SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL EVALUATION OF NOVEL SERIES OF HYDROXAMIC ACIDS

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ABSTRACT
This study aims to prepare a novel series of non-cyclic hydroxamic acids which they are Currently occupies a wide fields because of their remarkable biological activities, through the synthesis of nitrones by the condensation of aldehydes and derivatives of aromatic or aliphatic hydroxylamine as a first step, then these nitrones were oxidized by lead Tetraacetate (LTA) to give N-acetoxy amide derivatives as a second step, which were hydrated in the acid middle to obtain the approval hydroxamic acids as a last step. Structures of the synthesized compounds were established by elemental analyses, FT-IR, 1HNMR. All the newly synthesized compounds were screened for their antibacterial and antifungal activities. The bioassays indicated that most of the synthesized compounds exhibited moderate to significant antibacterial and antifungal activities.

KEYWORDS: Nitrones , Acetoxy amide derivatives, hydroxamic acids, biological tests, elemental analyses.

INTRODUCTION
Hydroxamic acids refers to a class of chemical organic compounds having general formula R–CO–NHOH and R–CO–NR’OH. These compounds are weak organic acids with low toxicity. [1-2] Hydroxamic acids find many applications in chemistry and biology and have been the subject of many experimental investigations. Hydroxamic acids are very well known for their antibacterial[3-5], antifungal[6-7], antitumor[8-10], anti tuberculous[11] and antimalarial[12] properties. They have also been applied as food additives[13], growth factors[14], metal chelators[15], rare earth mineral collectors[16], inhibitors of various enzymes such as peroxidases[17], ureases[18], matrix metalloproteases[19], hydrolases[20], cyclooxigenases[21] and reagents for solvent extraction and spectrophotometric determination of metals[22].

In the present investigation, we carried out the synthesis of novel series of non-cyclic hydroxamic acids According to consecutive reactions. Where in the first step, we were prepared the nitrones by condensation of aldehydes and derivatives of aromatic or aliphatic hydroxylamine, then in the second step, these nitrones were oxidized by lead Tetraacetate (LTA) to give N-acetoxy amide derivatives, which were hydrated in the acid middle in the last step to give the approval hydroxamic acids. All these synthesized compounds were evaluated for their antibacterial and antifungal activity.

EXPERIMENTAL
MATERIAL AND METHODS
All solvents and reagents were purchased from Sigma Aldrich and Fluka. All melting points were measured on open capillary method. IR spectra were recorded for KBr on Perkin-Elmer FT-IR spectrometer and absorption frequencies (ν) are stated in cm⁻¹. The NMR spectra were recorded using Bruker DRX 400 spectrometer at 400 MHz for ¹HNMR with tetramethylsilane as the internal standard CDCl₃ solution were recorded on a Bruker DT-400 MHz spectrometer, and Chemical shifts (δ) are reported in parts per million (ppm). Reactions were monitored by thin layer chromatography (TLC) on silica gel, plates were visualizing with ultraviolet light or iodine.

General procedure for preparation of nitrones [23-24]
All nitrones are obtained by condensation of aldehydes with derivatives of aromatic or aliphatic hydroxylamine.

Preparation of nitrone 3a: A solution of N-benzyl hydroxylamine (0.5 g, 0.003 mol) in THF (10 ml) was taken and cooled to 0–5 °C in an ice bath. N-dimethylamine benzaldehydes (0.47g, 0.003 mol) in THF (5 ml) and Triethylamine (0.32 ml, 0.003 mol) in THF (2ml) were added drop by drop and respectively with magnetic stirring to the cold reaction mixture. The resulting mixture was allowed to stir at 0–5 °C for un hour. The solvent was evaporated under vacuum and the
product was purified by recrystallization from ethanol/petroleum ether to obtain the nitrone 3a.

The nitrone 3b was prepared similarly by using different arylaldehyde (3-hydroxy-4-methoxybenzaldehyde).

Nitrone 3a: Yield 54%; orange solid; Rf = 0.57 ; mp: 151. IR (KBr, ν, cm⁻¹): 1604 (C=N), 1183(N→O), 1231 (C=N), 1366 (C-H). ¹HNMR (CDCl₃, δ, ppm): 7.30-7.70 (m,5H), 8.15 (m, 2H), 6.71 (s,1H), 5.03 (s,2H), 3.09 (s,3H).

Nitrone 3b: Yield 68%; yellow solid; Rf = 0.69; mp: 134. IR (KBr, ν, cm⁻¹): 1687 (C=N), 1149(N→O), 1252(C-O), 3084(O-H). ¹HNMR (CDCl₃, δ, ppm): 7.21-7.60 (m,5H), 7.91(m,2H), 6.93(s,1H), 4.92(s,2H), 3.77(s,3H), 3.39(s,1H).

Preparation of nitrone 3c
A solution of N-methylhydroxylamine hydrochloride (0.5 g, 0.005mol) in THF(10 ml) was taken and cooled to 0–5 °C in an ice bath. N-dimethylaminobenzaldehyde (0.89g, 0.005 mol) in THF (5 ml) and Triethylamine (0.62 ml, 0.005 mol) in THF (2ml) were added drop by drop and respectively with magnetic stirring to the cold reaction mixture. The resulting mixture was allowed to stir at 0–5 °C for 1 h. The solvent was evaporated under vacuum and the product was purified by recrystallization from ethanol/petroleum ether to obtain the nitrone 3c. The nitrone 3d was prepared similarly by using different arylaldehyde (3-hydroxy-4-methoxybenzaldehyde).

Nitrone 3c
Yield 78%; orange solid; Rf = 0.65 ; mp: 177. IR (KBr, ν, cm⁻¹): 1583(C=N), 1146(N→O), 1279 (C=N), 1444(C-H). ¹HNMR (CDCl₃, δ, ppm):7.73 (dJ=8.2Hz, 1H), 7.23(d,J=7.6 Hz, 1H), 6.65(s,1H), 3.76(s,3H), 3.06 (s,3H).

Nitrone 3d
Yield 81%; yellow solid; Rf = 0.73 ; mp: 189. IR (KBr, ν, cm⁻¹): 1679 (C=N), 1118(N→O), 1244 (C-O), 3103 (O-H). ¹HNMR (CDCl₃, δ, ppm): 7.5-8.10 (m,3H), 6.71(s,1H), 3.89(s,3H), 4.00 (s,1H), 2.28 (s,3H).

General procedure for preparation of N-acetoxy amide\(^{[26-29]}\) (4a-4d)
The nitrones (3a-3d) (0.002 mol) were dissolved in benzene. To each solution, Lead Tetra acetate (LTA) was gradually added with magnetic stirring. The resulting mixture was allowed to stir at room temperature for 3 h. It was set-aside at room temperature overnight. The formed product was filtered to separate the precipitate and the solvent was evaporated under vacuum. The residue was purified by recrystallization from ethanol/petroleum ether to obtain solid or oil product (4a-4d) Depending on the type of nitrone.

**Compound 4a:** Yield 76%; dark orange solid; Rf = 0.59; mp: 120. IR (KBr, ν, cm⁻¹): 1595 (C=O), 1793 (-OOCOCH₃). ¹HNMR (CDCl₃, δ,ppm): 7.36(m,5H), 7.77-7.90(m.,2H), 3.11(s,3H), 1.26 (s,3H), 3.83(s,2H).

**Compound 4b:** Yield 73%; Brown solid; Rf = 0.66 ; mp: 194. IR (KBr, ν, cm⁻¹): 1657 (C=O), 1811 (-OOCOCH₃). ¹HNMR (CDCl₃, δ,ppm): 7.59 (m,5H), 7.13-7.90 (m, 3H), 3.46 (s,2H), 3.11(s,3H), 5.03(s,1H),3.72(s,3H).

**Compound 4c:** Yield 84%; dark orange solid; Rf = 0.61; mp: 118. IR (KBr, ν, cm⁻¹): 1661 (C=O), 1792 (-OOCOCH₃). ¹HNMR (CDCl₃, δ,ppm): 6.67- 8.04 (m, 2H), 3.86 (s,3H), 1.89(s,3H), 3.1(s,3H).

**Compound 4d:** Yield 81%; brown solid; Rf = 0.58; mp: 203. IR (KBr, ν, cm⁻¹): 1650 (C=O), 1801 (-OOCOCH₃). ¹HNMR (CDCl₃, δ,ppm): 6.98-8.30 (m,3H), 4.95 (s,1H), 3.14(s,3H), 3.33 (s,3H),2.34(s,3H).

**General procedure for preparation of hydroxamic acids\(^{[26-29]}\) (5a-5d)**
The N- acetoxy amide (4a-4d) (0.005 mol) were dissolved in HCL (10%). Each reaction mixture was heated to reflux for 3 hours. After cooling, the resulting mixture was washed with 10 % sodium-bi-carbonate solution (Na₂CO₃), treated with diethyl ether and then it was dried with anhydrous sodium sulphate. The ether was evaporated and the residue obtained was recrystallized from ethanol/petroleum ether to give the compounds (5a-5d).

**Compound 5a:** Yield 55%; orange solid; Rf = 0.52 ; mp: 210. IR (KBr, ν, cm⁻¹): 1657 (C=O), 3083 (O-H). ¹HNMR (CDCl₃, δ,ppm): 7.24(m,2H), 7.53-7.78 (m.,5H), 3.28 (s,2H), 9.76 (s, 1H), 3.14 (s,3H).

**Compound 5b:** Yield 49%; brown solid; Rf = 0.64 ; mp: 233. IR (KBr, ν, cm⁻¹):1666 (C=O), 3056 (O-H). ¹HNMR (CDCl₃, δ,ppm): 7.22(m,3H),3.10(s, 1H),7.33-8.13 (m.,5H), 4.04 (s,2H), 9.82(s, 1H), 3.82(s,3H).

**Compound 5c:** Yield 55%; yellow solid; Rf =0.51; mp: 212. IR (KBr, ν, cm⁻¹): 1646(C=O), 3312 (O-H). ¹HNMR (CDCl₃, δ,ppm): 7.53-7.78 (m.,3H), 9.66 (s,1H), 2.10(s, 3H), 3.98(s,3H).

**Compound 4d:** Yield 62%; yellow solid; Rf =0.73 ; mp: 149. IR (KBr, ν, cm⁻¹):1650(C=O), 3343 (O-H). ¹HNMR (CDCl₃, δ,ppm): 9.56(s,1H), 7.12-7.93 (m.,3H), 3.79(s,3H),3.17(s, 1H), 2.50(s,3H).
Antimicrobial activity
The in vitro biological screening effect of the synthesized compounds was tested against Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter*, *serratia*, and Gram positive bacteria *Staphylococcus aureus*, *streptococcus*, and the fungi *Candida albicans* and *candida kefyre* by disc diffusion method.[28-29] Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar. The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 mins. The tests were conducted at 1000 μg/disc. The loaded discs of 10 mm diameter were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. The plates were incubated for 24 h at 37°C for bacteria and 48 h at 37°C for fungi. Zone of inhibition was recorded in millimeters and the experiment was repeated twice.

RESULTS AND DISCUSSION
In the present work, a novel series of non-cyclic hydroxamic acids (5a-5d) were synthesized through the Synthesis of non-cyclic nitrones (3a-3d) by the condensation of aldehydes and derivatives of aromatic or aliphatic hydroxylamine as shown by (the scheme 01) below.

![Scheme 01](image)

Then the nitrones (3a-3d) were oxidized by lead Tetra–acetate (LTA) to give N-acetoxy amide derivatives (4a-4d) as shown by (the scheme 02) below.

![Scheme 02](image)

The N-acetoxy amide derivatives (4a-4d) were hydrated in the acid middle for to get the hydroxamic acids (5a-5d) as shown by (the scheme 03) below.

![Scheme 03](image)
Infrred spectra.

Spectrum (KBr, cm\(^{-1}\)) of the N-acetoxy amide derivatives (4a-4d) showed the absence of absorption band corresponding to (C=\(\equiv\)N group which related to nitrone (3a-3d), while exhibiting characteristic new absorption bands between the range (1792-1811) cm\(^{-1}\) corresponding to (OCOCH\(_3\)) group and between the range (1720-1725) cm\(^{-1}\) for the (C=O) amide. Also, hydroxamic acids (5a-5d) characterized by the appearance of new bands between (3200-3150) cm\(^{-1}\) for (O-H) and disappearance of absorption band corresponding to (OCOCH\(_3\)) group, which confirmed the formation of hydroxamic acids (5a-5d).

Nuclear Magnetic Resonance Spectra

The \(^1\)HNMR spectra of N-acetoxy amide derivatives (4a-4d) showed the absence of absorption signal corresponding to NH group which related to nitrone (3a-3d), while exhibiting characteristic absorption signals corresponding to the (OCOCH\(_3\)) group at (2-2.5) ppm. Additionally, the \(^1\)HNMR spectra of hydroxamic acids (5a-5d) appeared new signal corresponding to the OH group at (9.56-9.82) ppm.

Antimicrobial activity

The antibacterial activity of the prepared compounds against Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* sp, *serratia* sp and *Gram positive bacteria Staphylococcus aureus*, *stertpocoque* sp showed that most prepared compounds exhibited a moderate to good activity as shown in Table 01. In nitrones series, compound 3a exhibited good activity against bacteria *Enterobacter* sp and *Escherichia coli* as compared with other synthesized nitrones. Whereas, The nitrone 3b showed no antibacterial activity on the most of the species of bacteria used, in the case of *Staphylococcus aureus*, it was effective moderately.

In N-acetoxy amide derivatives series, compounds 4a and 4b showed good activity against bacteria *Pseudomonas aeruginosa* as compared with 4c which had moderate activity. Whereas, compound 4d exhibited good activity against bacteria *Escherichia* as compared with other synthesized N-acetoxy amide derivatives.

In hydroxamic acids series, compounds 5a and 5b showed lower activity against the species of bacteria used as compared with other synthesized hydroxamic acids. Whereas, compound 5c showed good activity against bacteria *Staphylococcus aureus* and *stertpocoque* sp. While, Compound 5d exhibited good activity against bacteria *serratia* sp.

The antifungal activity of the prepared compounds was studied on *Candida albicans* and *candida ksfere*.

The results of fungicidal screening show that among all the tested hydroxamic acids, compounds 5a and 5c showed good activity while the compound 5d was effective moderately whereas, the compound 5b showed no antifungal activity on the species of fungi used in this study as shown in Table 02.

Table 01: antibacterial activity of prepared compounds against the species of bacteria used

<table>
<thead>
<tr>
<th>Compounds</th>
<th>E.coli</th>
<th>S.aureus</th>
<th>Serratia.sp</th>
<th>P. aeruginosa</th>
<th>Enterobacter.sp</th>
<th>Sterptocoque. sp</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>11</td>
<td>08</td>
<td>12</td>
<td>13</td>
<td>&lt;6</td>
<td>07</td>
</tr>
<tr>
<td>3b</td>
<td>&lt;6</td>
<td>10</td>
<td>13</td>
<td>&lt;6</td>
<td>&lt;6</td>
<td>&lt;6</td>
</tr>
</tbody>
</table>
Table 02: Antifungal activity of hydroxamic acids (5a-5d) against the species of fungi used

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Diameter of inhibition zone (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Candida albicans</td>
</tr>
<tr>
<td>5a</td>
<td>24</td>
</tr>
<tr>
<td>5b</td>
<td>&lt;6</td>
</tr>
<tr>
<td>5c</td>
<td>46</td>
</tr>
<tr>
<td>5d</td>
<td>13</td>
</tr>
</tbody>
</table>

CONCLUSION
The present research includes the preparation of hydroxamic acids by three stages:
a) Synthesis of nitrones from condensation of aldehydes and derivatives of aromatic or aliphatic hydroxylamine.
b) Synthesis of N-acetoxy amide derivatives from nitrones which oxidized by LTA.
c) Synthesis of hydroxamic acids from N-acetoxy amide derivatives which hydrated in the acid middle.

The data showed that most synthesized compounds exhibited a moderate to good antibacterial and antifungal, against the species of bacteria and fungi used. These results are promising and open up the prospects in the synthesis of new biomolecules.

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