Preparation Of Some Hydroxamic Acid Derivatives and Study of their Biological Activity as Anti-Cancer and Anti-Bacterial Agents

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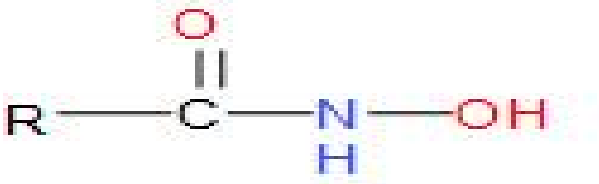
Abstract

In this research, the following hydroxamic acid compounds, N1, N2 -dihydroxyphthalamide and N1, N3 -dihydroxymalonamide, were prepared. And that is through the direct interaction between the primary esters of these compounds with hydroxylamine hydrochloride and using sodium hydroxide as a base medium PH (12-13) and the formation of hydroxamic acid was detected using the ferric ion as a primary reagent, and we infer that through the appearance of a pinkish-red color. The compounds after reacting with acetyl chloride to the following derivatives N 1, N2 -Di Acetyl phtalamide, N 1, N2 -Di Acetyl malonamide, these compounds were purified using the recrystallization process, and the formation of these compounds was also detected using the ferric ion, as there was no change in the color of the solution, as well as the melting points were set and these were diagnosed The compounds prepared using the spectrum of FT.IR)) (H.NMR), and (C13 NMR). The biological activity of these prepared compounds N1, N2 -Di Acetyl phtalamide, N1, N2 -Di Acetyl malonamide was studied by using tissue culture of cell cultures. breast cancer type (MCF-7) By promoting this type of cancer cells in a culture medium (ROMI-1640) and using the compound (MTT) method, these compounds gave high efficacy against cancer cells at a concentration of less than (50 micromoles) and a value of TC 50 = 26.85 mg/ml for compound N 1 , N2 -Di Acetyl phtalamide with a value of TC 50 = 22.52 mg/ml for the compound, N 1, N2 -Di Acetyl malonamide. The results showed that derivatives of hydroxamic acids are powerful against cancer cells, especially breast cancer type (MCF-7), and the effect of these compounds has also been studied. prepared on two types of positive and negative bacteria (E scherichiacoli Staphylococcus), where this study showed that the prepared compounds had a high inhibition of bacteria at a concentration (500 mg/ml).

1. Introduction

Hydroxamic acid is considered one of the natural and synthetic weak organic acids (1), and it is one of the most widely studied chemical compounds due to its enormous applications in various fields (2), as the general formula of hydroxamic acid is as follows:



Whereas, R is a saturated and unsaturated alkyl group, or a substituted aryl group. In general, the R is an organic residue, CO is a carbonyl group, and an amine hydroxyl group, and the active group retains its properties regardless of the composition of the rest of the molecule (3).

Despite the important properties of hydroxamic acids, it is one of the least compounds whose properties have been described, which constitutes difficulty in determining the correct composition of hydroxamic acid, because it is in three identical forms in the chemical compositions (tautomeric forms) and as shown in the structural formulas (4) as shown in Figure (1).

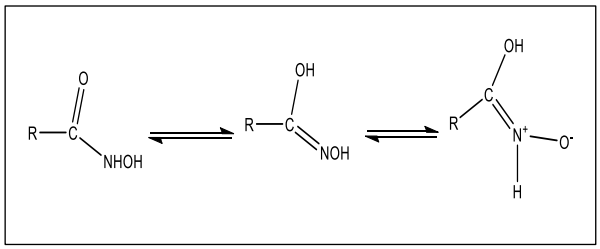


Figure (1) General formulas for hydroxamic acid

The chemical properties of hydroxamic acids have been neglected for a long time, but a number of recent research have shown the composition and chemical behavior of these compounds (5).

Recent developments in the chemistry of hydroxamic acid stimulated the isolation and separation of some natural compounds found primarily in fungi and algae, which are effective antibiotics against growth factors or cell division in malignant tumors (6). Hydroxamic acid also plays an important role in iron absorption during metabolic processes.

2. Experimental

Materials used

Hydroxyl amine hydrochloride: Sigma Aldrich (99.0 %), Ethyl Malonate: Aldrich (99.5%), Di butyl

5doi.org/10.31838/hiv23.01.137 Received: 15.09.22, Revised: 21.10.22, Accepted: 12.12.22

Phthalate: Aldrich (98.0%), Acetyl chloride: Merck (98.0%), Sodium Hydroxide: Aldrich (98.0%), Ethanol Absolute: Alpha Chemika (99.9%), Pyridine: Sigma Aldrich (98.0%).

Instruments

Sensitive Balance, Magnetic hote Plate Stirrer, FT-IR Spectrophotometer, H^1, C^{13}-NMR, FT-IR Spectrophotometer

Preparation of hydroxamic acid N 1, N2 -dihydroxyphthalamide from the ester (Di butyl phthalate) (D3)

In a circular flask with a capacity of (50) ml and equipped with a magnetic stirrer and a condenser, equal molar amounts were mixed in a ratio (1:1) of the ester Methyl benzoate and Hydroxyl amine hydrochloride, where (0.15 Mol) (10 grams) of Hydroxyl amine hydrochloride was dissolved in (50) ml of methanol and heated slightly To complete the dissolving process, after dissolving, (0.15 Mol) is added in (19) ml of methyl benzoate, then the second solution is added to the first, then a strong base such as NaOH or KOH is added to the solution until the PH becomes from 12-13 Then the flask is connected to a reflective condenser and thermal escalation is performed for half an hour. After the end of the escalation period, the reaction mixture is cooled in an ice bath, then the precipitate is filtered, then washed and dried to be recrystallized after that. and H1N.M.R, C13. N.M.R. (7)

In the same way, the compound N 1, N 3 -di hydroxy malonamide was prepared(D5)

Preparation of hydroxamic acid derivatives.

Preparation of N1, N2 -Di Acetyl phtalamide. (DA3)

0.1 Mol ((4.0 g) of N-hydroxy benzamide was dissolved in 50 ml of pyridine in a conical flask with a capacity of ((75 ml)), the reaction mixture was cooled using an ice bath, then the acetyl chloride solution was added to the mixture in drops, while maintaining that the temperature did not rise The temperature of the mixture was about (50C) and the mixture was stirred at room temperature for (6) hours. The mixture was filtered and the precipitate was taken, then a recrystallization process was performed for it, and the derivative was confirmed from the first detection procedure by taking a small amount of the precipitate in a test tube and adding drops of a solution containing ions to it Ferric, where the color of the solution did not change compared to hydroxamic acid compounds that give a reddish-pink color (8) . The melting point was determined, and the resultant was diagnosed spectrophotometric ally by FTIR spectrum and H1N.M. R, C13. N.M. R

In the same way it was prepared N1, N2 -Di Acetyl malonamide. (DA5) -

Evaluation of the effectiveness of some prepared hydroxamic acids as anti-cancer.

Tissue culture

The biological activity of some prepared hydroxamic acids (N 1, N2 dihydroxyphthalamide and N 1, N 3 -dihydroxymalonamide) was investigated in this study using MCF-7 breast cancer cells. This type of cancer cells was cultured in RPMI culture medium. – 1640 prepared from the Italian company Eroclon with 10% of fetal bovine serum and 100 units/ml of penicillin and µ100 g/ml of streptomycin. The cells were passed using Trypsin – EDTA replanted at a confluence of 80% twice a week in an incubator in Humid atmosphere at a temperature of 37°C and 5% carbon dioxide (9,10) Examination of efficacy against cancer cells. The efficacy against cancer cells was measured using the method well known in the field known as Method using an MTT compound 3-[4,5-dimethylthiazol-2-yl-2,5-diphenyltetrazolium bromide] (MTT)

Where the MTT solution (provided by the Chinese company Macklin) was prepared with a concentration of (5 mg / L) by dissolving the MTT powder in a solution of Phosphate Buffer Saline (PBS).

Well sterilized. Then, 1 x 104 cancer cells were implanted in each hole of the 96-hole plastic cell well. And then adding certain concentrations of some prepared hydroxamic acids, which are N 1, N2 -dihydroxyphthalamide and N 1, N 3 –di hydroxy malonamide (12.5, 200, 100, 50, 25) micro molar and dissolved in the dimethyl sulfoxide solvent (Di methyl Sulphoxide - DMSO). For a period of 24 hours or 48 hours, three holes were used for each substance to be measured for the purpose of making sure of the result when measuring its effectiveness against cancer cells. After the end of the incubation period, the culture medium was removed from all the holes, then the holes were washed with PBS solution to remove all cancer cells that were not attached to the bottom of the holes. Then (28) micrometers of the prepared MTT solution, whose concentration was (2 mg/L), were added and the cells were incubated for 2.5 hours at 370 C. After that, the plate containing these 96 holes was incubated in a dark incubator for 3 hours at a temperature of 37 0 C, before adding ((130 microliters of solvent ((DMSO) that works to dissolve the unreacted MTT, which is a blue color solution, followed by an incubator 37 0 C for 15 minutes

The compound or substance is effective against cancer cells when the survival rate of cancer cells is less than 50%. (11,12)

Evaluation of the effectiveness of some prepared hydroxamic acids as antibacterial.

The biological activity of some prepared hydroxamic acid compounds was studied. This study included the use of isolated and laboratory-diagnosed pathogenic bacteria varieties using chemical and microscopic tests. It includes two types of Gram-positive bacteria and Gram-negative

bacteria. These isolates are among the causes of many diseases. Two types of pathogenic bacteria were used. Which:

1. Escherichia coli
2. Staphylococcus aureus

Culture Media:

According to the instructions of the company producing the culture medium, the nutrient agar medium was used, and this medium was used to feed and multiply germs of different types (12,13) where we weighed 5.6 grams of Nutrient agar powder in the sensitive balance and dissolved it in 200 ml of distilled water in a glass beaker, then We stir the beaker so that the powder dissolves and homogenizes with distilled water, then we close the beaker, and then we carry out the sterilization process by placing the beaker for half an hour with the Autoclave device, which is a pressure device that controls temperature, pressure and time required for sterilization by placing it for half an hour at a temperature (120 c) and under pressure (15 pounds / inch) after cooling the medium, it was poured into sterilized dishes ((Petri disk) and left to solidify, and then the dishes were placed in the incubator (Incubators) for a period of 24 hours at (37 0C).(13,14)

Preparation of the concentrations of the prepared compounds:

Concentrations of some prepared hydroxamic acids were prepared using the DMSO solvent and concentrations of (62.5, 125, 250, 500) mg/ml.

3. Results and discussion

Through the infrared spectrum ((FT-IR), the prepared compounds were identified, which are N 1, N2 -dihydroxyphthalamide and N 1, N 3 -dihydroxymalonamide, and N1, N2 -Di Acetyl phtalamide and N1, N2 -Di Acetyl malonamide it indicated the appearance of an absorption band for the active groups of the formed material, which is represented by the appearance of vibration stretch absorption bands for the carbonyl group Amides ((C=O), in addition to the primary amine adsorption band ((NH) and the appearance of a vibrational adsorption band for the hydroxyl group ((O-H) and, in addition to the aromatic adsorption bands (C-H arom), and the aliphatic adsorption bands ((C-H aliph), as shown in Table (1) The FT-IR values of the prepared compounds.(15 ,16)

Table (1) values of absorption bands in the infrared spectrum for the prepared hydroxamic and derivatives of hydroxamic acids compounds.

Major FTIR Absorption cm-1						
V C=C	V O-H	V N-H	V C=O	V C-H aliphatic.	V C-H arom.	Symb.
1576.45	3300.80	3380.56	1740.45	--	3060.70	D3
----	3203.14	3387.49	1656.07	2908.78	--	D5

V C=C	V N-H	V C=O	V C-H aliphatic.	V C-H arom.	Symb.
1482.91	3436.85	1786.92	2958.11	3085.37	DA3
----------	3329.59	1712.35	2956.03	----------	DA5

Whereas, the structural formula of the compounds was confirmed by proton nuclear magnetic resonance spectroscopy, H1-NMR, as shown in the figures shown below. (17 ,18)

Whereas, the compound N 1, N2 -dihydroxyphthalamide prepared as shown in Figure (2) showed a single signal at [δ=(3.69) ppm, (s, 2H), -CH2-] belonging to the protons of the methyl group, and as indicated by the complementary area of validity. Its yield, which is equal to three protons, and a single signal appeared at [δ=(7.83) ppm, (s,1H), -C=O-NH-] belonging to the proton of the amide group. Monomeric at [δ=(9.56) ppm ,(s,1H) , -NH-OH] belonging to a proton of the hydroxyl group with an integral area of one proton.

As for the compound N 1, N2 -dihydroxyphthalamide prepared as shown in Figure (3), it showed multiple signals between [δ=(7.43-7.49) ppm, (m,4H), -Ar-H.] belonging to the protons of the benzene ring as indicated. The integral space for the validity of its yield, which is equal to four protons, and a single signal appeared at [δ=(7.88) ppm ,(s,1H), -C=O-NH-] belonging to the proton of the amide group, as indicated by the integral area of the validity of its yield, which is equal to one proton And a single signal appeared at [δ=(10.31) ppm ,(s,1H) , -NH-OH] belonging to the proton of the hydroxyl group with an integral area of one proton.

As for the prepared (AD3) compound, a single signal at [δ=(1.97) ppm ,(s,6H), -(CH3)2.] refers to the protons of the two methyl groups, and as indicated by the complementarity area of the correctness of its return, which is equal to six protons, and a single signal appeared at [δ=(3.50) ppm ,(s,2H), -CH2-] refers to the proton of the methyl group, and as indicated by the complementarity area for the validity of its return, which is equal to two protons, and a single signal appeared at [δ=(11.31) ppm,(s,2H) , -C=O-NH] refers to the proton of the amide group with a complementary space of two protons.

As for the prepared (AD5) compound, a single signal at [δ=(2.33) ppm, (s,6H), -(CH3)2.] belongs to the protons of the two methyl groups, and as indicated by the complementarity area of the validity of its return, which is equal to six protons, and a multiple signal appeared between [δ=(7.59-7.80) ppm ,(m,4H), -Ar-H.] refers to the protons of the aromatic ring, and as indicated by the integral area of the correctness of its return, which is equal to four protons, and a single signal appeared at [δ=(11.92) ppm, (s,2H) , -C=O-NH] refers to the two protons of the amide group, with an integral area of two protons.

The prepared compounds were also identified

using carbon-13C-NMR spectroscopy, as described:(19,20)

While the compound (D3) showed a signal at [δ=162.20ppm, (-C=O)] due to carbon (C7,7/), while the carbon atoms of the aromatic ring were observed within the range [δ=121.31-136.59 ppm, (C aromatic ring)] refers to the aromatic ring.

As for compound (D5), it showed a signal at [δ=164.95ppm, (-C=O)] belonging to carbon (C1,1/), and a signal at [δ=35.44ppm, (-CH2-)] belonging to carbon (C2).

The compound ((DA3) showed a signal at [δ=170.62ppm, (-C=O)] belonging to a carbon (C8,8/), and a signal at [δ=168.09ppm, (-C=O)] belonging to a carbon (C7 ,7/) As for the carbon atoms of the aromatic ring, their signals were observed within the range [δ=127.34-133.61 ppm, (C aromatic ring)] refer to the aromatic ring, and a signal at [δ=23.66ppm, (-CH3)] belongs to carbon (C9,9/).

As for the compound ((DA5), it showed a signal at [δ=171.96ppm, (-C=O)] belonging to a (C2,2/) carbon, and a signal at [δ=168.58ppm, (-C=O)] belonging to a carbon (C3,3/), and a signal at [δ=44.21ppm, (-CH2-)] belongs to a carbon (C4), and a signal at [δ=25.31ppm, (-CH3)] refers to a carbon (C1,1/) .

Based on the foregoing, this study aims to prepare an effective drug or treatment against cancer cells in general and against breast cancer cells in particular.

Based on the foregoing, this study aims to prepare an effective drug or treatment against cancer cells in general and against breast cancer cells in particular.

It is known that breast cancer cells are of several types, and in this study, one type of breast cancer cells was studied, which is MCF-7 type, and the effect of some prepared hydroxamic acid derivatives on this type of cancer cells was studied using certain concentrations of hydroxamic acids dissolved in ((DMSO, which works as a good solvent for the prepared citrus derivatives. The figures below show the anti-cancer activity with the prepared citrus derivatives (DA3, DA5).

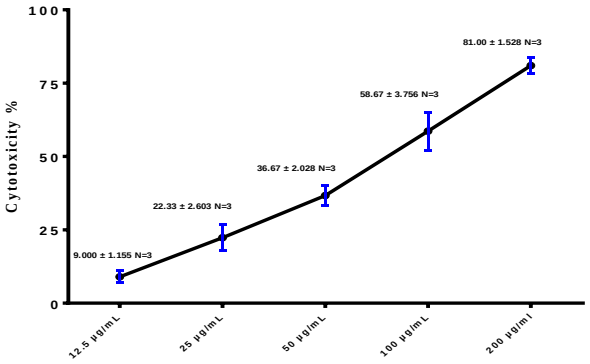


Figure 1: Cytotoxicity of DA3 in MCF-7 cells after 72 hr. IC50 = 26.85 µg/ml

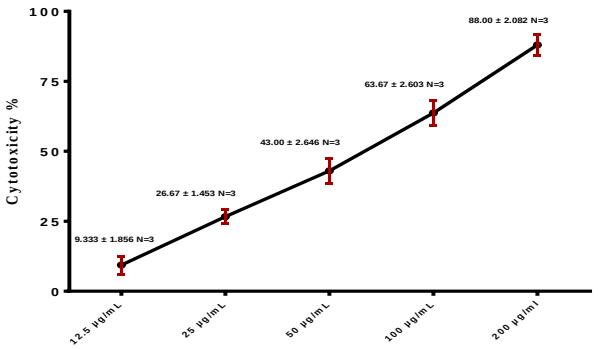
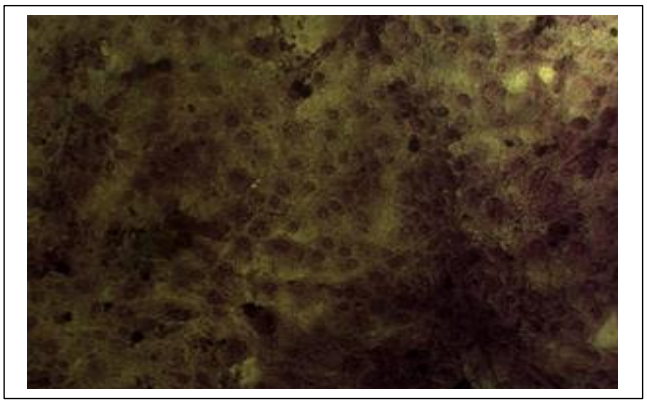
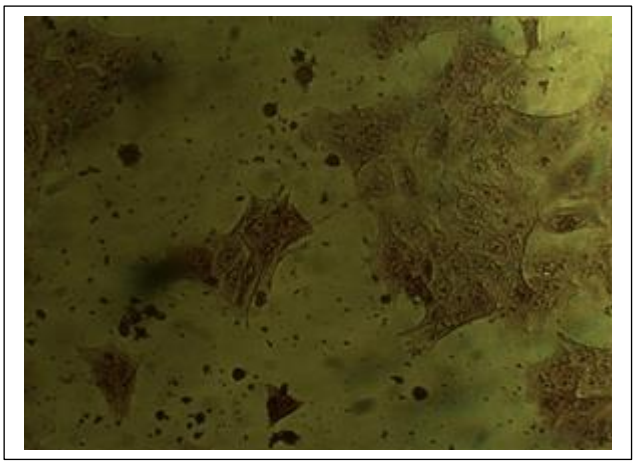


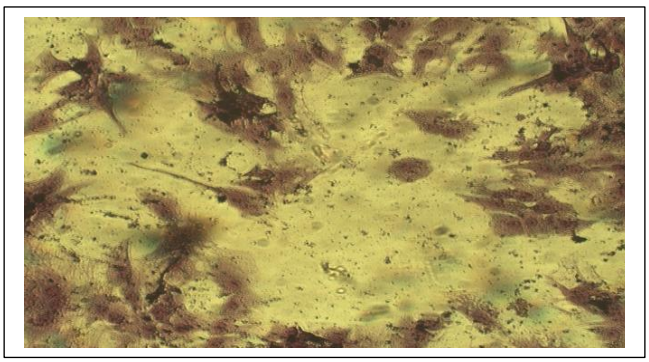
Figure 2: Cytotoxicity of DA5 in MCF-7 cells after 72 hr. IC50 = 22.52 µg/ml



Control untreated MCF-7. Magnification power



Morphological changes of MCF-7cells after treated with DA3. Magnification power 40x



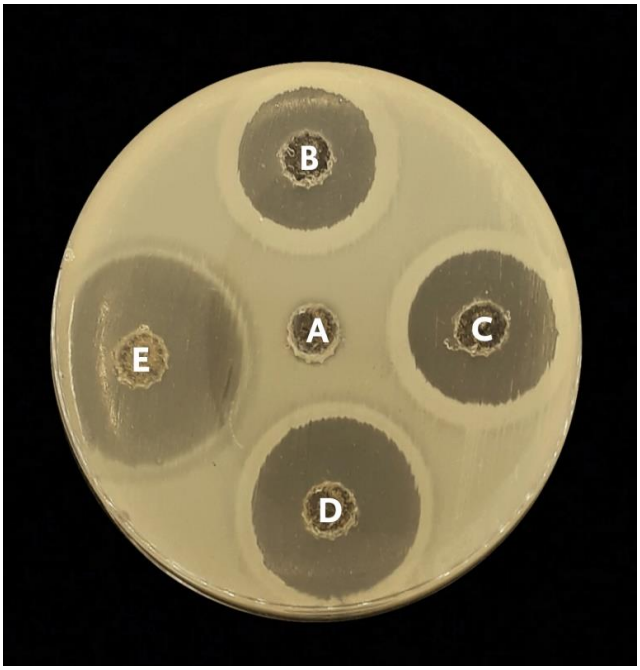
Morphological changes of MCF-7cells after treated with DA5. Magnification power 40x

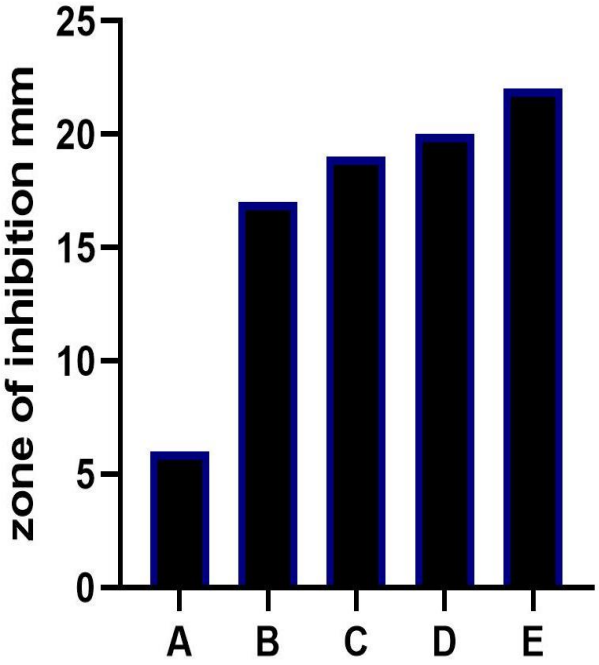
From the figures, it is clear that the prepared compounds (DA3, DA5) gave effectiveness against cancer cells at a concentration less than (50 micro molar) with a value of (26.85 µg/ml IC50 = for the compound of DA3) and with a value of (IC50 =

22.52 µg/ml for the compound (DA5), and the results showed that Hydroxamic acid derivatives gave a strong effect against cancer cells, especially breast cancer type MCF-7, due to its ability to penetrate the lipid membrane of the cancer cell and go to the specific location of the cancer cell.

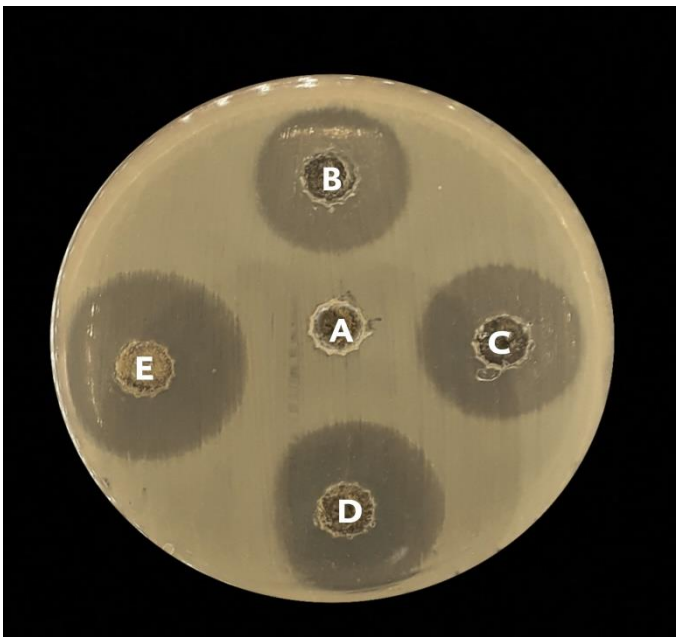
High antibacterial properties of some prepared hydroxamic acid derivatives.

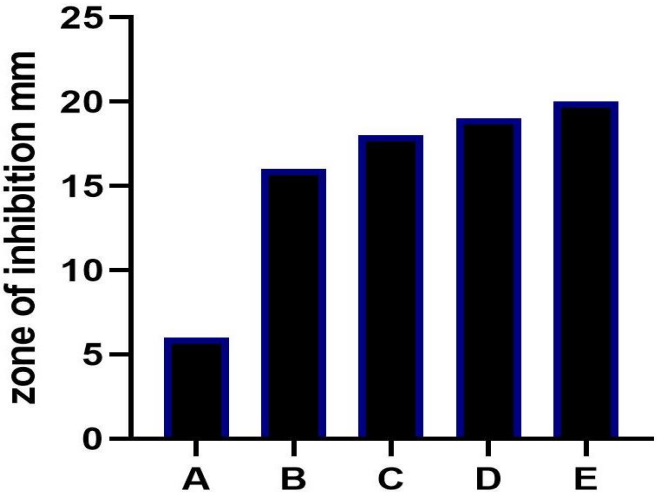
The effect of some hydroxamic acid derivatives prepared in this research was studied on two types of positive and negative bacteria, namely: Escherichia coli and Staphylococcus.



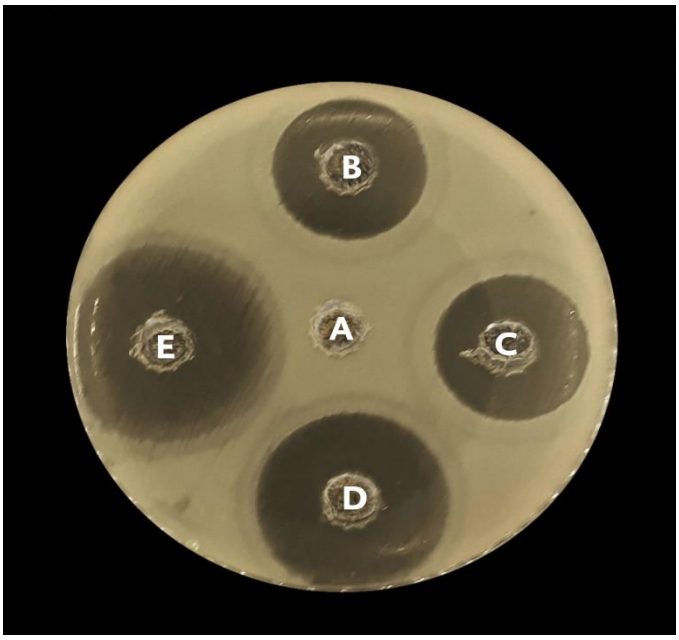


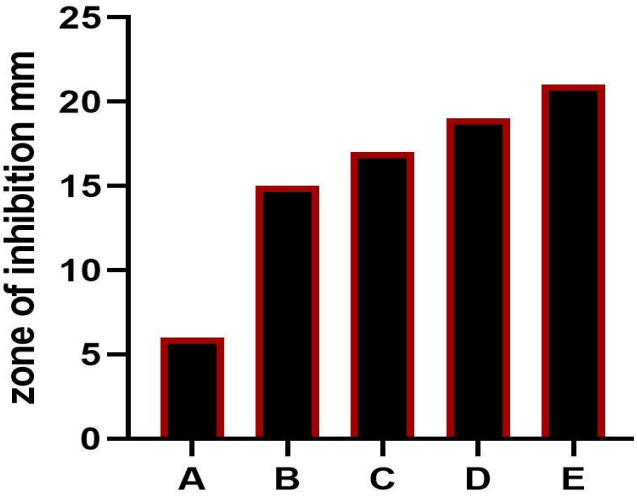
Antibacterial activity of (DA3) against Escherichia coli. A, control. B, 62.5 mg/ml. C, 125 mg/ml. D, 250 mg/ml. E, 500 mg/ml



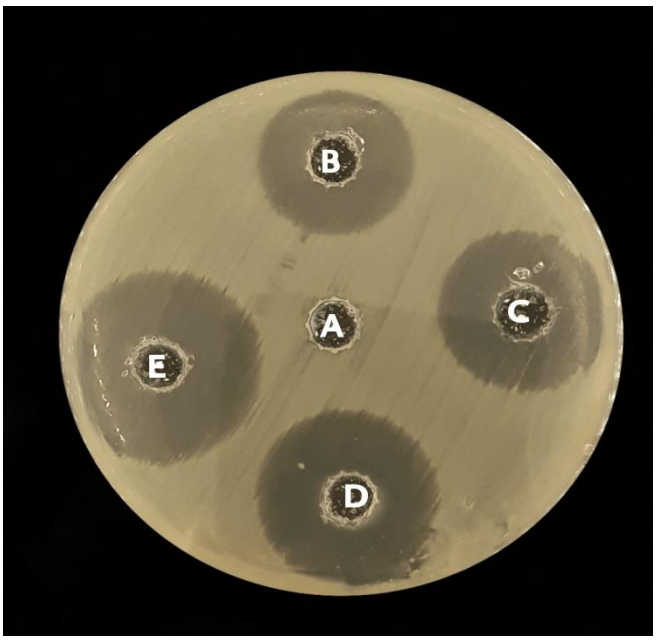


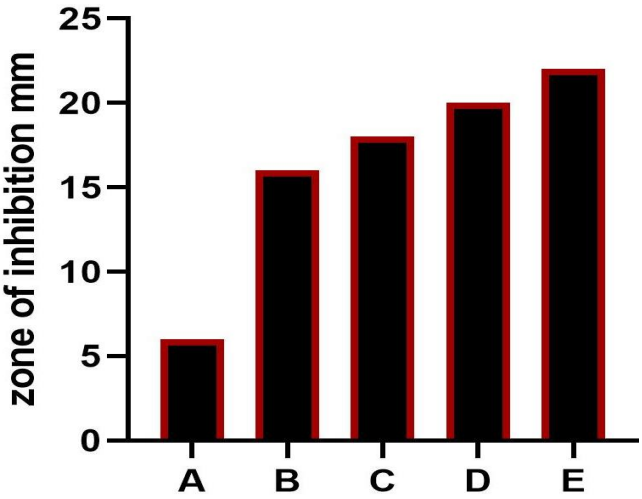
Antibacterial activity of (DA3) against Staphylococcus. A, control. B, 62.5 mg/ml. C, 125 mg/ml. D, 250 mg/ml. E, 500 mg/ml.





Antibacterial activity of (DA5) against Escherichia coli. A, control. B, 62.5 mg/ml. C, 125 mg/ml. D, 250 mg/ml. E, 500 mg/ml.





Antibacterial activity of (DA5) against Staphylococcus. A, control. B, 62.5 mg/ml. C, 125 mg/ml. D, 250 mg/ml. E, 500 mg/ml.

From the above forms, it was shown that the hydroxamic acid derivatives prepared in this research had a high inhibition of the bacteria used at a concentration of 500 mgml.

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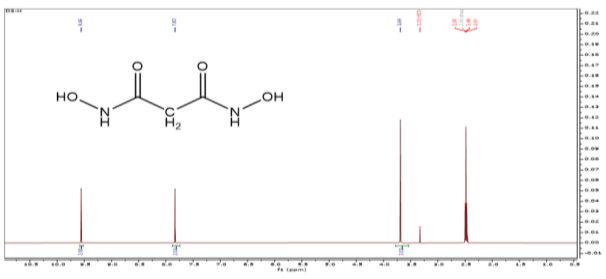
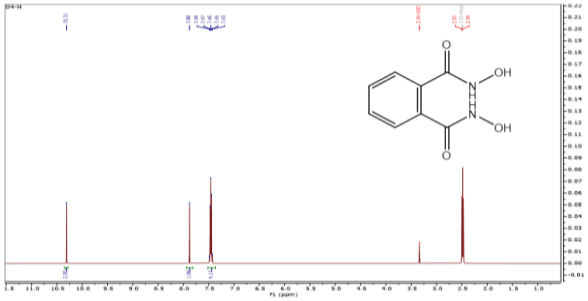
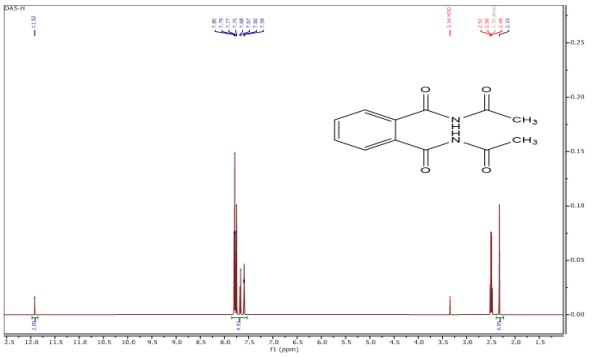
5. Appendix



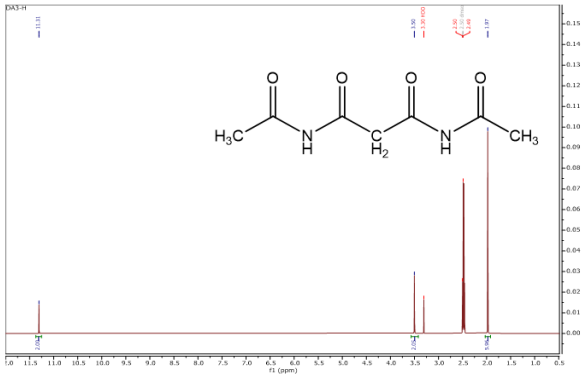
1H-NMR spectrum D5



1H-NM spectrum D3



H¹- NMR spectrum DA3



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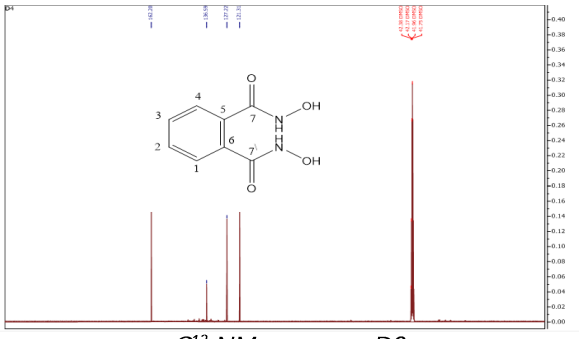

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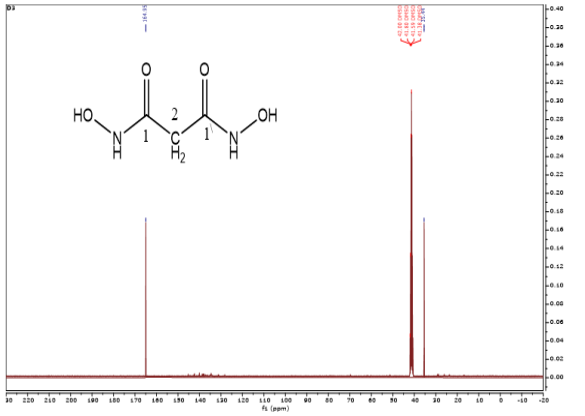

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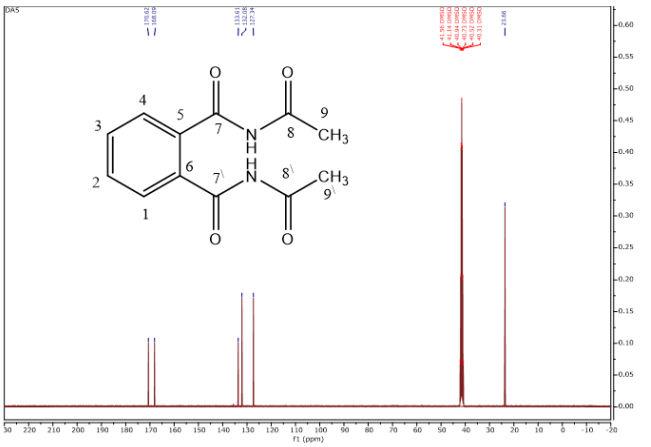

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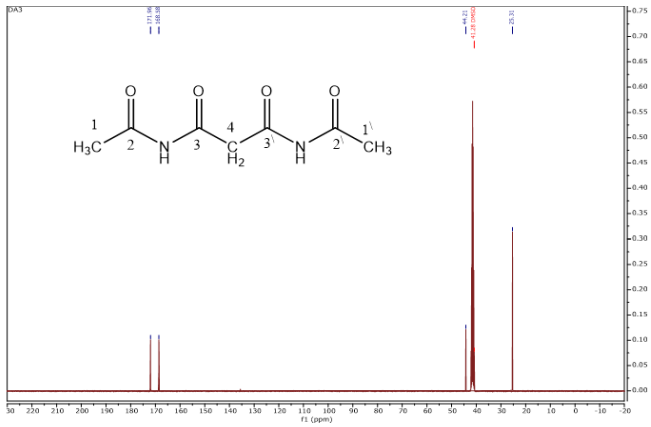

C[13]-NM spectrum DA5