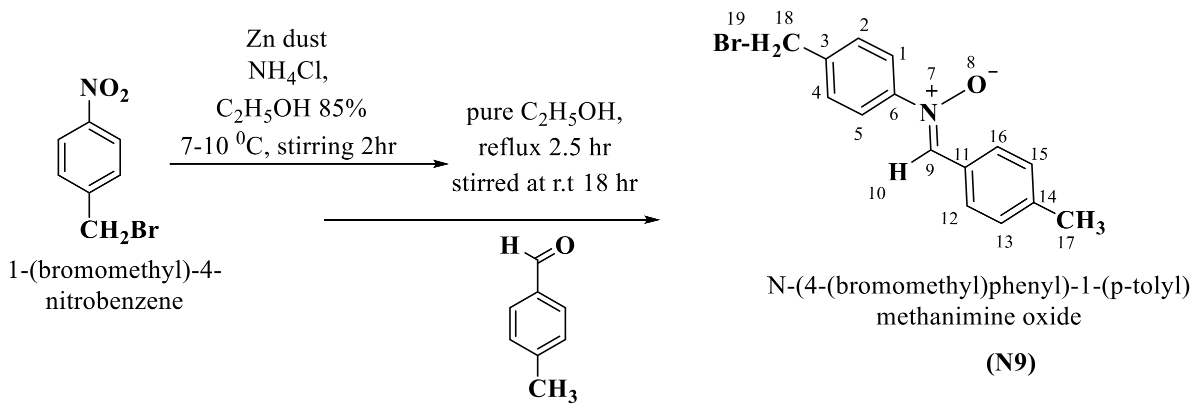
In this study, the starting materials for the synthesis of these nitrones (N1-N9) were nitrobenzene compounds in which bearing electron donating and electron withdrawing groups. Both types converted to corresponding phenylhydroxyl amine by Zn dust in aqueous solution and 85% of ethanol in presence of NH_4Cl as a weak acid. Synthesized phenylhydroxylamines were converted to new nitrones after condensation reaction with various substituted benzaldehydes (scheme 3 and scheme 4).



N1 : X_1= Cl, X_2=Cl, X_3= H, X_4= H
N2 : X_1 = Cl, X_2= Cl, X_3= p-CH_3, X_4= H
N3 : X_1 = Cl, X_2= Cl, X_3= o-NO_2, X_4= H
N4 : X_1 **= Cl,** X_2= Cl, X_3 = m-(OCH_3), X_4= p-(OCH_3)

N5 : X_1= CH_3, X_2=CH_3, X_3= H, X_4= H
N6 : X_1 = CH_3, X_2= CH_3, X_3= p-CH_3, X_4= H
N7 : X_1 = CH_3, X_2= CH_3, X_3= o-NO_2, X_4= H
N8 : X_1 **=** CH_3, X_2= CH_3, X_3 = m-(OCH_3), X_4= p-(OCH_3)

Scheme 3: General pathway for the synthesis of nitrones (N1- N8) by procedure A



Scheme 4: General pathway for the synthesis of nitrone (N9) by procedure B

According to the FT-IR characterization, the peaks that were important for the detection of nitrones include (N^+-O^-) absorption bands in the range of (1079-1068) cm^{-1}, and also (C=N) peaks in the range of (1598-1564) cm^{-1}. N-(3,4-dimethylphenyl)-1- (p-tolyl)metanimine oxide, in which bearing CH_3 group (electron donating group) in the (Ar-C=N) moiety, and two CH_3 groups in (Ar-N=C) moiety, the peak of (C=N) bond were appeared in the (1598.99) cm^{-1}, and it was the highest frequency for this bond when compared with another synthesized nitrones (N6).

N-(3,4-dichlorophenyl) -1-(2-nitrophenyl) methanimine oxide in which bearing NO_2 group (electron withdrawing group) in the (Ar-C=N) moiety, and two chlorine substitutions (electron withdrawing groups) in (Ar-N=C) moiety, the peak of (N^+–O^-) bond were appeared in the (1078.43) cm^{-1}, and it was the highest frequency for this bond when compared with another synthesized nitrones (N3).

Aromatic regions (Ar-H) were observed in the range (3100-3000) cm^{-1} and (aliphatic C-H) in the range (3000-2900) cm^{-1} were observed in the compounds in which these groups were present (Figure 3, 4 and 5).

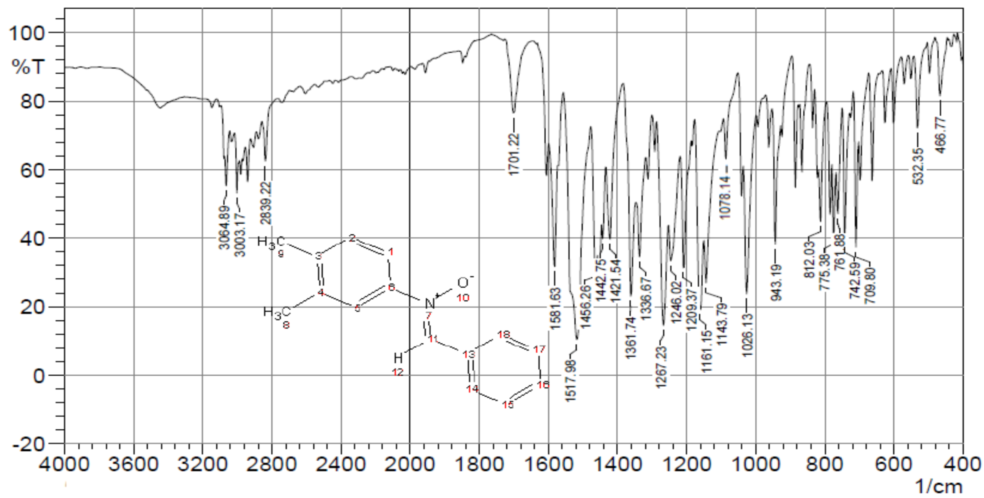


Figure 3: FT-IR of N-(3,4-dimethylphenyl)-1-phenylmethanimine oxide (N5)

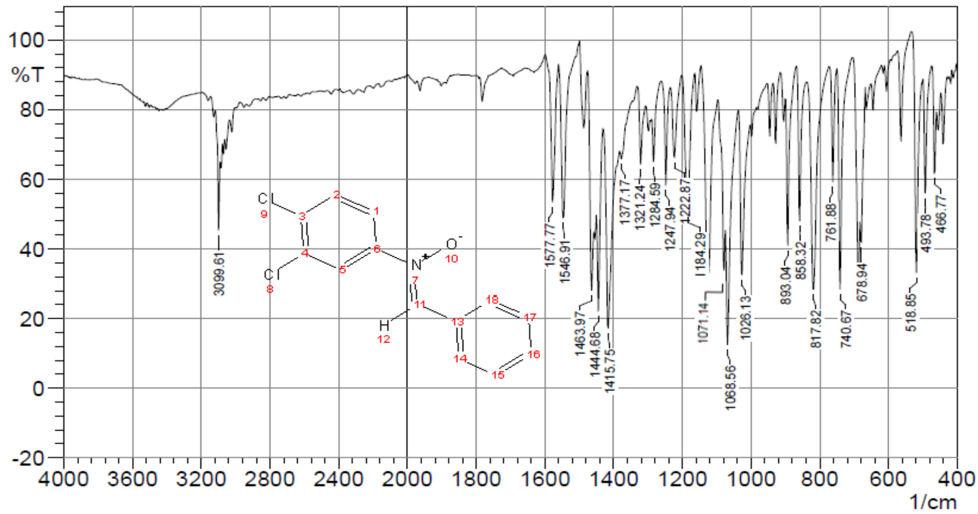


Figure 4: FT-IR of N-(3,4- dichlorophenyl)-1-phenylmethanimine oxide (N1)

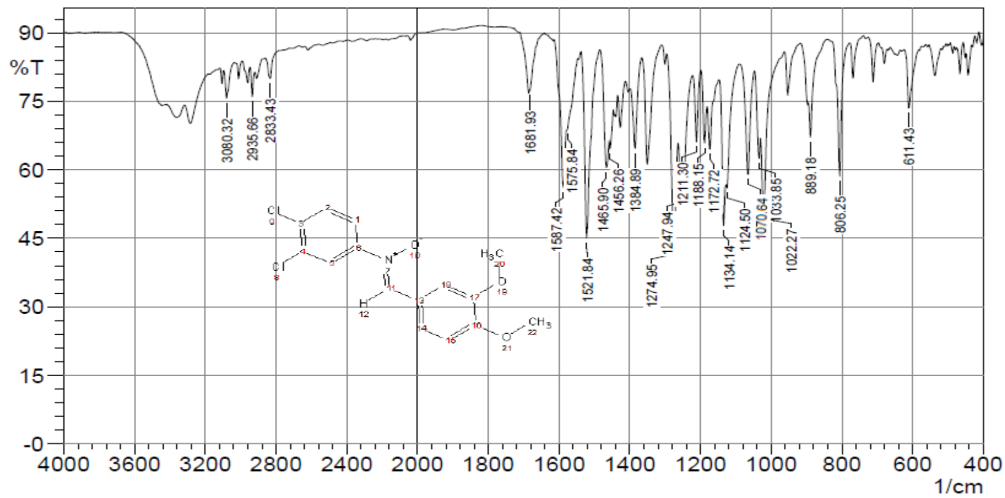


Figure 5: FT-IR of N-(3,4-dichlorophenyl)-1-(3,4-dimethoxyphenyl)methanimine oxide (N4)

In the case of ^{1}H-NMR, there were three types of protons in the synthesized nitrones. The first type of protons were aromatic protons, which were present in all nitrones and included hydrogens in two aromatic rings (H-Ar-N=C) and (H-Ar-C=N).The second type was related to the proton attached to the carbon in (H-C=N), which was also present in all the synthesized nitrones. The last protons were related to the aliphatic substituents protons, which was present in some compounds and were as substituents on aryl rings. All proton signals, whether those related to aromatic regions or (H-C=N) as a singlet, and shifting was depending on the type of substitution, were observed in all synthesized nitrones. The spectrum of protons of aliphatic, either methyl (2-3 ppm) or methoxy (3-4 ppm) groups were clearly observed in the synthesized nitrones in which these substituents were present (figure 6, 7 and 8).

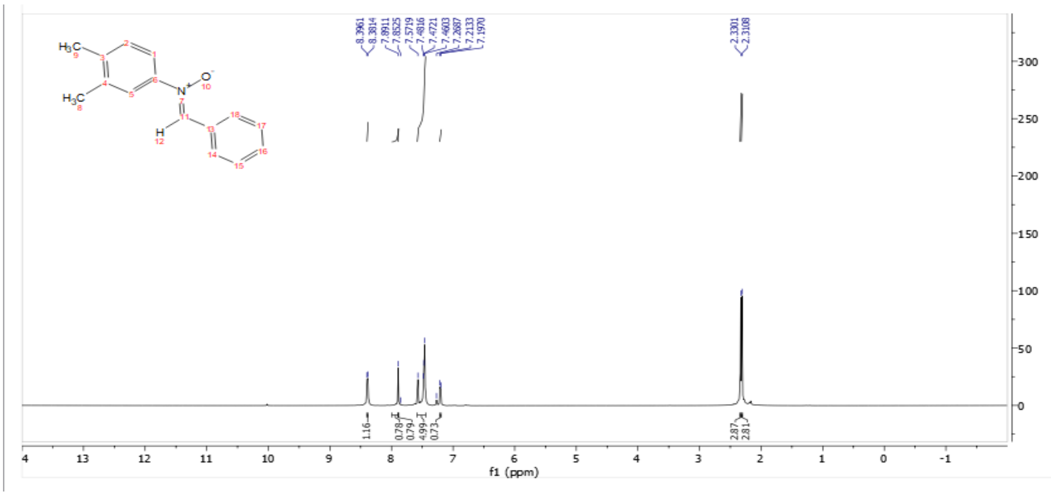


Figure 6: ^{1}H-NMR of N-(3,4-dimethylphenyl)-1-phenylmethanimine oxide (N5)

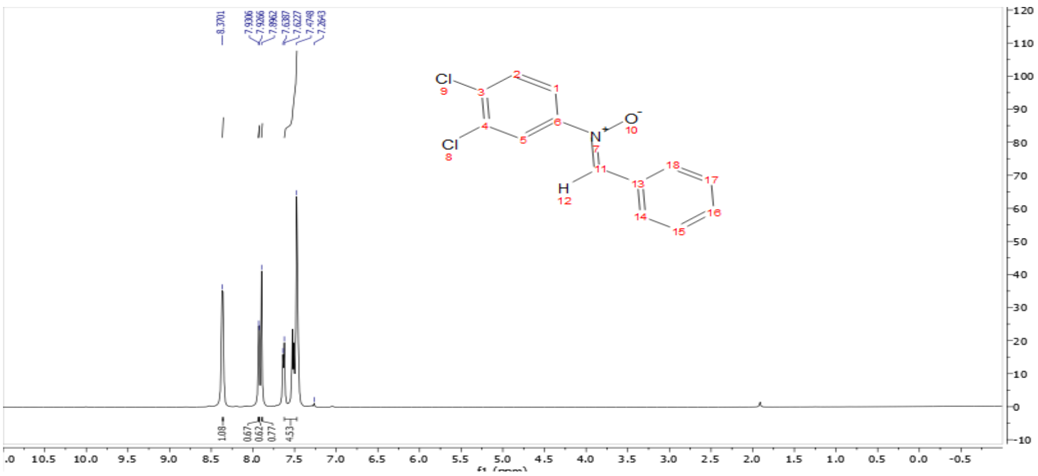


Figure 7: ^{1}H-NMR of N-(3,4- dichlorophenyl)-1-phenylmethanimine oxide (N1)

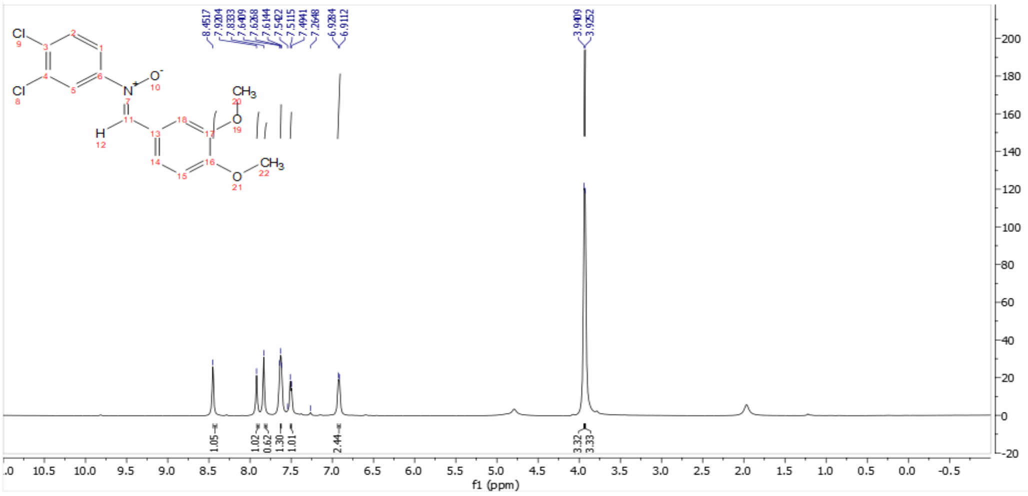


Figure 8: ^{1}H-NMR of N-(3,4-dichlorophenyl)-1-(3,4-dimethoxyphenyl)methanimine oxide (N4)

At ^{13}C NMR, all peaks related to those carbons present in synthesized nitrones was observed, whether they were aromatic or aliphatic (N5, N1, N4), (Figures 9, 10 and 11) (Al Adhreai *et al*., 2022).

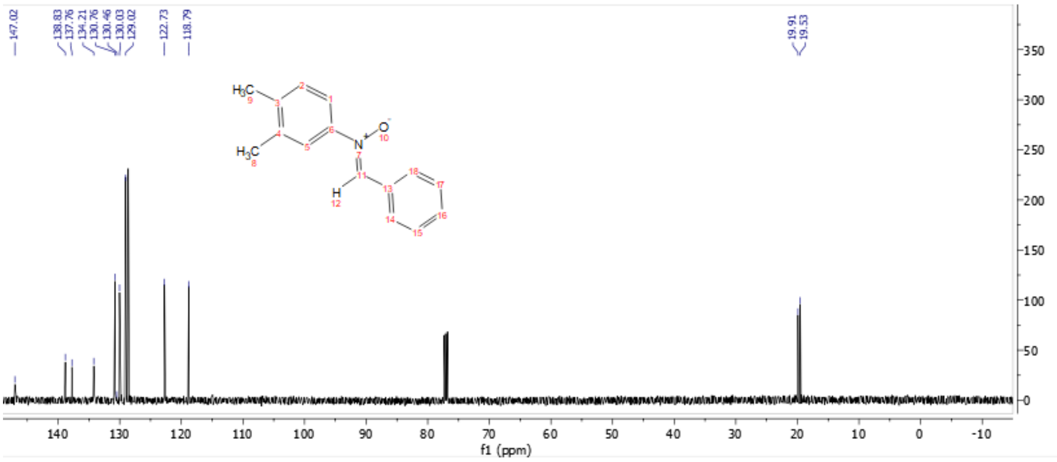


Figure 9: ^{13}C NMR of N-(3,4-dimethylphenyl)-1-phenylmethanimine oxide (N5)

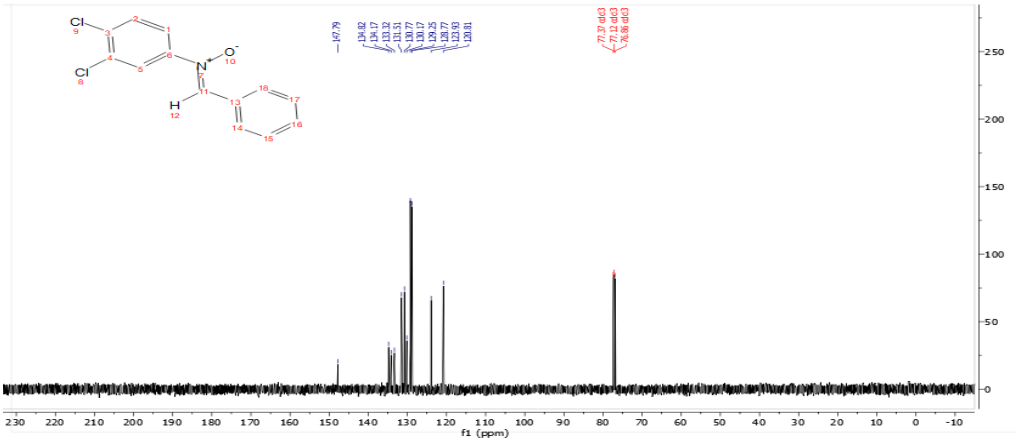


Figure 10: ^{13}C NMR of N-(3,4- dichlorophenyl)-1-phenylmethanimine oxide (N1)

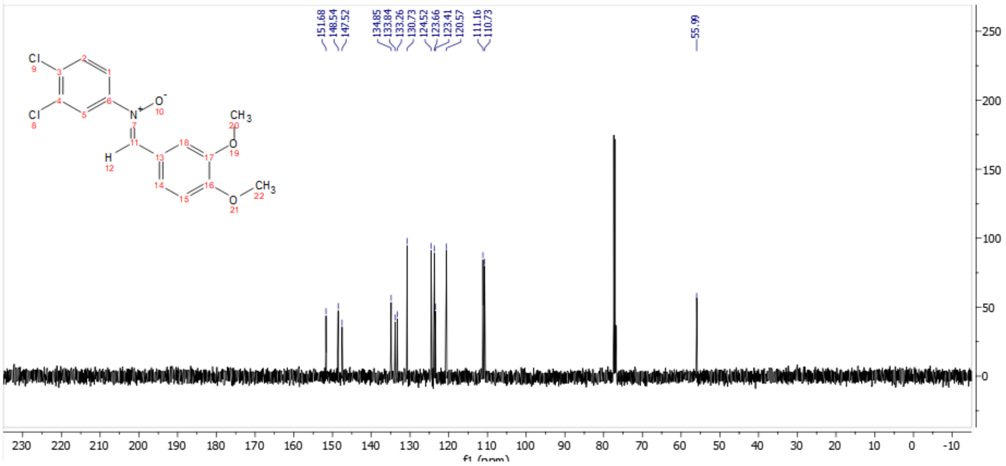


Figure 11: ^{13}C NMR of N-(3,4-dichlorophenyl)-1-(3,4-dimethoxyphenyl)methanimine oxide (N4)

In the (N1-N4), the aromatic nitro compounds that had two Cl groups, after converted to corresponding phenylhydroxylamine and condensation reaction with four types of substituted benzaldehydes (benzaldehyde, 3-chloro benzaldehyde, 2-nitrobenzaldehyde and 3,4-dimethoxybenz aldehyde), the higher yields were obtained when benzaldehyde was (2-nitrobenzaldehyde). The reason for this was the presence of the nitro group in the ortho position of benzaldehyde. It was an electron withdrawing group and increased electrophilicity of benzaldehyde and when a nucleophile (exist pair of electron on N atom) such as N-(3,4-dichlorophenylhydroxylamine) reacted with this benzaldehyde, the yield was higher than other three substituents of benzaldehydes (section 2.6, N3).

The lowest yield in this reaction was observed when N- (3,4-dichlorophenylhydroxylamine) reacted with (3,4-dimethoxy benzaldehyde) due to the electron donating of methoxy groups (-OCH_3) in the benzaldehyde, which reduced the electrophilicity of the benzaldehyde and slower reaction occurred when attacked by nucleophile (section 2.7, N4).

In N5, N6, N7 and N8 all the topics described for (N1-N4), were applied to these synthesized nitrones, and therefore, it had the highest yield in N7 (section 2.10).

In these reactions when used two starting materials, one of them 1,2-dichloro-4-nitrobenzene and the other 1,2-dimethyl-4-nitrobenzene (scheme 3), their differences were in the types of substitutitionsa. In 1,2-dichloro-4-nitrobenzene there were two Cl groups, which were electron withdrawing groups. These groups were causes a lower yield when compared to 1,2-dimethyl-4-nitrobenzene (compare yields of N1 and N5, N2 and N6, N3 and N7, N4 and N8). Due to the presence of two electron donating groups CH_3 in 1,2-dimethyl-4-nitrobenzene with the same substituted benzaldehydes, the yield of synthesized nitrones was higher compared to 1,2-dichloro-4-nitrobenzene (West & Davis, 1989) .

Although N9 could not be prepared by method A and was prepared by method B, its yield was lower than other nitrones (scheme 4).

According to the studies of HK Kim (Kim *et al*., 1970) and Mariana C. Ferraz (Ferraz *et al*., 2017) on nitrone derivatives, it has been clearly shown that substitutions have a significant effect on the biological activities of nitrones. Their activities were considered accordingly, for two types of bacteria: *E. coli* as Gram negative bacteria, *S. aureus* as Gram-positive bacteria and also were considered as antifungal against *Candida albicans* fungi. The results were interesting. In general, the activity of synthesized nitrones in these two concentrations against *Candida albicans* was better than their activity against the mentioned bacteria. In nitrones (N1-N4), the presence of two methoxy groups in (Ar-CH=N) moiety caused inactivation of (N4) against *E.coli*, and when NO_2 was present in (Ar-CH=N) moiety, the activity of nitrone was improved against *E.coli* bacteria and fungi (N3). The presence of CH_3 in mentioned moiety prevented the growth of E.coli and S. *aureus* bacteria and *Candida albicans* in nitrone (N2).

In nitrones (N5-N8), their activity was better than the previous nitrones, and the presence of two methoxy groups in (Ar-CH=N) moiety caused the activity of nitrone (N8) to be high against both bacteria, and the presence of CH_3 in the (Ar-CH=N) moity decreased the activity of nitrone (N6) against *E.oli* bacteria.

Nitron (N9) was inactive against *E.coli* and also did not appear inhibition zone against *S.aureus* at the lower concentration. This nitrone was resistant only against fungus *Candida albicans* (table 1).

Table 1: Antibacterial and antifungal of synthesized nitrones in different zone (mm)

N	microorganism	500 µg / mL in (DMSO)	1000 µg / mL in (DMSO)
N1	*Escherichia coli*	20	22
	Staphylococcus aureus	20	23
	Candida albicans	19	20
N2	*Escherichia coli*	16	18
	Staphylococcus aureus	16	17
	Candida albicans	25	26
N3	*Escherichia coli*	15	22
	Staphylococcus aureus	16	25
	Candida albicans	25	28
N4	*Escherichia coli*	NI	NI
	Staphylococcus aureus	19	20
	Candida albicans	15	20
N5	*Escherichia coli*	20	25
	Staphylococcus aureus	26	35
	Candida albicans	22	23
N6	*Escherichia coli*	12	13
	Staphylococcus aureus	15	16
	Candida albicans	15	26
N7	*Escherichia coli*	18	20
	Staphylococcus aureus	20	22
	Candida albicans	25	30
N8	*Escherichia coli*	25	30
	Staphylococcus aureus	28	30
	Candida albicans	20	22
N9	*Escherichia coli*	NI	NI
	Staphylococcus aureus	NI	12
	Candida albicans	12	13

The inhibition zone of levofloxacin against *E.coli* bacteria= 36 mm and against *S.aureus*= 34 mm.
The inhibition zone of clotrimazole against *Candida albicans*= 33 mm
NI: not inhibition

CONCLUSION

In this study, the data obtained by FT-IR, ^{1}H-NMR and ^{13}C NMR spectra confirmed the structure of the new synthesized nitrones (N1-N9). In the prepared nitrone derivatives, both substituted phenylhydroxylamine derivatives and substituted benzaldehydes have a significant effect on their properties and yields. For this reason nitrone (N7) was prepared with the highest yield (81%) in the condensation reaction between substituted phenylhydroxyamine bearing electron donating groups (N-(3,4-dimethylphenyl) hydroxyl amine) and substituted benzaldehyde with electron withdrawing groups (2-nitrobenzaldehyde). The biological activities of synthesized nitrones showed that most of them were active against *S. aureus* and *E. coli* bacteria and all of them showed antifungal activity against *Candida albicans.*

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