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# Synthesis, Characterization and Study of Biological Activity of Some New Nitrone and Isoxazolidine compounds

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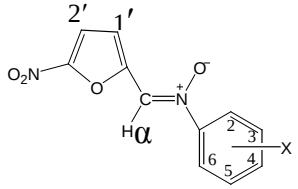
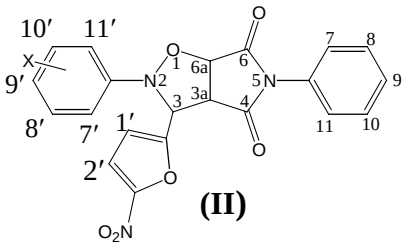
***Absract***

Two series of new compounds of nitrofuran derivatives **I** and **II** were prepared. The first included nitrone compounds synthesized from the reaction of 5-nitrofurfural with N-arylhydroxylamines. The second series, concerned with the synthesis of isoxazolidines by 1,3-dipolar cycloaddition reaction of nitrones with N-phenylmaleimide. The synthesized compounds were characterized by elemental analysis, UV-visible spectroscopy, FT-IR spectroscopy and $^{1}$H-NMR spectroscopy. All prepared compounds were screened for their antibacterial activity against two bacterial species, *Escherichia coli* and *Staphylococcus aureus*, as well as fungi (*Aspergillus niger*). The activity data showed that most compounds had good activity as compared with standard drugs. The results of $LD_{50}$ of some selected compounds showed that some are moderately toxic and others are non toxic in the range of graded doses.



X= H(N1), p-Br(N2), p-Cl(N3), o-Cl(N4), m-Me(N5) and o-OMe(N6)

**(I)**



X= H(N1), p-Br(N2), p-Cl(N3), o-Cl(N4), m-Me(N5) and o-OMe(N6)

**(II)**

**Keywords:** Nitrones, Isoxazolidines, 1,3-Dipolar cycloaddition, Biological activity, $LD_{50}$

## *1. Introduction*

Nitrones are important synthetic intermediates that have been used extensively in organic chemistry[1]. Some nitrones have been used as therapeutic agents in the treatment of a wide variety of diseases arising from oxidative damage induced by free radicals particularly in biological systems[2,3]. Isoxazolidine compounds are known to possess biological activities, such as antibacterial, antifungal, anti-inflammatory activities[4-6]. There are many synthetic methods for the synthesis of nitrones that have been reported by several ways[7-13] . Nitrones are easily available from aldehydes or ketones with N-monosubstituted hydroxylamines. This reaction proceeds smoothly and in high yields. Nitrones can react as 1,3-dipolar species with a large variety of dipolarphiles to give different products. One of the most synthetic applications of nitrones is their use as 1,3-dipoles in cycloaddition reactions to olefins for the preparation of isoxazolidines. isoxazolidines are known to posses biological activity.

## 2. Experimental chemical part

Melting points of the compounds were determined using a Gallenkamp melting point apparatus. IR spectroscopy analyses were recorded on FT-IR 8400S SHIMADZU(Japan) as KBr disk and the UV-visible spectra( $10^{-4}$ M/ethanol) were recorded SPECTROSCAN80D u.v-visible spectrometer in Chemistry Department-College of Science-University of Basrah/Iraq. $^{1}$H-NMR spectra were recorded using Bruker model ultra shield 300MHz (Switzerland), and CHNS analysis were recorded using EuroVector model EA3000A (Italy) in the analytical Laboratory of AL-ALBAYET University, Jordan.

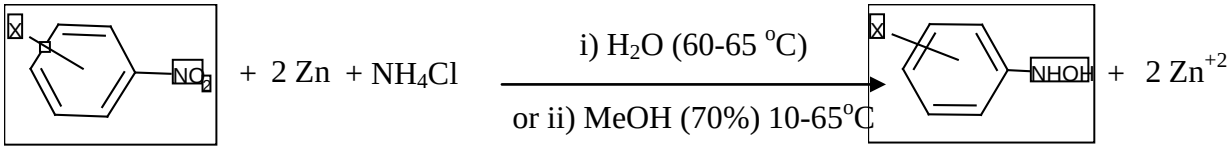
### 2.1 Preparation of starting materials

### 2.1.1 General procedure preparation of N-(x-phenyl) hydroxylamine[14]: P1, P2 andP3

(0.47 mole) of ammonium chloride, 800 ml of water and the appropriate nitro aromatic compound, namely, nitrobenzene, m-nitrotoluene or o-methoxynitrobenzene (0.41 mole) were placed in a reaction vessel. The mixture was stirred vigorously by means of a mechanical stirrer, and 62 g of zinc dust was added during (15-20) minutes. As the reduction proceeded, the temperature rised to 60-65 $^{0}$C, stirring was continued for 15 minutes in which all the zinc dust had been added, at the end of which the reaction was complete, as indicated by the fact that the temperature of the mixture ceased to rise. The hydroxylamine was filtered and purified by dissolving it in benzene and was precipitated with petroleum ether.

### 2.1.2 General procedure preparation of N-(x-phenyl) hydroxylamine[14]: P4 and P5

(0.23 mole) of ammonium chloride, 400 ml of methanol (70%) and an appropriate nitro aromatic compound, namely, p-bromonitrobenzene or p-chloronitrobenzene (0.2 mole) were placed in a 1l round flask. The round flask was cooled to 10 $^{o}$C by immersing it in an ice-salt bath and 31 g of zinc dust was added during 15 minutes. After adding all the zinc dust, the reaction mixture was heated to 60 $^{o}$C for 45 minutes. After that, the hot solution was filtered with suction to remove the zinc oxide. The solid was washed with 50 ml of hot water. The filtrate was saturated with salt and cooled for 30 minutes. The hydroxylamine was filtered and purified by dissolving it in benzene and was precipitated with petroleum ether. The characterizations of N-arylhydroxylamies are summarized in Table 1.




X= H(P1), m-Me(P2), o-OMe(P3) p-Br(P4) and p-Cl(P5)

**Table 1: The characterizations of the N-arylhydroxylamines**

| Compd. | Crystals shape | M.P °C | Yield % |
|---|---|---|---|
| **P1** | Lightyellow needle | 80-81 | 62 |
| **P2** | Light paleyellow sheet | 68-70 | 71 |
| **P3** | Pale lead needle | 77-79 | 60 |
| **P4** | Light white sheet | 96-98 | 53 |
| **P5** | White sheet | 88-89 | 60 |

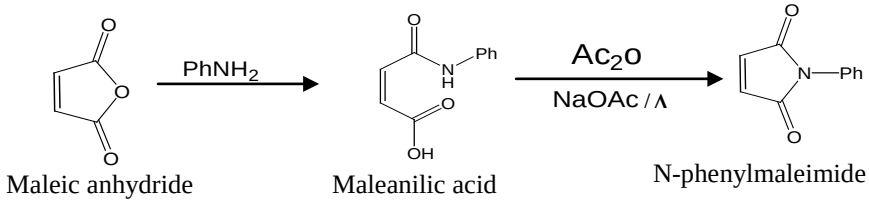
### 2.1.3 Preparation of N-phenylmaleimide [15, 16]

**A- Maleanilic acid:**

In a 1l two-necked round bottom flask provided with a reflux condenser and dropping funnel, 30 gm (2 mole) of maleic anhydride and 382 ml of diethyl ether were placed. Stirring was started by using a magnetic stirrer and when all the maleic anhydride was dissolved, a solution of 28 ml (29.1 g, 2 mole) of aniline in 30 ml of diethyl ether run in through the dropping funnel. The resulting thick suspension was stirred at room temperature for 1hr then cooled to 15-20 °C in an ice bath. The product was obtained by suction filtration in the form of a fine cream-colored powder with m.p 201-202 °C, suitable to be used in the next step without purification. The yield was 57 g (97%).

**B- N-phenylmaleimide:**

103 ml of acetic anhydride and 10 g of anhydrous sodium acetate were placed in a 500 ml round flask provided with a reflux condenser. 48 g of the maleanilic acid obtained as described in method A was added and the resulting suspension was dissolved by swirling and heating on a steam bath for 30 minutes. The reaction mixture was cooled almost to room temperature in a cooled water bath and then poured into 200 ml of ice water. The precipitated product was removed by suction filtration, washed three times with 75 ml of portions of ice water and once with 75 ml of petroleum ether (b.p 30-60 °C) and dried. The product was purified by reacrystallization from cyclohexane to yield 32 g deep yellow needle crystals (with yield 75%) with m.p 88-89 °C.



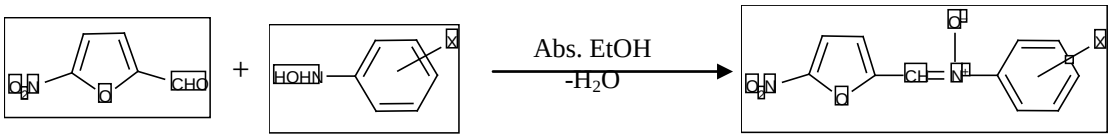

Maleic anhydride — Maleanilic acid — N-phenylmaleimide

***3 Preparation of compounds***

**3.1 Preparation of nitrones**

**3.1.1General procedure preparation of C-(5-nitro-2-furyl)-N-(x-phenyl) nitrone[1]: N1-N3, N5 and N6**

In a 100 ml round flask, 0.02 mole of 5-nitrofurfural and 15 ml of absolute ethanol were placed. This solution was stirred and warmed to 50 $^{o}$C, and a solution of the appropriate N-arylhydroxylamine, namely, N-phenylhydroxylamine, N-(p-bromophenyl)hydroxylamine, N-(p-chlorophenyl)hydroxylamine, N-(m-methylphenyl)hydroxylamine or N-(o-methoxyphenyl)hydroxylamine (0.02 mole) in 15 ml of absolute ethanol was added. After the addition is complete, the reaction mixture was kept with stirring in the dark overnight at room temperature. The reaction mixture was filtered by suction filtration and the product was recrystalized from absolute ethanol to yield the desired nitrones. The properties of the prepared nitrones are represented in Table 2.




X= H(N1), p-Br(N2), p-Cl(N3), m-Me(N5) and o-OMe(N6)

**3.1.2 Preparation of C-(5-nitro-2-furyl)-N-(o-chlorophenyl) Nitrone[17]: N4**

(18 mmole) of o-chloronitrobenzene and 1.23 g (23 mmole) 0f ammonium chloride were dissolved or dispersed (depending on the solubility) in 25 ml of 60% aqueous ethanol. The solution was cooled to 10 $^{o}$C, and then 65 mmole of zinc dust was added portion wise, with continuous stirring over a period of 1.5hr.During this addition, the temperature was maintained below 15 $^{o}$C. The solid was removed by filtration and washed with three portions of 8 ml of boiling ethanol. 2.82 g (20 mmole) of 5-nitrofurfural was added to the combined alcoholic filtrates and the mixture was stirred at room temperature for 20 hrs. The precipitated nitrone was filtered and recrystalized from absolute ethanol to give 3.5g (64%) yellow fine needle crystals with m.p 179-181 $^{o}$C, as shown in Table 2.

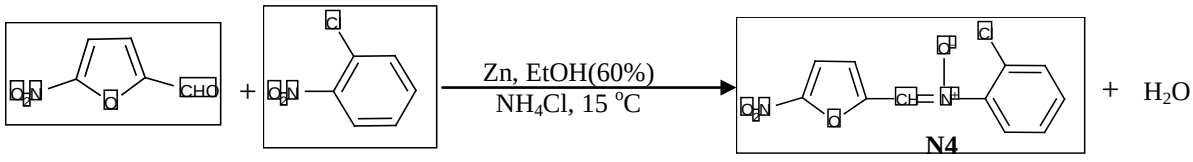
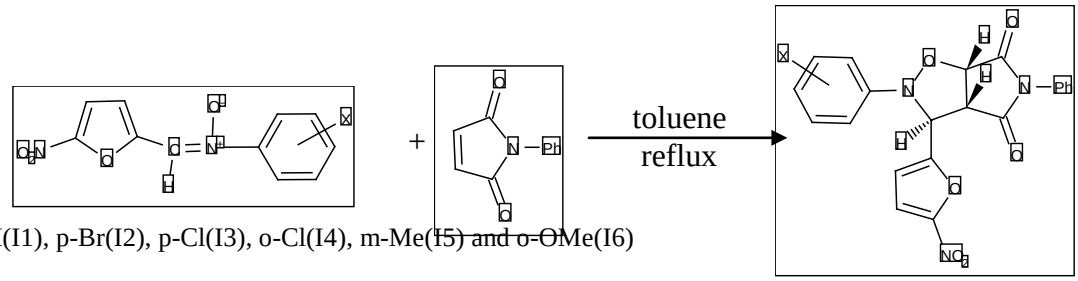



**Table 2: The characteristics of nitrones**

| Yield (%) | Crystals shape | M.p $^{o}$C | Compd. |
|---|---|---|---|
| 82 | Deep yellow fine needle | 178-180 | **N1** |
| 78 | Yellow fine needle | 170-172 | **N2** |
| 74 | Yellow needle | 163-165 | **N3** |
| 64 | Yellow fine needle | 179-181 | **N4** |
| 80 | Deep yellow needle | 135-137 | **N5** |
| 90 | Yellow needle | 164-166 | **N6** |

### 3.2 Preparation of isoxazolidines[18]: 2-(x-phenyl)-4,6-dioxo-phenyl-3-(5-nitro-2-furyl)-2,5-iazo-1-oxobicyclo-[3.3.0] octane

In a round flask provided with a reflux condenser, 0.38 mmole of nitrone was dissolved in 20 ml of toluene. Then 0.4 mmole of N-phenylmaleimide was added and the reaction mixture was refluxed. The reaction was monitored by TLC. The solvent was concentrated by evaporation under reduced pressure with rotary evaporator and the mixture was cooled overnight. The solid was filtered and recrystalized from toluene to give the desired isoxazolidine. The characterizations of the products illustrated in Table 3.




X= H(I1), p-Br(I2), p-Cl(I3), o-Cl(I4), m-Me(I5) and o-OMe(I6)

**Table3:The characteristics of the isoxazolidines**

| Compound | m.p °C | Reflux time (hr) | Crystals | Yield (%) | TLC (eluent) | $R_f$ |
|---|---|---|---|---|---|---|
| **I1** | 188-191 | 5 | White fine needle | 71 | Hexane:EtAc (2.5:1.5) | 0.70 |
| **I2** | 182-184 | 4.5 | White powder | 65 | Hexane:EtAc (2.5:1.5) | 0.62 |
| **I3** | 187-189 | 5.5 | White powder | 61 | Hexane:EtAc (2.5:1.5) | 0.66 |
| **I4** | 205-207 | 5 | Yellowish powder | 55 | Benzene:$CHCl_3$(3.5:3.6) | 0.58 |
| **I5** | 164-166 | 5 | White powder | 70 | Cyclohexane: EtAc (3:2) | 0.65 |
| **I6** | 176-178 | 6.5 | Cream powder | 80 | Hexane:EtAc (2.5:1.5) | 0.70 |

## *4. Experimental biological part*

### 4.1 Antibacterial activity[19- 21]

The antibacterial activity of the control substance (dimethylsulfoxide, DMSO), the standered drugs (nitrofurazone and nitrofurantoin) and prepared compounds were evaluated using the disc diffusion method. A stock solution of 1000 µg/ml was prepared by dissolving each compounds in DMSO. Filter paper (Whatmann No. 4) 6-mm-diameter discs were with solution of test compounds. The plates were incubated at optimum growth temperature (37 $^{o}$C) for 24 hrs and then the zone of microbial growth inhibition around the discs was measured (in mm). The test bacteria included *Escherichia coli* and *Staphylococcus aureus*

### 4.2 Antifungal activity[22]

The antifungal activity of the investigated compounds, compared with Fluconazole as standard drug, was tested against pathogenic fungal, *Aspergilus Niger*, at a concentration of (1000 µg/ml). The medium used in this respect was Sabouraud dextrose as the growth medium. Wells (6 mm in diameter) were cut using (Cork borer) and 100 µl of each compound was added to each well.

### 4.3 Median Lethal Dose ($LD_{50}$) [23]

The short-term toxicity, study in term of $LD_{50}$ of some active compounds, was determined in Swiss albino mice BALB/c, weighting about 25 gm, one month old and were housed under controlled conditions.

## 5. Results and discussion.

The results of elemental analysis (C. H. N.) of the prepared compounds in this study are summarized in the Tables 4. From the table, it was found that the results are in a good agreement with the calculated values, this indicates that the prepared compounds are as expected.

**Table 4: Elemental analysis of prepared compounds**

| Compd. | Molecular formula | Calculated | | | Found | | |
|---|---|---|---|---|---|---|---|
| | | C% | H% | N% | C% | H% | N% |
| **N1** | $C_{11}H_8N_2O_4$ | 56.9 | 3.47 | 12.06 | 56.87 | 4.02 | 12.16 |
| **N3** | $C_{11}H_7ClN_2O_4$ | 49.55 | 2.64 | 10.5 | 50.17 | 2.57 | 10.78 |
| **N4** | $C_{11}H_7ClN_2O_4$ | 49.55 | 2.64 | 10.5 | 50.11 | 2.85 | 10.23 |
| **N5** | $C_{12}H_{10}N_2O_4$ | 58.53 | 4.09 | 10.37 | 58.23 | 4.48 | 11.65 |
| **I1** | $C_{21}H_{15}N_3O_6$ | 62.22 | 3.72 | 10.36 | 62.66 | 3.72 | 10.16 |
| **I2** | $C_{21}H_{14}BrN_3O_6$ | 52.08 | 2.91 | 8.67 | 52.36 | 3.31 | 8.69 |
| **I3** | $C_{21}H_{14}ClN_3O_6$ | 57.35 | 3.20 | 9.55 | 57.56 | 3.24 | 9.10 |
| **I4** | $C_{21}H_{14}ClN_3O_6$ | 57.35 | 3.20 | 9.55 | 57.56 | 3.17 | 9.59 |
| **I5** | $C_{22}H_{17}N_3O_6$ | 63.00 | 4.08 | 10.01 | 62.98 | 4.06 | 9.80 |
| **I6** | $C_{22}H_{17}N_3O_7$ | 60.69 | 3.93 | 9.65 | 60.53 | 3.88 | 9.80 |

UV-visible spectra of nitrones showed distinguished absorption bands within the region (375-390 nm) attributed to the $\pi\rightarrow\pi^*$ transitions of nitrone group. The other three bands at the regions (215-220 nm), (247-255 nm) and (305-315 nm), attributed to the electronic transitions $\pi\rightarrow\pi^*$ of the aromatic system[24-27]. On the other hand, the absorption spectra of isoxazolidines showed a disappearance of the absorption band related to nitrone group with the appearance of only two absorption

bands which attributed to the electronic transitions $\pi\pi^*$ of aromatic system which confirmed the formation of isoxazloidines[28]

The IR spectra (Table5 ) of nitrones showed two absorption bands in the ranges (1136-1163 $cm^{-1}$) and (1500-1600 $cm^{-1}$) which assigned to stretching vibrations of N→O and C=N groups, respectively[24].The absence of OH (3200-3400 $cm^{-1}$) and C=O (1702 $cm^{-1}$) absorption bands in the IR spectra confirmed the formation of compounds via condensation reaction of nitrofurfural with N-arylhydroxyl amines.

The IR spectra (Table6 ) of isoxazolidines showed new absorption bands at (1014-1388 $cm^{-1}$) assigned to isoxazolidine ring in addition to a very strong absorption band at (1718-1727 $cm^{-1}$) which attributed to the carbonyl group[29, 30].

**Table 5:data of the FT-IR spectra (cm-1) of nitrones**

| Compound | N→O (Str.) | C=N C=C (Str.) | C=C (Str.) | C-O-C (Str.) | $NO_2$ (Str.) | | Ar-H | | AliphaticC-H (Str.) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Sym. | Asym. | (Str.) | (O. O. P.) Bend. | |
| **N1** | 1153 s | 1571* | 1481 s | 1016 m<br>1240 m | 1339 vs | 1520 s | 3053 w<br>3130 w | 812 m | ——— |
| **N2** | 1153 s | 1598 m | 1481 vs | 1014 vs<br>1247 vs | 1346 vs | 1539 s | 3060 w<br>3150 w | 806 s | ——— |
| **N3** | 1153 m | 1600 w | 1479 s | 1012 m<br>1242 m | 1358 vs | 1527 m | 3057 w<br>3159 w | 806 m | ——— |
| **N4** | 1163 m | 1551 m | 1479 s | 1026 m<br>1244 m | 1350 vs | 1514 m | 3090 w<br>3153 w | 812m | ——— |
| **N5** | 1136 m | 1526 s | 1483 s | 1014 m<br>1245 m | 1350 vs | 1525 s | 3070 w<br>3149 w | 814 m | 2879 w<br>2925 w |
| **N6** | 1157 m | 1609 m | 1483 s | 1016 m<br>1249 m | 1344 vs | 1539 m | 3055 w<br>3151 w | 806 m | 2838 w<br>2935 w |

**Table 6: data of the FT-IR spectra ($cm^{-1}$) of isoxazolidines**

| Compoud | C-N C-O N-O (str.) | C=O (Str.) | C-O-C (Str.) | C=C (Str.) | $NO_2$ | | ArH | | Aliphatic C-H (str.) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Sym. | Asym. | (Str.) | (O. O. P.) bend. | |
| **I1** | 1014-1388 m-s | 1727 vs | 1014 m<br>1236 m | 1496 s<br>1593 m | 1354 s | 1529 m | 3070 w<br>3139 w | 808 m | 2850 w<br>2933 w |
| **I2** | 1018-1388 m-s | 1720 vs | 1018 m<br>1235 m | 1494 s<br>1589 m | 1359 s | 1529 m | 3134 w | 812 m | 2850 w<br>2975 w |
| **I3** | 1016-1385 m-s | 1726 vs | 1016 m<br>1242 m | 1496 vs<br>1591 m | 1371 vs | 1528* | 3130 w | 818 m | 2876 w<br>2987 w |
| **I4** | 1018-1386 m-s | 1721Vs | 1018 vs<br>1244 vs | 1497 vs<br>1589 s | 1357 vs | 1525 vs | 3070 w | 810 m | 2884 w<br>2851 w<br>2992 w |
| **I5** | 1020-1387 m-s | 1721 vs | 1020 s<br>1240 s | 1500 vs<br>1595 s | 1367 vs | 1529* s | 3062 w<br>3130 w | 800 m | 2864 w<br>2991 w<br>2921 w |
| **I6** | 1020-1385 m-s | 1718 vs | 1020 m<br>1238 s | 1496 s<br>1591 m | 1356 s | 1528* | 3130 w | 808 m | 2850 w<br>2996 w |

The $^1$H-NMR spectra of nitrones as shown in Table 7 showed singlet signal in the region (8.612-9.140 ppm) attributed to the proton H-α[31]. Protons of the furan ring. Protons H-1′ and H-2′ showed second-order system AB[27,28,32], the ratio Δν/J is less than 8. So that, the proton H-1' gave a doublet signal at (7.490-7.893 ppm) and the proton H-2' also appeared as a doublet signal at (7.927-8.050 ppm) with coupling constant ($J_{1',2'}$= 3.9-4.2 Hz).

The spectra also showed that protons of the phenyl rings substituted in para-position exhibited spin system type AA′XX′[28].

$^1$H-NMR spectra of isoxazolidines showed that these compounds have only one type of geometrical form which is *anti*-isomer. The results as in Table 8 showed according to the values of coupling constants and dihedral angles ($\theta \approx 90^o$) between the protons H-3 and H-3a and the protons H-3a and H-6a is $J_{3a,6a} \approx$ 7.5 Hz[27,28,33] . Disappearance of the signal of proton H-α confirms the formation of isoxazolidine ring. In all spectra, observed a doublet signal at (6.604-6.970 ppm) which may attributed to the proton H-1', and also a doublet signal at (7.380-7.729 ppm) characterized to the proton H-2' with coupling constant ($J_{1',2'}$= 3.6-3.9 Hz). Due to the ratioΔν/J is greater than 8 the spin system for protons H-1' and H-2' may described as AX[28].

**Table 7: data of $^1$H-NMR spectra [δ (ppm), J (Hz)] of nitrones**

| Compound | H-α (s) | H-1′ (d) | H-2′ (d) | $J_{1'-2'}$ | Aromatic C-H | Aliphatic C-H |
|---|---|---|---|---|---|---|
| **N1** | 9.070 | 7.862 | 7.955 | 3.9 | 7.542-7.593(m, H-3, H-4, H5) 7.985-8.032(m, H-2, H-6) | —— |
| **N2** | 9.140 | 7.893 | 7.984 | 3.9 | 7.815(m, $J_{2,3}$=$J_{5,6}$=9, H-2, H-6) 8.005(m, $J_{2,3}$=$J_{5,6}$=9, H-3, H-5) | —— |
| **N3** | 9.105 | 7.860 | 7.944 | 3.9 | 7.643(d, $J_{2,3}$=$J_{5,6}$= 9, H-2, H-6) 8.038(d, $J_{2,3}$=$J_{5,6}$= 9, H-3, H-5) | —— |
| **N5** | 9.035 | 7.856 | 7.933 | 4.2 | 7.445(t, $J_{4,5}$=$J_{5,6}$= 7.8, H-5) 7.383(d, $J_{4,5}$= 7.5, H-4) 7.785(d, $J_{5,6}$= 8.1, H-6) | 2.339 |
| **N6** | 8.612 | 7.878 | 7.927 | 3.9 | 7.651(dd, $J_{5,6}$= 7.8, $J_{4,6}$= 1.5, H-6) 7.541(td, $J_{3,4}$=$J_{4,5}$= 8.1 Hz, $J_{4,6}$= 1.5, H-4) 7.313(d, $J_{3,4}$= 8.4, H-3) 7.128(t, $J_{4,5}$=$J_{5,6}$= 7.8, H-5) | 3.915 |

**Table 8: data of $^1$H-NMR spectra [δ (ppm), J (Hz)] of isoxazolidines**

| Compd. | H-3(s) | H3a(d) | H6a(d) | $J_{3a,6a}$ | H1′(d) | H2′(d) | $J_{1',2}$ | Aromatic C-H | Aliphatic C-H |
|---|---|---|---|---|---|---|---|---|---|
| **I1** | 5.783 | 4.334 | 5.242 | 7.5 | 6.606 | 7.380 | 3.7 | 6.601-6.650 (m, H-8, H-10)<br>7.341-7.419 (m, H-7, H-9, H-11)<br>7.185-7.319(m, 5H) | -------- |
| **I2** | 6.240 | 4.434 | 5.412 | 7.5 | 6.949 | 7.708 | 3.9 | 7.207(d, , $J_{7',8'}$=$J_{10',11'}$=9, H-7′, H-11′)<br>7.477(d, , $J_{7',8'}$=$J_{10',11'}$ =9, H-8′, H-10′)<br>7.399-7.417(m, , H-7, H-9, H-11)<br>6.705-6.729(m, H-8, H-10) | -------- |
| **I3** | 6.260 | 4.461 | 5.443 | 7.5 | 6.970 | 7.729 | 3.9 | 7.284(d, , $J_{7',8'}$ =$J_{10',11'}$ =9, H-7′, H-11′ )<br>7.381(d, $J_{7',8'}$ =$J_{10',11'}$ =9, H-8′, H-10′ )<br>7.413-7.464(m, H-7, H-9, H-11)<br>6.742-6.787(m, H-8, H-10) | ------- |
| **I4** | 5.613 | 4.460 | 5.600 | 7.5 | 6.916 | 7.646 | 3.9 | 7.485-7.617(m, H-′7, H-8′, H-9′, H-10′)<br>7.106-7.303(m, H-7, H-8, H-9, H-10, H-11) | ------- |
| **I5** | 6.175 | 4.422 | 5.377 | 7.5 | 6.955 | 7.717 | 3.9 | 6.639-6.671(m, H-8, H-10) 7.351-7.433(m, H-7,H-9,H-11)<br>7.163(t, $J_{8',9'}$ =J7′,8′=7.8,H-8′) 6.835(d, J8′,9′=7.5, H-9′)<br>7.009-7.072(m, H-7′, H-11′) | 2.210 |
| **I6** | 5.762 | 4.380 | 5.517 | 7.5 | 6.805 | 7.610 | 3.6 | 6.786-6.841(m, H-10′)<br>7.016-7.050(m, H-8, H-8′, H-10)<br>7.426-7.541(m, H-7, H-9, H-11)<br>7.142-7.166(d, H-7′, H-9′) | 3.866 |

The antibacterial activity of the prepared compounds (Tables 9 and 10) against Gram positive bacteria *Staphylococcus aureus* (ATCC 25923) and Gram negative *Escherichia coli* (ATCC 25922) showed that most prepared compounds exhibited a good activity as compared with standard drugs. In nitrones series, compound N6 exhibited good activity against bacteria (*S. aureus*, 25mm and *E. coli*, 18mm) as compared with N4 which had lower activity (*S. aureus*, 9mm and *E. coli*, 10mm). For isoxazolidines, I5 compound showed good activity against (*S. aureus*, 20mm and *E. coli*, 16mm) as compared with I4 which had lower activity against bacteria (*S. aureus*, 15mm and *E.coli*, no inhibition).

The antifungal activity of the prepared compounds against the pathogenic fungus *Aspergillus niger* (Table 11) showed that most prepared compounds had good activity as compared with standard drugs. Nitrones exhibited good activity as compared with isoxazolidines in which compound N4 showed good activity (24mm) in nitrone series. For isoxazolidines, compounds I3 and I5 had the higher activity (15mm).

**Table9: *In vitro* antibacterial activity and minimum inhibitory concentration(MIC) of prepared compounds against *Escherichia coli* (ATCC 25922)**

| **Compound** | ***E. coli* [ATCC 25922]** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Minimum inhibitory concentration (MIC) (mm)** | | | | | | |
| **Concentration (µg/ml)** | **1000** | **500** | **400** | **300** | **150** | **50** | **MIC** |
| **N1** | 11 | 8 | NI | NI | NI | NI | 500 |
| **N2** | 13 | 10 | 8 | NI | NI | NI | 400 |
| **N3** | NI | NI | NI | NI | NI | NI | NI |
| **N4** | 10 | 7 | NI | NI | NI | NI | 500 |
| **N5** | 13 | 9 | NI | NI | NI | NI | 500 |
| **N6** | 18 | 14 | 10 | 7 | NI | NI | 400 |
| **I1** | NI | NI | NI | NI | NI | NI | NI |
| **I2** | NI | NI | NI | NI | NI | NI | NI |
| **I3** | 13 | 8 | NI | NI | NI | NI | 500 |
| **I4** | NI | NI | NI | NI | NI | NI | NI |
| **I5** | 16 | 13 | 9 | NI | NI | NI | 400 |
| **I6** | 15 | 11 | 8 | NI | NI | NI | 400 |
| **Nitrofurazone** | 30 | ----- | ----- | ----- | ----- | ----- | ----- |
| **Nitrofurantoin** | 20 | ----- | ----- | ----- | ----- | ----- | ----- |

**Table 10:** ***In vitro*** **antibacterial activity and minimum inhibitory concentration (MIC) of prepared compounds against** ***Staphylococcus aureus*** **(ATCC25922)**

| **Compound** | ***S. aureus*** **[ATCC 25923]** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Minimum inhibitory concentration (MIC) (mm)** | | | | | | |
| **Concentration (µg/ml)** | **1000** | **500** | **400** | **300** | **150** | **50** | **MIC** |
| **N1** | 13 | 10 | 8 | NI | NI | NI | 400 |
| **N2** | 11 | 7 | NI | NI | NI | Ni | 500 |
| **N3** | 20 | 16 | 11 | 9 | NI | NI | 300 |
| **N4** | 9 | NI | NI | NI | NI | NI | NI |
| **N5** | 20 | 14 | 10 | 8 | NI | NI | 300 |
| **N6** | 25 | 19 | 14 | 10 | 8 | NI | 150 |
| **I1** | NI | NI | NI | NI | NI | NI | NI |
| **I2** | 15 | 10 | NI | NI | NI | NI | 500 |
| **I3** | 18 | 13 | 10 | 7 | NI | NI | 200 |
| **I4** | NI | NI | NI | NI | NI | NI | NI |
| **I5** | 20 | 15 | 10 | 8 | NI | NI | 300 |
| **I6** | 19 | 12 | 10 | NI | NI | NI | 400 |
| **Nitrofurazone** | 25 | ----- | ----- | ----- | ----- | ----- | ----- |
| **Nitrofurantion** | 21 | ----- | ----- | ----- | ----- | ----- | ----- |

NI= No Inhibition

**Table 11: Antifungal activity of prepared compounds and standard drugs against** ***Aspergillus niger*** **fungs**

| **Compound (1000µg/ml** | **Diameter of inhibition zone in (mm)** |
|---|---|
| **N1** | 19 |
| **N2** | 14 |
| **N3** | 17 |
| **N4** | 24 |
| **N5** | 13 |
| **N6** | 13 |
| **I1** | NI |
| **I2** | 10 |
| **I3** | 9 |
| **I4** | NI |
| **I5** | 9 |
| **I6** | NI |
| **Fluconazole** | 16 |
| **Nitrofurazone** | NI |
| **Nitrofurantoin** | NI |

The results of $LD_{50}$ showed that compounds N5 and N6 are moderately toxic substances, while compounds I3 and I5 are non toxic in the range of graded doses (Table12). according to the classification of Klassen and Doull.

**Table12: The number and percentage of mortalities of mice and LD50 values**

| Group number | Dose (mg/kg) | Mortality | | | Total No. of mortality | Mortality (%) | $LD_{50}$ (g/kg) |
|---|---|---|---|---|---|---|---|
| | | First day | Second day | Third day | | | |
| **Nitrone (N5)** | | | | | | | |
| **1** | 1000 | 0 | 0 | 0 | 0 | 0 | **2.4** |
| **2** | 1500 | 1 | 0 | 0 | 1 | 20 | |
| **3** | 2250 | 0 | 2 | 0 | 2 | 40 | |
| **4** | 2500 | 2 | 0 | 1 | 3 | 60 | |
| **5** | 3000 | 2 | 2 | 0 | 4 | 80 | |
| **6** | 4000 | 3 | 2 | 0 | 5 | 100 | |
| **Nitrone (N6)** | | | | | | | |
| **1** | 2500 | 0 | 1 | 0 | 1 | 20 | **3.5** |
| **2** | 4000 | 2 | 1 | 0 | 3 | 60 | |
| **3** | 4500 | 2 | 1 | 1 | 4 | 80 | |
| **4** | 5000 | 2 | 1 | 2 | 5 | 100 | |

## ***6. Conclusion.***

The present research includes the preparation of nitrones and their cycloaddition with N-phenylmaleimide. The data showed that most synthesized compounds exhibited a good antimicrobial activity, antibacterial and anifungal, against Gram-positive and Gram-negative bacteria and clinical pathogenic fungus as compared with the used standard drugs.The toxicity of some selected compounds (N5 and N6) is moderate according to Klassen and Doull toxicity scale. While compounds I4 and I5 showed no toxicity in the range of graded doses.

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# تحضير، تشخيص ودراسة الفعالية البيولوجية لبعض مركبات النايترون والايزوكسازولدين الجديدة

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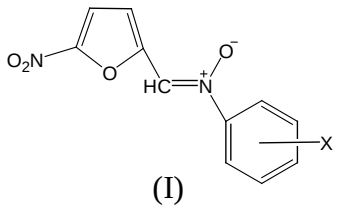
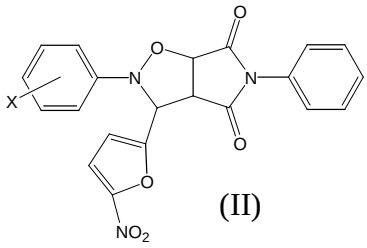
**المستخلص**

تضمنت الدراسة الحالية تحضير سلسلتين من المركبات الجديدة لمشتق النايتروفيوران. تضمنت السلسلة الأولى تحضير ستة من مركبات النايترون من تفاعل 5-نايتروفورفورال مع مشتقات ال N-أريل هيدروكسيل امين. أما السلسلة الثانية تضمنت تحضير مركبات الايزوكسازولدين من تفاعل الإضافة الحلقية نوع 3,1- ثنائي القطب لسلسلة النيترونات مع N-فنيل ماليميد.



(I)

X=H(N1), p-Br(N2), p-Cl(N3), o-Cl(N4)
m-Me(N5) and o-OMe(N6)



(II)

X=H(I1), p-Br(I2), p-Cl(I3), 0-Cl(i4)
m-Me(I5) and o-OMe(I6)

درست الفعالية البيولوجية للمركبات قيد الدراسة ضد البكتيريا باستخدام العزلات القياسية الموجبة والسالبة لصبغة الغرام(*Escherichia coli* ATCC 25922 , *Staphylococcus aureus* ATCC 25923) وأيضا الفعالية المضادة للفطريات باستخدام العزلات السريرية *Aspergillus Niger* إذ أظهرت النتائج إن اغلب المركبات ذات فعالية مضادة للبكتريا مقارنة بالمركبات الدوائية القياسية

بينت دراسة السمية إن بعض المركبات هي مواد متوسطة السمية بينما لم يمتلك البعض الاخرأية سمية ضمن المدى من الجرعات المعطاة للحيوانات المختبرة.