Synthesis of two novel series of azoaldonitrones and preliminary evaluation of their antibacterial activity

Zeid Hassan Abood Ammar Abd AL.Hussain awad

Email :zeid.ab2013@yahoo.com

Department of Chemistry, College of Science, Kerbala University

(NJC)

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Abstract

In this work two series of new azoaldonitrones have been synthesized. At first, azoaldehydes [A_1-A_{10}] were prepared via coupling reactions between the diazonium salts of the primary aromatic amines (2-amino-5-mercapto-1,3,4-thiadiazole, 3-aminopyridine,4-methoxyaniline, 2,4-dichloroaniline, 4-chloroaniline, 2-chloroaniline, 3-bromoaniline, 3-nitroaniline, 4-nitroaniline and benzidine, respectively) and alkaline solution of 2-hydroxybenzaldehyde as coupling reagent. Next, the resulting azoaldehydes [A_1-A_{10}] were introduced in acid-catalyzed condensation reactions with *N*-phenylhydroxylamine in absolute ethanol to obtain ten new azoaldonitrones [N_1-N_{10}] respectively. Later, treatment of azoaldehydes [A_1-A_5] with *N*-benzylhydroxylamine under the same conditions afforded five new azoaldonitrones [A_{11}-A_{15}] respectively. The structures of the synthesized azoaldonitrones were confirmed by (C.H.N.S.) elementary analysis and the spectroscopic methods including FT-IR and ^{1}H NMR. The synthesized azoaldonitrones [N_1-N_{15}] were tested for their antibacterial activity against two pathogenic strains of bacteria *Staphylococcus aureous* (Gram-positive) and *Escherichia coli* (Gram-negative). The results revealed that azoaldonitrones [N_1] and [N_{11}] which are containing thiadiazole moiety showed higher activity against both strains of bacteria, while compound [N_6] appeared higher activity only against Gram-positive bacteria. Moreover, most of the prepared azoaldonitrone compounds showed medium activity against Gram-positive bacteria and weak activity against Gram-negative bacteria.

Key words: Azoaldehydes, Aldonitrones, Antibacterial activity

الخلاصة

تم من خلال هذا العمل تحضير سلسلتين جديدتين من مركبات الالدونايترون الحاوية على مجموعة الازو . في البداية تم تحضير الديهايدات حاوية على مجموعة الازو [A_1–A_{10}] عن طريق تفاعلات ازدواج مابين املاح الدايازونيوم للامينات الاولية الاروماتية (2– امينو–5– مركبتو–1،3،4– ثايادايازول، 3– امينو بيريدين، 3– ميثوكسي انيلين، 2،4– ثنائي كلوروانيلين، 4 – كلوروانيلين، 2– كلوروانيلين، 3– برومو انيلين، 3– نايتروانيلين، 4– نايترو انيلين، بنزيدين) على التتالي، ومحلول قاعدي لمركب 2– هيدروكسي بنزالديهايد ككاشف ازدواج . بعد ذلك تم ادخال الازوالديهايدات الناتجة [A_1–A_{10}] في تفاعلات تكثيف محفزة بحامض مع مركب *N*– فنيل هيدروكسيل امين في الايثانول المطلق فتم الحصول على عشر الدونايترونات جديدة حاوية على مجموعة الازو [N_1–N_{10}] على التتالي . ان معاملة الازوالديهايدات [A_1–A_5] لاحقا مع *N*– بنزيل هيدروكسيل امين تحت نفس الشروط اعطت خمس الدونايترونات جديدة حاوية على مجموعة الازو [N_{11}–N_{15}] على التتالي .

شخصت تراكيب مركبات الازوالدونايترون [N_1–N_{15}] المحضرة بوساطة التحليل الكمي الدقيق لعناصر (C.H.N.S.) والطرائق الطيفية المتضمنة مطيافية الاشعة تحت الحمراء والرنين النووي المغناطيسي للبروتون. تم اختبار الفعالية البايولوجية لجميع مركبات الازوالدونايترون المحضرة [N_1–N_{15}] ضد سلالتين من البكتريا هما (*Staphylococcus aureous*) الموجبة لصبغة كرام و (*Escherichia coli*) السالبة لصبغة كرام وقد دلت النتائج المستحصلة على ان مركبات الازوالدونايترون [N_1] و [N_{11}] الحاوية على حلقة ثايادايازول قد اظهرت فعالية بايولوجية عالية ضد كلا السلالتين البكتيريتين الموجبة والسالبة لصبغة كرام ، بينما اظهر مركب الازوالدونايترون [N_6] فعالية بايولوجية عالية ضد البكتريا الموجبة لصبغة كرام فقط ، في حين اظهرت اغلب المركبات المحضرة فعالية متوسطة تجاه البكتريا الموجبة لصبغة كرام وضعيفة تجاه البكتريا السالبة لصبغة كرام.

الكلمات المفتاحية:ازوالديهايد، الدونايترون ، فعالية ضد البكتريا

Introduction

Nitrones have been known for more than a century due to their wide range of biological activities include antibacterial activity$^{(1,2)}$, antifungal, anti-inflammation, anti-tuberculosis$^{(3-5)}$, prevent the onset of streptozotocin-induced Diabetes$^{(6)}$ and used as useful reagents or intermediates in the synthesis of a variety of nitrogen-containing compounds which find application as agro-chemicals and pharmaceuticals$^{(7)}$. Nitrones were originally developed as free radical-trapping agents in free radical chemistry. Two decades later, nitrones were found to protect biological systems from oxidative stress. Nitrones had been tested as therapeutic agents for neural and systemic dysfunctions including atherosclerosis, stroke, and Alzheimer`s disease $^{(8-10)}$. One of the most commonly used nitrones spin traps are α-phenyl-*N*-*t*-butyl nitrone (PBN) $^{(11)}$, 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and 5-diethoxyphosphoryl-5-methyl-1-pyrroline N-oxide (DEPMPO) $^{(12-15)}$ are among the most commonly used spin-trapping reagents, which not only have contributed to the understanding of free radical mediated processes in biochemical systems, but have found applications as therapeutic agents in the treatment of pathological disorders caused by unregulated production of reactive oxygen species (ROS)(16), including ischemia-reperfusion injury (17), neurodegeneration, and aging processes(18,19). PBN also shows very interesting pharmacological effects (20). Azo compounds showed variety of interesting biological activities including antibacterial (21) and pesticidel (22) activities. The azo dyes possess antiseptic and antiprotozoal (23, 24) properties and also promote wound healing (25).

Experimental

1. General

The chemicals used in this work were obtained from Fluka, sigma aldrich, GCC, Merck, BDH and S.D.Fine and were used without another purification. Column chromatography was performed with silica gel (40-60 mesh). Silica TLC plates were used with an aluminum backing (0.2 mm, 60 F_{254}). The reactions were monitored by TLC and visualized by development of the TLC plates with an alkaline potassium permanganate dip or with iodine vapor. Melting points were determined on an Electro thermal Stuart SMP 30 capillary melting point apparatus. Infrared spectra were recorded on SHIMADZU FTIR-8400S Infrared Spectrophotometer as potassium bromide discs. ^{1}H NMR spectrum of aldonitrone [N_1] was obtained on Bruker Avance III 400 spectrometer 400 MHz in DMSO-d_6 as solvent and TMS as an internal standard at the University of New South Wales, Sydney, Australia, aldonitrones [N_2, N_3, N_4, N_5, N_6, N_7 and N_{10}] were collected on NMR spectrometer, Bruker 2009 spectrometer at 400 MHz in DMSO-d_6 as solvent and TMS as an internal standard at Kashan University, Iran, and for aldonitrones [N_{11}-N_{15}] were recorded on NMR spectrometer, Bruker Avance III spectrometer at 400 MHz in DMSO-d_6 as solvent and TMS as an internal standard at Esfahan University, Iran. Elemental Analysis (C. H. N.S.) was carried out with Perkin Elmer 300A Elemental Analyzer at Esfahan University, Iran. Autoclave was used to sterilize agar media, supplied from Prestige Medical-England. Incubator was used to maintain different temperature required for the growth of organism, supplied from Binder - Germany. The method selected for the synthesis of *N*-phenylhydroxylamine is based on research of Salman and Majeed (26), *N*-benzylhydroxylamine was made to research of Almosawy (27) and azo compounds [A_1-A_{10}] were prepared following the method described by Acton (28), their physical properties and other characteristic were listed in table (1)

2.General procedure for the Synthesis of aldonitrones [N_1- N_9] (26)

Azoaldehydes [A_1- A_9] (0.002 mol) were dissolved in absolute ethanol (20 mL) containing *p*-toluenesulphonic acid (0.0002 mol, 0.0344 g) and three equivalents of magnesium sulphate (0.006 mol, 0.72 g) as drying agent , then *N*-phenylhydroxylamine (0.002 mol, 0.218 g) dissolved in absolute ethanol (10 mL) was added drop wise . The reaction mixture was refluxed with stirring on a water bath at 70 °C for (12- 20 h.) and monitored by TLC .The mixture was then filtered to remove magnesium sulphate and allowed to cool down to room temperature. The solution was concentrated and the crude product was purified by recrystallization from suitable solvent and then by column chromatography.Table (2) shows physical properties and other characteristics for the synthesized azoaldonitrones [N_1-N_9]. The (C.H.N.S.) elementary analysis of azoaldonitrones [N_1- N_7] was listed in table (4).

3. Synthesis of aldonitrone derivative [N_{10}] (26)

Azoaldehyde derivative [A_{10}] (0.002 mol , 0.9 g) was dissolved in absolute ethanol (20mL) containing *p*-toluenesulphonic acid (0.0004 mol , 0.0688 g) and six equivalents of magnesium sulphate (0.012 mol , 1.44 g) as drying agent , then *N*-phenylhydroxylamine (0.004 mol, 0.436 g) dissolved in absolute ethanol (10 mL) was added drop wise . The reaction mixture was refluxed with stirring on a water bath at 70 ^{o}C for (22 h.) and monitored by TLC .The mixture was then filtered to remove magnesium sulphate and allowed to cool down to room temperature . The solution was concentrated and the crude product was recrystallized from mixture of (2-propanole : n-hexane, 1: 4). Table (2) shows physical properties and other characteristics for the synthesized azoaldonitrone [N_{10}].

4. General procedure for the Synthesis of aldonitrones [N_{11}-N_{15}] (27)

Azoaldehyde derivatives [A_1-A_5] (0.002 mol) were dissolved in absolute ethanol (20 mL) containing *p*-toluenesulphonic acid (0.0002 mol, 0.0344 g) and three equivalents of magnesium sulphate (0.006 mol , 0.72 g) as drying agent , then *N*-benzylhydroxylamine (0.002 mol, 0.246 g) dissolved in absolute ethanol (10 mL) was added drop wise . The reaction mixture was refluxed with stirring on a water bath at 70 ^{o}C for (12-16 h.) and monitored by TLC. The mixture was then filtered to remove magnesium sulphate and allowed to cool down to room temperature. The solution was concentrated and the crude product was purified by recrystallization from suitable solvent and then by column chromatography. Table (3) shows physical properties and other characteristics for the synthesized azoaldonitrones [N_{11}-N_{15}]. The (C.H.N.S.) elementary analysis of azoaldonitrones [N_{11}-N_{15}] was listed in table (4).

Table (1): physical properties and other characteristics for the synthesized azoaldehyde derivatives [A_1-A_{10}]

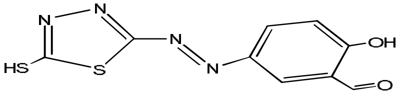
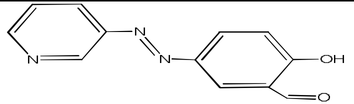
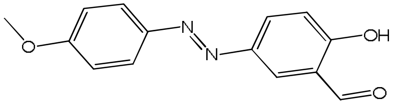
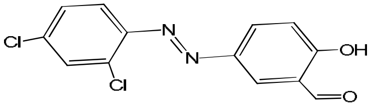
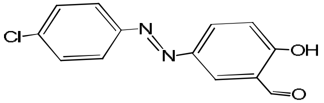
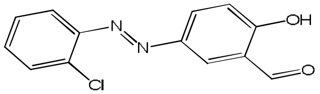
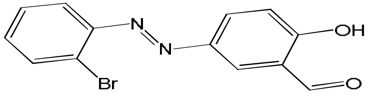
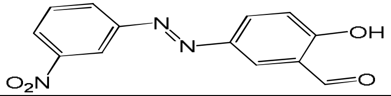
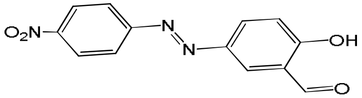
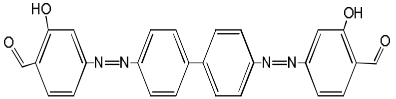
Com. no.	Structure	Molecular formula	Color	M.Wt. g/mol	Rec.solvent	Yield %	M.P.°C	R_f
A_1		$C_9H_6N_4O_2S_2$	orange	266.29	dioxane : n-heptane 1 : 2	58	189-191	0.46 n-hexane : Et_2O 1 : 4
A_2		$C_{12}H_9N_3O_2$	orange	227.22	ethanol	50	171-173	0.56 n-hexane : EtOAc 4 : 1
A_3		$C_{14}H_{12}N_2O_3$	dark brown	256.25	benzene	68	119-121 Lit.(29).120	0.72 n-hexane : Et_2O 1 : 3
A_4		$C_{13}H_8N_2O_2Cl_2$	red	295.12	n-hexane : EtOAc 4 : 1	79	160-162 (dec.)	0.68 n-hexane : Et_2O 1 : 2
A_5		$C_{13}H_9N_2O_2Cl$	greenish yellow	260.67	n-hexane : EtOAc 4 : 1	90	131-133 Lit.(30) 130	0.78 n-hexane : Et_2O 1 : 3
A_6		$C_{13}H_9N_2O_2Cl$	red	260.67	n-hexane : EtOAc 4 : 1	91	173-175	0.66 n-hexane : Et_2O 1 : 2
A_7		$C_{13}H_9N_2O_2Br$	yellow	305.12	n-hexane : EtOAc 4 : 1	72	152-154	0.67 n-hexane : Et_2O 1 : 1
A_8		$C_{13}H_9N_3O_4$	red	271.23	ethanol	75	104-106 Lit.(30) 105	0.74 n-hexane : EtOAc 2 : 3
A_9		$C_{13}H_9N_3O_4$	brown	271.23	ethanol	77	113-115 Lit.(30) 112	0.65 n-hexane : Et_2O 1 : 2
A_{10}		$C_{26}H_{18}N_4O_4$	brown	450.38	ethanol	54	193 Lit.(31) 190	0.62 n-hexane : Et_2O 1 : 1

Table (2): physical properties and other characteristics for the synthesized azoaldonitrones [N_1-N_{10}]

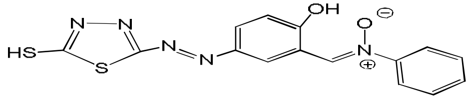
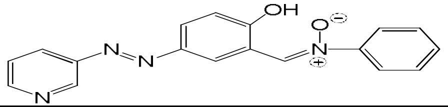
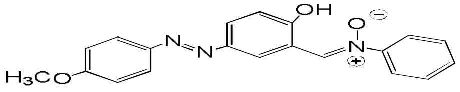
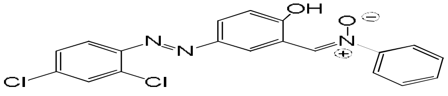
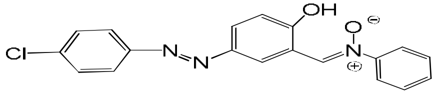
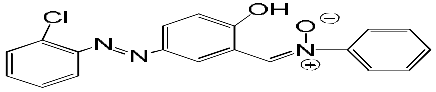
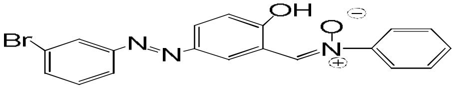
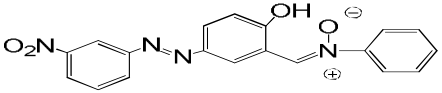
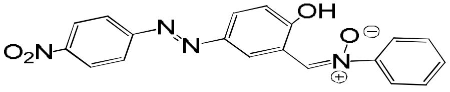
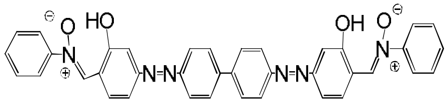
Com. no.	Structure	Molecular formula	M.Wt. g/mol	Color	RN. time (h.)	Rec.solvent	Yield %	M.P.ºC	$\mathbf{R}_f$
N_1		$C_{15}H_{11}N_5O_2S_2$	357.41	red	15	ethanol	50	123-124	0.35 n-hexane : Et_2O 1 : 4
N_2		$C_{18}H_{14}N_4O_2$	318.32	purple	15	ethyl acetate	53	118-120	0.49 n-hexane : EtOAc 1 : 4
N_3		$C_{20}H_{17}N_3O_3$	347.36	dark red	13	n-hexane : benzene 3:1	64	112-113	0.40 n-hexane : Et_2O 1 : 3
N_4		$C_{19}H_{13}N_3O_2Cl_2$	386.23	red	15	n-hexane : EtOAc 4:1	60	139	0.55 n-hexane : Et_2O 1 : 2
N_5		$C_{19}H_{14}N_3O_2Cl$	351.78	dark brown	16	n-hexane : EtOAc 4:1	57	131-132	0.57 n-hexane : Et_2O 1 :3
N_6		$C_{19}H_{14}N_3O_2Cl$	351.78	black	15	n-hexane : EtOA 4 : 1	56	150-152	0.51 n-hexane : Et_2O 1 : 2
N_7		$C_{19}H_{14}N_3O_2Br$	396.24	brown	17	n-hexane : EtOAc 4:1	63	119	0.58 n-hexane : Et_2O 1 : 1
N_8		$C_{19}H_{14}N_4O_4$	362.34	red	20	ethanol	58	118-120	0.54 n-hexane : EtOAc 2 : 3
N_9		$C_{19}H_{14}N_4O_4$	362.34	orange	18	ethanol	51	94-96	0.39 n-hexane : Et_2O 1 : 2
N_{10}		$C_{38}H_{28}N_6O_4$	632.66	dark brown	20	2-propanol : n-hexane 1:4	47	157-159	0.60 $CHCl_3$: benzene 1 : 3

Table (3): physical properties and other characteristics for the synthesized azoaldonitrones [N_{11}-N_{15}]

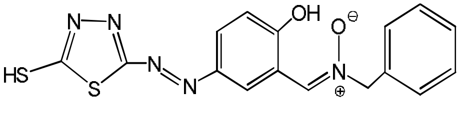
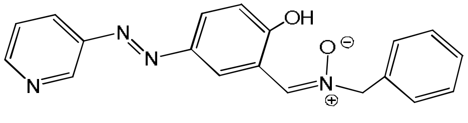
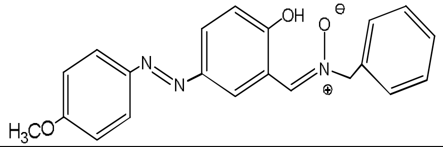
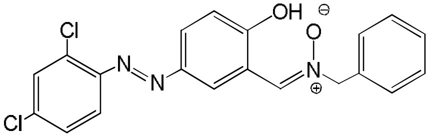
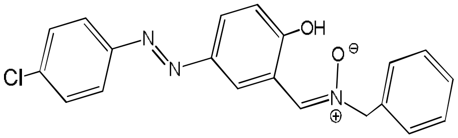
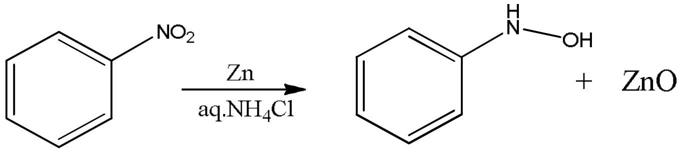
Com. no.	**Structure**	**Molecular formula**	**M.Wt.** g/mol	**Color**	**RN.time (h.)**	**Rec.solvent**	**Yield %**	**M.P.ºC**	**R_f**
N_{11}		$C_{16}H_{13}N_5O_2S_2$	371.43	orange	13	ethanol	52	151-153	0.35 n-hexane : Et_2O 1 : 4
N_{12}		$C_{19}H_{16}N_4O_2$	332.35	purple	12	ethyl acetate	66	147-149	0.49 n-hexane : EtOAc 1 : 4
N_{13}		$C_{21}H_{19}N_3O_3$	361.39	dark red	13	n-hexane : benzene 2 : 1	65	134-135	0.40 n-hexane : Et_2O 1 : 3
N_{14}		$C_{20}H_{15}N_3O_2Cl_2$	400.26	brown	15	n-hexane : EtOAc 2 : 1	60	131-133	0.55 n-hexane : Et_2O 1 : 2
N_{15}		$C_{20}H_{16}N_3O_2Cl$	365.81	yellow	16	ethyl acetate	51	142-144	0.57 n-hexane : Et_2O 1 : 3

Table (4): (C.H.N.S.) elementary analysis of the synthesized azoaldonitrones [N_1-N_7 and N_{11}-N_{15}]

Com. no .	Calculated %				Found %			
	C	H	N	S	C	H	N	S
N_1	50.41	3.10	19.59	17.94	49.90	3.12	20.39	17.42
N_2	67.91	4.43	17.60	---	68.01	4.56	17.34	---
N_3	69.15	4.93	12.10	---	68.08	4.80	11.78	---
N_4	59.08	3.39	10.88	---	58.95	3.42	10.02	---
N_5	64.87	4.01	11.94	---	63.68	3.78	11.65	---
N_6	64.87	4.01	11.94	---	64.92	4.11	11.02	---
N_7	57.59	3.56	10.60	---	57.72	3.84	10.37	---
N_{11}	51.74	3.53	18.85	17.27	52.01	3.71	18.38	16.71
N_{12}	68.66	4.85	16.86	---	68.08	3.94	16.11	---
N_{13}	69.79	5.30	11.63	---	68.97	4.89	10.72	---
N_{14}	60.01	3.78	10.50	---	59.46	3.08	9.71	---
N_{15}	65.67	4.41	11.49	---	66.10	4.69	10.83	---

Results and Discussion

N-Phenylhydroxylamine was synthesized through reaction of nitrobenzene with zinc powder in presence of ammonium chloride as catalyst (26) as shown in scheme [1].

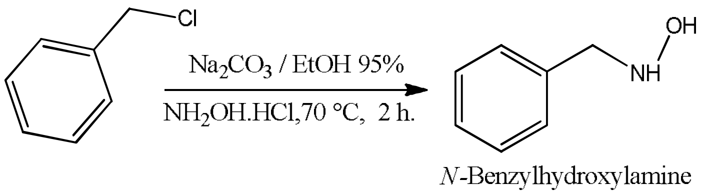


N-Phenylhydroxylamine

Scheme [1]

The synthesized *N*-phenylhydroxylamine showed identical melting point with that reported.

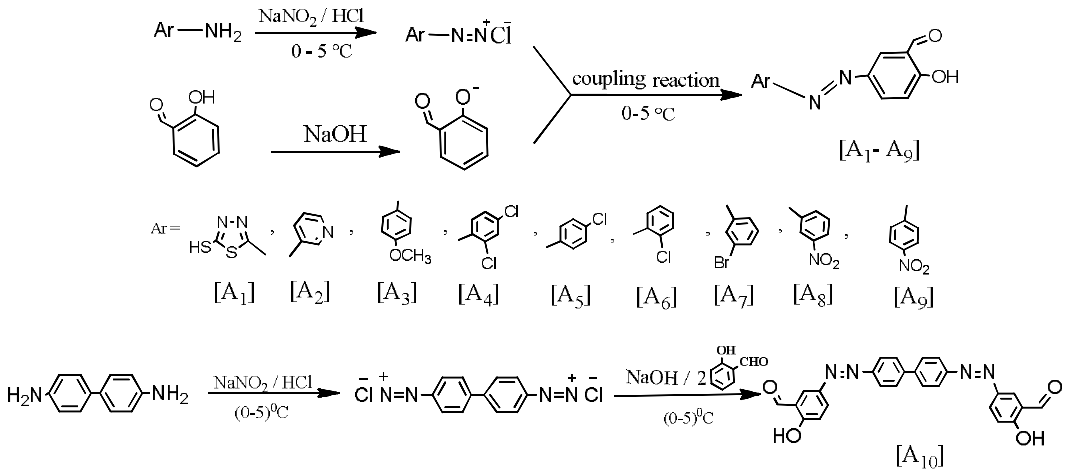
N-benzylhydroxylamine was synthesized via reaction of hydroxylamine hydrochloride with benzyl chloride in presence of anhydrous sodium carbonate in ethanol 95% (27)as indicated in scheme [2].



Scheme [2]

The resulting *N*-benzylhydroxylamine showed analogues melting point with that published.

A coupling reaction between the diazonium salts of the primary aromatic amines [2-amino-5-mercapto-1,3,4-thiadiazole, 3-aminopyridine, 4-methoxyaniline, 2,4-dichloroaniline, 4-chloroaniline, 2-chloroaniline, 3-bromoaniline, 3-nitroaniline, 4-nitroaniline and benzidine] respectively with phenoxide salt of 2-hydroxybenzaldehyde at (0-5) °C afforded different azoaldehyde derivatives [A_1-A_{10}] respectively (28). Scheme [3].



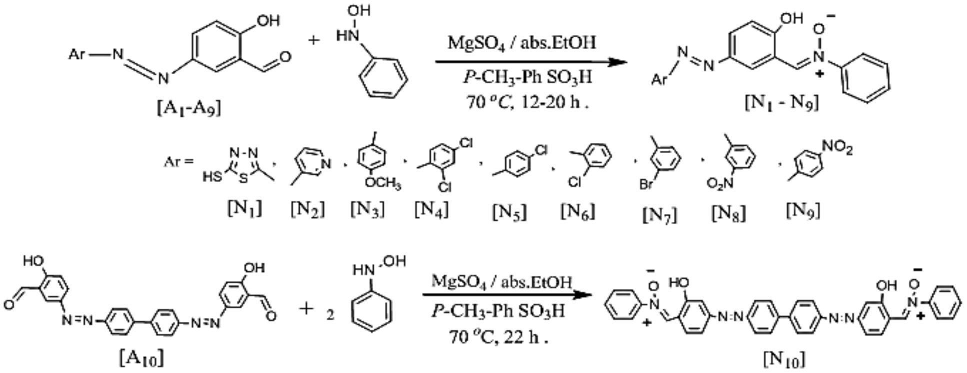
Scheme [3]

The synthesized azoaldehydes [A_1-A_{10}] were characterized by their melting points and FT-IR spectroscopy at $\bar{\upsilon}$ (cm^{-1}) (KBr). The FT-IR spectra of all derivatives are devoid the sharp weak bands at the general range (3400-3250) cm^{-1} attributed to asymmetric and symmetric stretching vibrations of ($-NH_2$) (32) group and appearance of strong band at the range (1635-1672) cm^{-1} attributed to the stretching vibration of (C=O) group. The intramolecular hydrogen bonding between carbonyl group oxygen atom and *o*-hydroxy group causes shifting of stretching vibrations of carbonyl groups towards lower frequencies (32). Other characteristic bands shown in table (5).

Table (5): FT-IR data of the synthesized azoaldehydes [A_1-A_{10}] in cm^{-1}

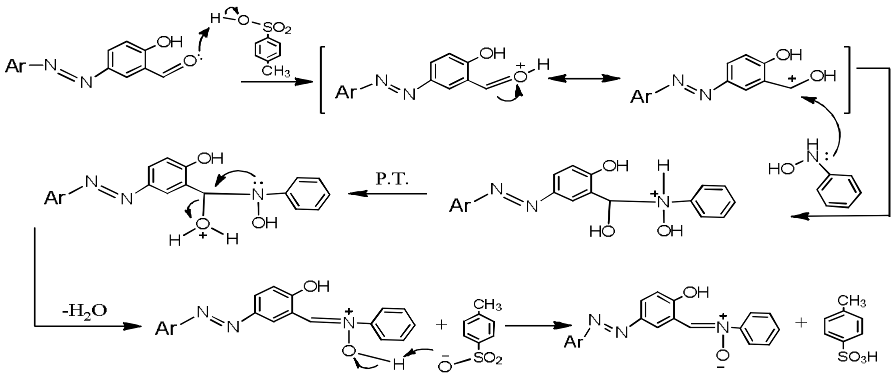
Com. no.	FT-IR bands
[A_1]	3284 ($\upsilon_{\text{O-H, phenolic}}$), 3099$_{br}$ ($\upsilon_{\text{N-H, thioketone form}}$ and $\upsilon_{\text{C-H, benzene ring, vibration coupling}}$), 2842 ($\upsilon_{\text{C-H, aldehyde}}$), 2613 ($\upsilon_{\text{S-H, thioenol form}}$), 1635 ($\upsilon_{\text{C=O}}$), 1550, 1539 and 1502 ($\upsilon_{\text{C=C, benzene ring}}$ and $\upsilon_{\text{(C=N), thiadiazole ring}}$), 1425 ($\upsilon_{\text{N=N}}$), 1392 ($\upsilon_{\text{C=S , thioketone form}}$), 835, 752 and 700 (δ o.o.p.$_{\text{C-H, benzene rings}}$).
[A_2]	3435 ($\upsilon_{\text{O-H}}$), 3037 ($\upsilon_{\text{C-H, aromatic rings}}$), 2835 and 2748 ($\upsilon_{\text{C-H, aldehyde}}$), 1672 ($\upsilon_{\text{C=O}}$), 1591, 1521 and 1477 ($\upsilon_{\text{C=C and C=N, aromatic rings}}$), 1411 ($\upsilon_{\text{N=N}}$), 902, 833, 744 and 715 (δ o.o.p. $_{\text{C-H, aromatic ring}}$).
[A_3]	3444 ($\upsilon_{\text{O-H}}$), 3060 ($\upsilon_{\text{C-H, benzene rings}}$), 2958 (υ as. C-H, CH_3), 2893 (υ s. C-H, CH_3),2732 and 2839 ($\upsilon_{\text{C-H, aldehyde}}$), 1660 ($\upsilon_{\text{C=O}}$), 1598, 1562 and 1500 ($\upsilon_{\text{C=C, benzene rings}}$), 1473 (δ as. $_{\text{C-H, CH3}}$), 1373(δ s. $_{\text{C-H, CH3}}$), 1247 ($\upsilon_{\text{as. C-O-C, ether}}$), 1026 ($\upsilon_{\text{s. C-O-C, ether}}$), 906, 848, 765 and 685 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[A_4]	3425 ($\upsilon_{\text{O-H}}$), 3064 ($\upsilon_{\text{C-H, benzene rings}}$), 2870 ($\upsilon_{\text{C-H, aldehyde}}$), 1656 ($\upsilon_{\text{C=O}}$), 1606, 1568, 1527 and 1477 ($\upsilon_{\text{C=C, benzene rings}}$), 1402 ($\upsilon_{\text{N=N}}$), 1099 ($\upsilon_{\text{C-Cl}}$) , 896, 837, 779 and 690 (δ o.o.p.$_{\text{C-H, benzene rings}}$).
[A_5]	3417 ($\upsilon_{\text{O-H}}$), 3224$_{br}$ ($\upsilon_{\text{O-H}}$ and $\upsilon_{\text{C-H, benzene rings, vib. coupling}}$), 2870 ($\upsilon_{\text{C-H, aldehyde}}$), 1668 ($\upsilon_{\text{C=O}}$), 1618, 1573, and 1477 ($\upsilon_{\text{C=C, benzene rings}}$), 1087 ($\upsilon_{\text{C-Cl}}$), 902, 837, 767 and 727 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[A_6]	3209 ($\upsilon_{\text{O-H}}$), 3092 ($\upsilon_{\text{C-H, benzene rings}}$), 2847 and 2740 ($\upsilon_{\text{C-H, aldehyde}}$), 1664 ($\upsilon_{\text{C=O}}$), 1616, 1575, 1521 and 1479 ($\upsilon_{\text{C=C, benzene rings}}$), 1419 ($\upsilon_{\text{N=N}}$), 1057 ($\upsilon_{\text{C-Cl}}$) , 898, 842, 821, 771, 744 and 678 (δ o.o.p.$_{\text{C-H , benzene rings}}$).
[A_7]	3196 ($\upsilon_{\text{O-H}}$), 3078 ($\upsilon_{\text{C-H, benzene rings}}$), 2874 ($\upsilon_{\text{C-H, aldehyde}}$), 1662 ($\upsilon_{\text{C=O}}$), 1620, 1575, 1485, 1471 and 1454 ($\upsilon_{\text{C=C, benzene rings}}$), 1408 ($\upsilon_{\text{N=N}}$), 1057 ($\upsilon_{\text{C-Br}}$) , 900, 883, 829, 792, 769, 738 and 675 (δ o.o.p.$_{\text{C-H , benzene rings}}$).
[A_8]	3448 ($\upsilon_{\text{O-H}}$), 3101 and 3012 ($\upsilon_{\text{C-H, benzene rings}}$), 2847 and 2735 ($\upsilon_{\text{C-H, aldehyde}}$), 1664 ($\upsilon_{\text{C=O}}$), 1608 and 1473 ($\upsilon_{\text{C=C, benzene rings}}$), 1531 (υ as. NO_2), 1361 (υ s. NO_2), 900, 868, 842, 819 and 732 (δo.o.p.$_{\text{C-H , benzene rings}}$).
[A_9]	3197 ($\upsilon_{\text{O-H}}$), 3055 ($\upsilon_{\text{C-H, benzene rings}}$), 2870 ($\upsilon_{\text{C-H, aldehyde}}$), 1666 ($\upsilon_{\text{C=O}}$), 1573 and 1521 ($\upsilon_{\text{C=C, benzene rings}}$), 1477 ($\upsilon_{\text{as.}}$ NO_2), 1373 ($\upsilon_{\text{s.}}$ NO_2), 902, 839, 810, 765 and 729 (δ o.o.p.$_{\text{C-H, benzene rings}}$).
[A_{10}]	3402 ($\upsilon_{\text{O-H}}$), 3036 ($\upsilon_{\text{C-H, benzene rings}}$), 2852 and 2730 ($\upsilon_{\text{C-H, aldehyde}}$), 1656 ($\upsilon_{\text{C=O}}$), 1616, 1597, 1572 and 1481 ($\upsilon_{\text{C=C, benzene rings}}$), 902, 825, 744 and 705 (δ o.o.p. $_{\text{C-H, benzene rings}}$).

A condensation reaction between the synthesized azoaldehydes [A_1-A_{10}] and *N*-phenylhydroxylamine in presence of *p*-toluenesulfonic acid as catalyst and magnesium sulphate as dehydrating agent in absolute ethanol results formation of azoaldonitrones [N_1-N_{10}] (26) as shown in scheme [4].



Scheme [4]

The reaction passes through losing H_2O molecule as shown in the following suggested mechanism. Scheme [5].



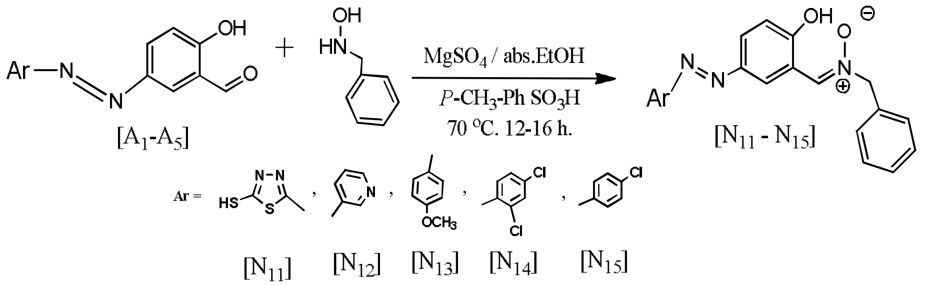
Scheme [5]

The (C.H.N.S.) elementary microanalysis of the prepared compounds [N_1-N_7], table (4) showed good agreement between the calculated and found values. The synthesized azoaldonitrones [N_1-N_{10}] were identified by FT-IR spectroscopy. ^1H NMR spectroscopy was used to confirm the structure of compounds [N_1-N_8] and [N_{10}]. FT-IR spectra at $\bar{\upsilon}$ (cm^{-1}) (KBr) of all synthesized compounds [N_1-N_{10}] illustrate good evidence that the formation of azoaldonitrones took place successfully through disappearing the sharp and strong band around (1635-1672) cm^{-1} which attributed to υ (C=O) and appearing less sharp and less intensity band at lower frequency (1591-1637) cm^{-1} assigned to υ (C=N) of nitrone group. Moreover, the spectra appeared bands at (1047-1180) may be due to $\upsilon(^{+}N{-}O^{-})$ bond in nitrone group. Other characteristic bands with their interpretation were listed in table (6).

Table (6): FT-IR data of the synthesized azoaldonitrones [N_1-N_{10}] in cm^{-1}

Com. no.	FT-IR bands
[N_1]	3390 ($\upsilon_{\text{O-H}}$), 3176 ($\upsilon_{\text{N-H, thioketone form}}$), 3095 ($\upsilon_{\text{C-H, benzene rings}}$), 2769 ($\upsilon_{\text{C-H, nitrone}}$), 1606 ($\upsilon_{\text{C=N, nitrone}}$), 1552, 1537 and 1479 ($\upsilon_{\text{C=C, benzene ring and }\upsilon\text{ (C=N), thiadiazole ring}}$), 1363 ($\upsilon_{\text{C=S, thioketone form}}$), 1058($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 873, 833, 750 and 673 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[N_2]	3425 ($\upsilon_{\text{O-H}}$), 3082 ($\upsilon_{\text{C-H, aromatic rings}}$), 2899 ($\upsilon_{\text{C-H, nitrone}}$), 1637 ($\upsilon_{\text{C=N, nitrone}}$), 1591, 1523, 1481 and 1456 ($\upsilon_{\text{C=C and C=N, aromatic rings}}$), 1055 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 831, 758 and 688 (δ o.o.p. $_{\text{C-H, aromatic rings}}$).
[N_3]	3423 ($\upsilon_{\text{O-H}}$), 3066 ($\upsilon_{\text{C-H, benzene rings}}$), 2981 (υ as. C-H, CH_3), 2907 (υ s. C-H, CH_3), 2837 ($\upsilon_{\text{C-H, nitrone}}$), 1600 ($\upsilon_{\text{C=N, nitrone}}$), 1510, and 1462 ($\upsilon_{\text{C=C, benzene rings}}$), 1251($\upsilon_{\text{as. C-O-C, ether}}$), 1178 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 1030 ($\delta_{\text{s. C-O-C, ether}}$), 837, 812, 792 and 680 (δ o.o.p. $_{\text{C-H, aromatic rings}}$).
[N_4]	3441 and 3394 ($\upsilon_{\text{O-H}}$), 3040 ($\upsilon_{\text{C-H, benzene rings}}$), 2852 ($\upsilon_{\text{C-H, nitrone}}$), 1593 ($\upsilon_{\text{C=N, nitrone}}$), 1579 and 1481 ($\upsilon_{\text{C=C, benzene rings}}$), 1101 ($\upsilon_{\text{C-Cl}}$), 1055 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 869, 821, 759, 730 and 690 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[N_5]	3429 ($\upsilon_{\text{O-H}}$), 3066 ($\upsilon_{\text{C-H, benzene rings}}$), 2877 ($\upsilon_{\text{C-H, nitrone}}$), 1597 ($\upsilon_{\text{C=N, nitrone}}$), 1489 ($\upsilon_{\text{C=C, benzene rings}}$), 1446 ($\upsilon_{\text{N=N}}$), 1093 ($\upsilon_{\text{C-Cl}}$), 1047 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 833, 767, 723 and 675 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[N_6]	3425 ($\upsilon_{\text{O-H}}$), 3072 ($\upsilon_{\text{C-H, benzene rings}}$), 2875 ($\upsilon_{\text{C-H, nitrone}}$), 1591($\upsilon_{\text{C=N, nitrone}}$), 1489 and 1442 ($\upsilon_{\text{C=C, benzene rings}}$), 1415 ($\upsilon_{\text{N=N}}$), 1128 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 1057 ($\upsilon_{\text{C-Cl}}$), 893, 827, 759, 725 and 692 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[N_7]	3414 ($\upsilon_{\text{O-H}}$), 3068 ($\upsilon_{\text{C-H, benzene rings}}$), 2901 ($\upsilon_{\text{C-H, nitrone}}$), 1593 ($\upsilon_{\text{C=N, nitrone}}$), 1570, 1485 and 1465($\upsilon_{\text{C=C, benzene rings}}$), 1400 ($\upsilon_{\text{N=N}}$), 1109 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 1049 ($\upsilon_{\text{C-Br}}$), 893, 817, 785, 759 and 680 (δ o.o.p .$_{\text{C-H, benzene rings}}$).
[N_8]	3448 ($\upsilon_{\text{O-H}}$), 3076 ($\upsilon_{\text{C-H, benzene rings}}$), 2868 ($\upsilon_{\text{C-H, nitrone}}$), 1595($\upsilon_{\text{C=N, nitrone}}$), 1549 (υ as. NO_2), 1485 and 1448 ($\upsilon_{\text{C=C, benzene rings}}$), 1415 ($\upsilon_{\text{N=N}}$), 1350 (υ s. NO_2), 1163 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 904, 842, 813, 765, 734 and 680 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[N_9]	3398 ($\upsilon_{\text{O-H}}$), 3081 ($\upsilon_{\text{C-H, benzene rings}}$), 2893 ($\upsilon_{\text{C-H, nitrone}}$), 1602 ($\upsilon_{\text{C=N, nitrone}}$), 1527 (υ as. NO_2), 1470 ($\upsilon_{\text{C=C, benzene rings}}$), 1350 (υ s. NO_2), 1180 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$, nitrone), 900, 835, 810, 732 and 680 (δ o.o.p.$_{\text{C-H, benzene rings}}$).
[N_{10}]	3435 and 3406 ($\upsilon_{\text{O-H}}$), 3073 ($\upsilon_{\text{C-H, benzene rings}}$), 2856 ($\upsilon_{\text{C-H, nitrone}}$), 1599 ($\upsilon_{\text{C=N, nitrone}}$), 1487 ($\upsilon_{\text{C=C, benzene rings}}$), 1111 ($\upsilon\ {}^{+}\text{N}-\text{O}^{-}$,nitrone), 895, 829, 763, 720 and 700 (δ o.o.p. $_{\text{C-H, benzene rings}}$).

Azoaldonitrones [N_{11}-N_{15}] were synthesized by the condensation reactions between the synthesized azoaldehydes [A_1-A_5] and *N*-benzylhydroxylamine in absolute ethanol in presence of *p*-toluenesulfonic acid as catalyst and magnesium sulphate ($MgSO_4$) as dehydrating agent (27) as indicated in scheme [6].



Scheme [6]

The reaction mechanism was described in scheme (5), except using *N*-benzylhydroxylamine instead of *N*-phenylhydroxylamine. The (C.H.N.S.) elementary microanalysis of the prepared compounds [N_{11}-N_{15}], table (4) showed good agreement between the calculated and found values. FT-IR spectra, at $\bar{\upsilon}$ (cm^{-1}) (KBr) of all synthesized compounds [N_{11}-N_{15}] provide good evidence that the formation of azoaldonitrones happened successfully via disappearing the sharp and strong bands around (1635-1672) cm^{-1} which assigned to υ (C=O) and appearing less sharp and less intensity band at lower frequency (1587-1600) cm^{-1} attributed to the str.vib. of (C=N) bond in nitrone group. Beside this, the spectra showed appeared bands at (1043-1136) cm^{-1} may be due to υ (${}^{+}N—O^{-}$) bond in nitrone group. Other characteristic bands with their interpretation were listed in table (7).

Table (7): FT-IR data of the synthesized azoaldonitrones [N_{11}-N_{15}] in cm^{-1}

Com. no.	FT-IR bands
[N_{11}]	3394 ($\upsilon_{\text{O-H}}$), 3173 ($\upsilon_{\text{N-H, thioketone form}}$), 3091 ($\upsilon_{\text{C-H, benzene rings}}$), 2773 ($\upsilon_{\text{C-H, nitrone}}$) 1600 ($\upsilon_{\text{C=N, nitrone}}$), 1594, 1535, 1494 and 1454 ($\upsilon_{\text{C=C, benzene rings and } \upsilon \text{ (C=N), thiadiazole ring}}$), 1367 ($\upsilon_{\text{C=S, thioketone form}}$), 1043 ($\upsilon\ {}^{+}_{\ }\text{N - O}^{-}_{\text{, nitrone}}$), 869, 840, 815, 746 and 702 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[N_{12}]	3419 ($\upsilon_{\text{O-H}}$), 3064 ($\upsilon_{\text{C-H, aromatic rings}}$), 2814 ($\upsilon_{\text{C-H, nitrone}}$), 1593 ($\upsilon_{\text{C=N, nitrone}}$), 1521, 1508, 1489 and 1456 ($\upsilon_{\text{C=C and C=N, aromatic rings}}$), 1415 ($\upsilon_{\text{N=N}}$), 1043 ($\upsilon\ {}^{+}_{\ }\text{N - O}^{-}_{\text{, nitrone}}$), 868 , 831, 767, 744 and 692 (δ o.o.p. $_{\text{C-H, aromatic rings}}$).
[N_{13}]	3421 ($\upsilon_{\text{O-H}}$), 3068 ($\upsilon_{\text{C-H, benzene rings}}$), 2958 ($\upsilon$ as. C-H, CH_3), 2937 (υ s. C-H, CH_3), 2837 ($\upsilon_{\text{C-H, nitrone}}$), 1600 ($\upsilon_{\text{C=N, nitrone}}$), 1510, 1462 and 1442 ($\upsilon_{\text{C=C, benzene rings}}$), 1251 ($\upsilon_{\text{as. C-O-C, ether}}$), 1134 ($\upsilon\ {}^{+}_{\ }\text{N - O}^{-}_{\text{, nitrone}}$), 1030 ($\delta_{\text{s. C-O-C, ether}}$), 835, 815, 769 and 692 (δ o.o.p. $_{\text{C-H, aromatic rings}}$).
[N_{14}]	3414 ($\upsilon_{\text{O-H}}$), 3090 ($\upsilon_{\text{C-H, benzene rings}}$), 2931 ($\upsilon_{\text{C-H, nitrone}}$), 1587 ($\upsilon_{\text{C=N, nitrone}}$), 1461 ($\upsilon_{\text{C=C, benzene rings}}$), 1136 ($\upsilon\ {}^{+}_{\ }\text{N - O}^{-}_{\text{ , nitrone}}$), 1101 ($\upsilon_{\text{C-Cl}}$), 868, 815, 769 and 692 (δ o.o.p. $_{\text{C-H, benzene rings}}$).
[N_{15}]	3435 and 3211 ($\upsilon_{\text{O-H}}$), 3066 ($\upsilon_{\text{C-H, benzene rings}}$), 2825 ($\upsilon_{\text{C-H, nitrone}}$), 1600 ($\upsilon_{\text{C=N, nitrone}}$), 1521 and 1479 ($\upsilon_{\text{C=C, benzene rings}}$), 1428 ($\upsilon_{\text{N=N}}$), 1105 and 1093 ($\upsilon_{\text{C-Cl}}$), 1043 ($\upsilon\ {}^{+}_{\ }\text{N - O}^{-}_{\text{ , nitrone}}$) 899 , 835, 817 and 771 (δ o.o.p. $_{\text{C-H, benzene rings}}$).

^{1}H NMR spectra of aldonitrones [N_1-N_8] and [N_{10}]

All synthesized aldonitrone compounds [N_1-N_{10}] just contain aromatic protons and proton of nitrone group in addition of phenolic proton, except compound [N_1] which also contains sulfhydryl (S-H) proton in structure of thioenol form and (N-H) proton in structure of thioketone form and compound [N_3] which also contains aliphatic protons of methoxy group (O-CH_3). It is well-known that signals of aromatic protons appeared in the down field region at narrow range of chemical shifts and consequently difficult to distinguish, so the theoretical chemical shifts values of the aromatic protons in the target compounds [N_1-N_8] and [N_{10}] were used to help us for interpretation of the found chemical shifts of them.

^{1}H NMR spectra of compounds [N_1-N_8] and [N_{10}] appeared signals of the aromatic protons at the general range of δ= (6.260-8.838) ppm (32). Proton of nitrone group is deshielded due to the higher electron withdrawal of nitrone group carbon atom, so its signal appears in the down field, the literature referred that the signal of proton of nitrone group in aldonitrones which have the general formula Ar-CH=NOAr appears near δ= 8.5 ppm (26, 33) due to the decreasing of the electronic density of nitrone group carbon atom as result for the conjugation with aromatic rings.

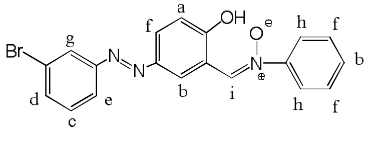
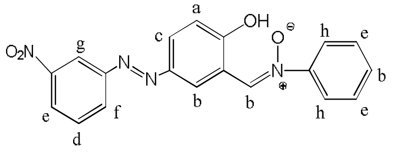
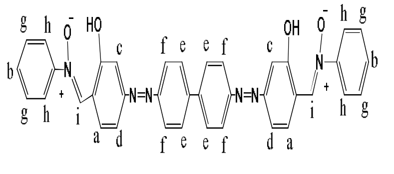
^{1}H NMR spectra of compounds [N_1-N_8] and [N_{10}] appeared signal of nitrone group proton at the general range of δ= (8.184-8.766) ppm, also the spectra of all compounds were devoid of aldehydic proton signal at the general range of δ= (9.50-10.10) ppm, so expect that the reactions happened and yielded aldonitrone compounds [N_1-N_8] and [N_{10}], this conclusion is assisted by the (FT-IR) and (C.H.N.S.) analysis data.

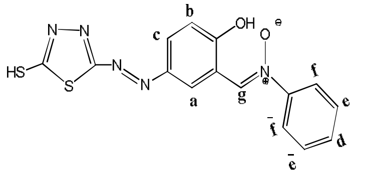
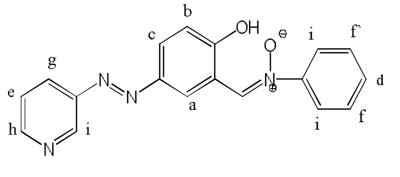
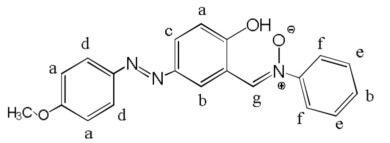
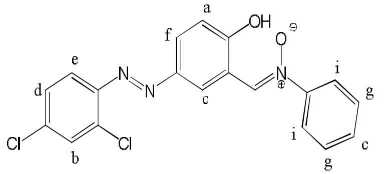
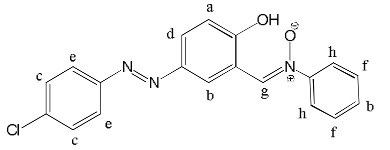
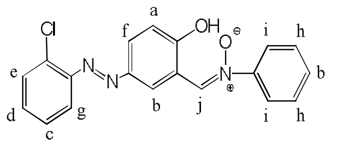
^{1}H NMR spectrum, (400 MHz, DMSO) of compound [N_1] appeared the following signals at δ (ppm): 2.500 (DMSO solvent)(34), 3.164 (s,1H, S-H, thioenol form), 3.347 (s, 1H, N-H, thioketone form), 3.387 (H_2O in DMSO). The singlet signal at 10.129 ppm and 13.161 ppm due to the phenolic (O-H) proton. The expanded spectrum showed appearance of nine signals in the down field region at the range (6.980-8.264) attributed to eight nonequivalent types of aromatic protons and nitrone group proton. The interpretation of these signals was carried out in association with the theoretical chemical shifts values of these aromatic protons and the literature value of nitrone group proton as follows :

6.980-7.017(t,1H, Ha), 7.193-7.275 (m,1H, Hb),7.302-7.340 (t,1H, Hc), 7.385-7.406 (d,1H, Hd), 7.441-7.478 (t,1H,He), 7.511-7.570 (q,1H, He`), 7.612-7.707 (m,1H, Hf ), 8.064-8.084 (d, 1H, Hf`), 8.245-8.264 (d, 1H, Hg, nitrone group).

The interpretation of ^{1}H NMR spectra for other aldonitrones [N_2-N_8] and [N_{10}] was carried out following the interpretation described for aldonitrone [N_1] and listed in table (8) .

Table (8): ^1H NMR data of the synthesized azoaldonitrones [N_1-N_8] and [N_{10}]

[N_7]		2.479 (DMSO solvent), 3.364 (H_2O in DMSO), 5.734 (s, 1H, 1×O-H phenolic), 6.725 (s, 1H, Ha), 7.479-7.499 (d,2H, 2×Hb), 7.537 (s, 1H, Hc), 7.578 (s, 1H, Hd), 7.705 (s, 1H, He), 7.771-7.791 (d, 1H, Hf), 7.870-7.888 (d, 2H, 2×Hg), 8.038(s, 1H, Hh), 8.209 (s,2H, 2×Hi), 8.398-8.437 (t,1H, Hj, nitrone group).
[N_8]		2.475 (DMSO solvent), 3.475 and 4.023 (H_2O in DMSO), 5.731 (s, 1H, 1×O-H phenolic), 7.079 (s, 1H, Ha), 7.456 (s, 2H, 2×Hb), 7.652 (s, 1H, Hc), 7.844 (s, 1H, Hd), 7.968 (s, 3H, 3×He), 8.101(s,1 H, Hf), 8.230 (s, 1H, Hg), 8.287 (s, 2H, 2×Hh), 8.435 (s, 1H, Hi, nitrone group).
[N_{10}]		2.477 (DMSO solvent), 3.339-4.519 (H_2O in DMSO), 5.734 and 9.157 (s, 2H, 2×O-H phenolic) , 7.024 (s,2H, 2×Ha), 7.167 (s, 2H, 2×Hb), 7.265 (s, 2H, 2×Hc), 7.338 (s, 2H, 2×Hd), 7.405 (s, 4H, 4×He), 7.479 (s, 4H, 4×Hf), 7.782 (s, 4H, 4×Hg), 7.886-7.940 (d, 4H, 4×Hh), 8.243 and 8.318 (s, 2H, 2×Hi, nitrone groups).

Com. no.	Structure	δ (ppm)
[N_1]		2.500 (DMSO solvent), 3.164 (s,1H, S-H, thioenol form), 3.347 (s, 1H, N-H, thioketone form), 3.387 (H_2O in DMSO), 6.980-7.017(t,1H, Ha), 7.193-7.275 (m,1H, Hb),7.302-7.340 (t,1H, Hc), 7.385-7.406 (d, 1H, Hd), 7.441-7.478 (t,1H, He), 7.511-7.570 (q,1H, He`), 7.612-7.707 (m,1H, Hf ), 8.064-8.084 (d, 1H, Hf`), 8.245-8.264 (d, 1H, Hg, nitrone group), 10.129 and 13.161 (s, 1H, 1×O-H phenolic).
[N_2]		2.477 (DMSO solvent), 3.389 (H_2O in DMSO), 6.260 (s,1H, Ha), 6.357 (s,1H, Hb),7.431 (s,1H, Hc), 7.490 (s, 1H, Hd), 7.711 (s,1H,He), 7.784 (s,2H, 2×Hf), 7.957 (s,1H, Hg), 8.407 (s, 1H, Hh), 8.525 (s, 3H, 3×Hi, due to closeness of their chemical shifts) 8.592 (s, 1H, Hj, nitrone group), 9.766 and 9.983 (s, 1H, 1×O-H phenolic).
[N_3]		2.480 (DMSO solvent), 3.359 (H_2O in DMSO), 3.817 (s, 3H, CH_3-O), 6.384 (br, 1H, 1×O-H phenolic), 7.012 (s, 3H, 3×Ha), 7.047 (s, 2H, 2×Hb), 7.069 (s, 1H, Hc), 7.253 (s, 2H, 2×Hd), 7.741 (s, 2H, 2×He), 7.760 (s, 2H, 2×Hf), 8.184 (s, 1H, Hg, nitrone group).
[N_4]		2.489 (DMSO solvent), 3.355-3.677 (H_2O in DMSO), 7.099 (s, 1H, Ha), 7.580 (s, 1H, Hb), 7.708 (s, 2H, 2×Hc), 7.803 (s, 1H, Hd), 7.878 (s, 1H, He), 7.962 (s, 1H, Hf), 8.148 (s, 2H, 2×Hg), 8.766 (s, 1H, Hh, nitrone group), 8.838 (s, 2H, 2×Hi), 13.50 (br, 1H, 1×O-H phenolic)
[N_5]		2.519 (DMSO solvent), 3.420-3.482 (H_2O in DMSO), 5.739 (br, 1H, 1×O-H phenolic), 7.086-7.103 (d,1H, Ha), 7.311 (s, 2H, 2× Hb), 7.446-7.468 (d, 2H, 2×Hc), 7.509 (s, 1H, Hd), 7.577 (s, 2H, 2×He), 7.774 (s, 2H, 2×Hf), 8.050 (s, 1H, Hg, nitrone group), 8.385 (s, 2H, 2×Hh).
[N_6]		2.509 (DMSO solvent), 3.393 (H_2O in DMSO), 6.850 (s, 1H, Ha), 6.932-7.130 (q, 2H, 2× Hb), 7.281 (s,1H, Hc), 7.373 (s, 1H, Hd), 7.477 (s, 1H, He), 7.509 (s,1H, Hf), 7.775-7.779 (d,1H, Hg), 7.793-7.797 (d, 2H, 2×Hh), 7.885 (s,2H, 2×Hi), 8.46 (s,1H, Hj, nitrone group), 8.480 (s, 1H, 1×O-H phenolic).

^{1}H NMR spectra of aldonitrones [N_{11}-N_{15}]

^{1}H NMR spectra of aldonitrones [N_{12}-N_{15}] illustrate good evidence that the synthesis of these compounds happened successfully by appearing the singlet signal of methylene group protons (-CH_2-) in the high field region at narrow range of δ= (2.296-2.297) ppm, beside this the spectra of these compounds were devoid of singlet signal in the down field at the range of δ= (9.5-10.1) ppm assigned to aldehyde group proton. ^{1}H NMR spectrum of aldonitrone [N_{11}] is also devoid of the singlet signal of aldehyde group proton at the range of δ= (9.5-10.1), so expect that the reaction took place successfully and produced compound [N_{11}]. The singlet signal of methylene group protons in compound [N_{11}] probably interacted with signal of DMSO solvent. The proton of nitrone group is deshielded due to the higher electron withdrawal of nitrone group carbon atom, so its signal appears in the down field near δ= 8.5 ppm according to literature (27) . All synthesized aldonitrones [N_{11}-N_{15}] appeared this signal at the range of δ= (7.813-8.481) ppm which is consider good evidence for the successes of the reactions and formation of aldonitrone compounds [N_{11}-N_{15}].

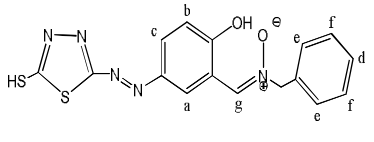
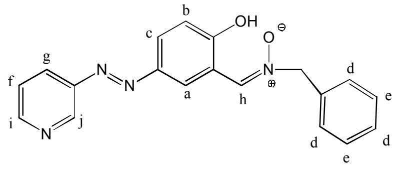
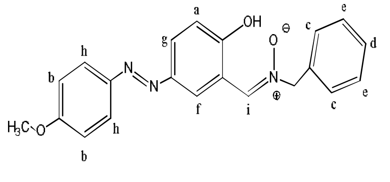
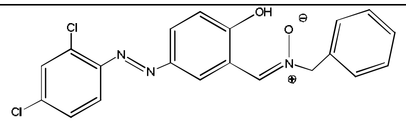
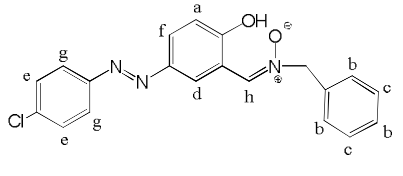
FT-IR spectral data and (C.H.N.S.) elementary analysis of these compounds are assisted and agreed with the ^{1}H NMR spectral data about success of synthesis of these aldonitrones [N_{11}-N_{15}].

The spectra of compounds [N_{11}-N_{15}] appeared signals of the aromatic protons in the down field region at the range of δ= (6.593-8.705) ppm. The theoretical chemical shifts values of these aromatic protons were used to help us for interpretation of their found chemical shifts.

^{1}H NMR spectrum, (400 MHz, DMSO) of compound [N_{11}] appeared the following signals at δ (ppm): 2.505-2.509 (DMSO solvent) (34), 3.171 (s, 1H, N-H, thioketone form), 3.393 (H_2O in DMSO). The singlet signal at (13.189) ppm assigned to the phenolic (O-H) proton, The expanded spectrum showed appearance of seven signals in the down field region at the range (7.114-8.015) ppm attributed to six nonequivalent types of aromatic protons and proton of nitrone group. The interpretation of these signals was performed according to the theoretical chemical shifts values of these aromatic protons and the literature value of nitrone group proton as follows :

7.114 (s, 1H, Ha), 7.363 (s, 1H, Hb), 7.436 (s, 1H, Hc), 7.578-7.597 (d, 1H, Hd), 7.947-7.966 (t, 2H, 2×He), 7.994-7.997 (d, 2H, 2×Hf), 8.015 (s, 1H, Hg, nitrone group). The interpretation of ^{1}H NMR spectra for other aldonitrones [N_{12}- N_{15}] was carried out following the interpretation described for aldonitrone [N_{11}] and listed in table (9) .

Table (9): ^{1}H NMR data of the synthesized azoaldonitrones [N_{11}- N_{15}]

Com. no.	Structure	δ (ppm)
[N_{11}]		2.505-2.509 (DMSO solvent), 3.171 (s, 1H, N-H, thioketone form), 3.393 (H_2O in DMSO), 7.114 (s, 1H, Ha), 7.363 (s, 1H, Hb), 7.436 (s, 1H, Hc), 7.578-7.597 (d, 1H, Hd), 7.947-7.966 (t, 2H, 2×He), 7.994-7.997 (d, 2H, 2×Hf), 8.015 (s, 1H, Hg, nitrone group), 13.189 (s, 1H, 1×O-H phenolic).
[N_{12}]		2.297 (s, 2H, $-CH_2-$), 2.510 (DMSO solvent, 4.001 (H_2O in DMSO), 5.080 (s, 1H, 1×O-H phenolic), 6.593(s, 1H, Ha), 6.729-6.738 (d, 1H, Hb), 7.119-7.139 (d, 1H, Hc), 7.485-7.505 (d, 3H, 3×Hd), 7.70 (d, 2H, 2×He), 7.755 (d, 1H, Hf), 7.96 (d, 1H, Hg), 8.461 (s, 1H, Hh, nitrone group), 8.553-8.565 (d, 1H, Hi), 8.699-8.705 (d, 1H, Hj).
[N_{13}]		2.296 (s, 2H, $-CH_2-$), 2.509-2.514 (DMSO solvent), 3.510-3.515 (H_2O in DMSO), 3.848 (s, 3H, $O-CH_3$), 6.884-6.904 (d, 1H, Ha), 7.080-7.102 and 7.115-7.136 (dd, 4H, 2×Hb and 2×Hc), 7.289 (s, 1H, Hd), 7.373 (s, 2H, 2×He), 7.479-7.500 (d, 1H, Hf), 7.775-7.780 (d, 1H, Hg), 7.792-7.797 (d, 2H, 2×Hh), 7.813(s,1H, Hi, nitrone group), 8.455 (s, 1H, 1×O-H phenolic).
[N_{14}]		2.296 (s, 2H, $-CH_2-$), 2.505-2.514 (DMSO solvent), 3.508 (H_2O in DMSO), 5.079 (s, 1H, 1×O-H phenolic), (d, 7.115-7.136 + d, 7.479-7.499 + 8.125, s, aromatic protons), 8.417 (s, nitrone group)
[N_{15}]		2.296 (s, 2H, $-CH_2-$), 2.509 (DMSO solvent), 3.397 (H_2O in DMSO), 6.873-6.896 (d, 1H, Ha), 7.139 (m, 3H, 3×Hb), 7.325 (t, 2H, 2×Hc and s, 1H, Hd), 7.488-7.506 (d, 2H, 2×He), 7.575-7.596 (d, 2H, 2×Hf), 7.773-7.794 (d, 2H, 2×Hg). The signal of nitrone group proton may be interacted with signals of aromatic protons, 8.466 (s, 1H, 1×O-H phenolic).

Antibacterial activity

Multiple drug resistant organisms, such as methicillin-resistant *Staphylococcus aureous,* vancomycin-resistant *Enterococci,* etc. are becoming common causes of infections in the acute and long term care units in hospitals. The emergence of these resistant bacteria has created a major concern and an urgent need to agents in structural classes distinct from known chemotherapeutic agents (35). The most essential feature of good chemotherapeutic agent is that, it must show a high degree of selective toxicity towards a microorganism, so that, it can be given in sufficient doses to inhibit or kill the microorganism throughout the body without harming the body cell. Azo and nitrone compounds constitute an important class of compounds possessing a wide range of biological activity (36, 37).

Antibacterial tests

In this work, the antibacterial test was carried out according to the disc diffusion method. All azoaldonitrone compounds [N_1-N_{15}] were assayed for their antibacterial activity in **vitro** against one strain of Gram-positive bacteria (*staphylococcus aureous*) and one strain of Gram-negative bacteria (*Escherichia coli*). Prepared agar and petridishes were sterilized by autoclaving for 15 min. at 121 °C. The agar plates were surface inoculated uniformly from the both culture of the tested microorganism. In the solidified medium suitably spaced apart holes were made all 6mm in diameter. These holes were filled with 40 μL of the prepared compounds (5mg of the compound dissolved in 1mL of DMSO solvent). These plates were incubated at 37 ^{o}C for 24 h. for both bacteria. The zones of microbial growth inhibition around the discs were measured in (mm). The results of preliminary screening tests are listed in table (10).

Table (10): The antibacterial activities of the synthesized azoaldonitrones [N_1-N_{15}]

Bacteria	*Staphylococcus aureous* (Gram-positive)	*Escherichia coli* (Gram-negative)
Com. no.	Diameter of inhibition zone in (mm)	
N_1	11	19
N_2	12	7
N_3	7	8
N_4	13	-
N_5	12	-
N_6	20	-
N_7	14	-
N_8	-	-
N_9	15	-
N_{10}	12	-
N_{11}	17	11
N_{12}	-	-
N_{13}	-	7
N_{14}	13	-
N_{15}	14	7
DMSO	-	-

Key of symbols: Highly active = +++ (inhibition zone > 15 mm), Moderately active = ++ (inhibition zone 11-15 mm), Slightly active = + (inhibition zone 5-10 mm), Inactive = - (inhibition zone < 5 mm).

From the data obtained, it is found clearly that compounds [N_6] and [N_{11}] show higher activity against Gram-positive bacteria (*Staphylococcus aureous*). Compounds [N_1], [N_2], [N_4], [N_5], [N_7], [N_9], [N_{10}], [N_{14}] and [N_{15}] appeared medium activity. Compound [N_3] showed weak activity, while the other compounds [N_8], [N_{12}] and [N_{13}] show no activity against this type of bacteria.

In case of Gram-negative bacteria (*Escherichia coli*), it is found that only compound [N_1] which contains thiadiazole ring shows higher activity. Compound [N_{11}], which also contains thiadiazole ring show medium activity. Compounds [N_2, [N_3], [N_{13}] and [N_{15}] appeared weak activity, while the other compounds [N_4-N_{10}], [N_{12}] and [N_{14}] show no activity against this type of bacteria.

Conclusions

1. The synthesized aldonitrones have relatively high stability due to the extending conjugation with azo group.
2. Rate of reaction of *N*-benzylhydroxylamine with the synthesized azoaldehydes is relatively

more than the reaction rate of *N*-phenylhydroxylamine with the same azoaldehydes.

3. The synthesized azoaldehydes substituted with electron - donating groups react relatively faster than that substituted with electron withdrawing groups with both *N*-phenyl and benzylhydroxylamine since the electron donating group (especially by resonance) behaves as electron donor while nitrone group behaves as electron acceptor, this lead to high delocalization of π-electron density through conjugated system and formation of a charge transfer complex, consequently the stability of the resulting azoaldonitrones are increased.

4. The synthesized azoaldehydes substituted with electron withdrawing halogen react relatively faster than that substituted with electron with drawing nitro group since the halogen possesses mesomeric effect which contributes in delocalization of π-electron density leads to increase stability of the resulting azoaldonitrone compound while the nitro group does not possess like this effect.

5. Number of the synthesized azoaldonitrone compounds which appeared activity against Gram-positive (*Staphylococcus aureous*) is more than that in case of Gram-negative (*Escherichia coli*).

6. Azoaldonitrone derivative [N_1] showed the highest activity against Gram-negative (*Escherichia coli*) than the others due to presence of 1,3,4-thiadiazole moiety in its structure.

7. Azoaldonitrone derivative [N_6] showed the highest activity against Gram-positive (*Staphylococcus aureous)* than the others.

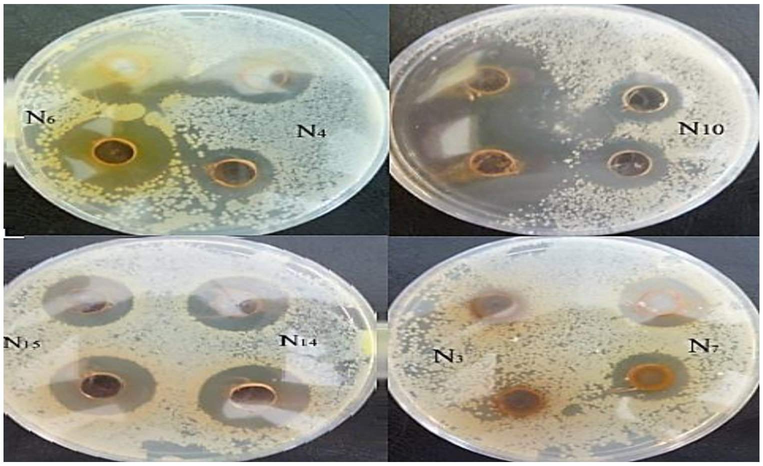


Fig. (1): Antibacterial activity of compounds [N_3, N_4, N_6, N_7, N_{10}, N_{14} and N_{15}] against *Staphylococcus aureous*

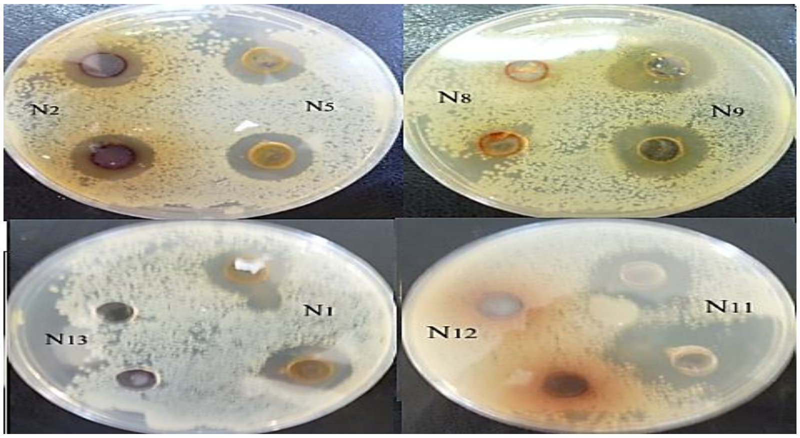


Fig. (2): Antibacterial activity of compounds [N_1, N_2, N_5, N_8, N_9, N_{11}, N_{12} and N_{13}] against *Staphylococcus aureous*

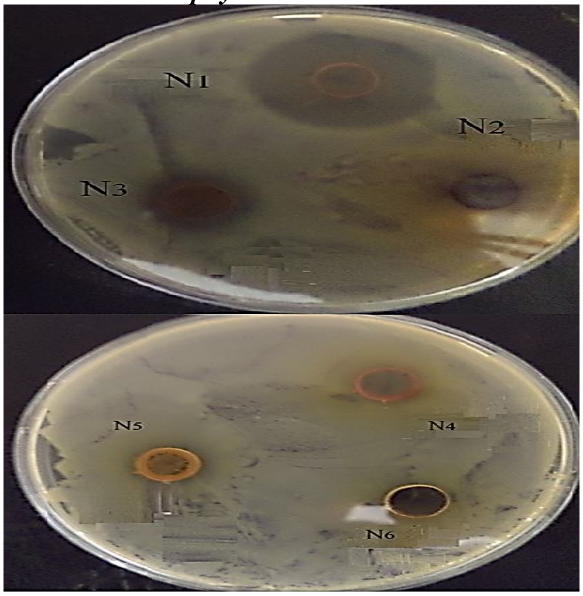


Fig. (3): Antibacterial activity of compounds [N_1, N_2, N_3, N_4, N_5 and N_6] against *Escherichia coli*

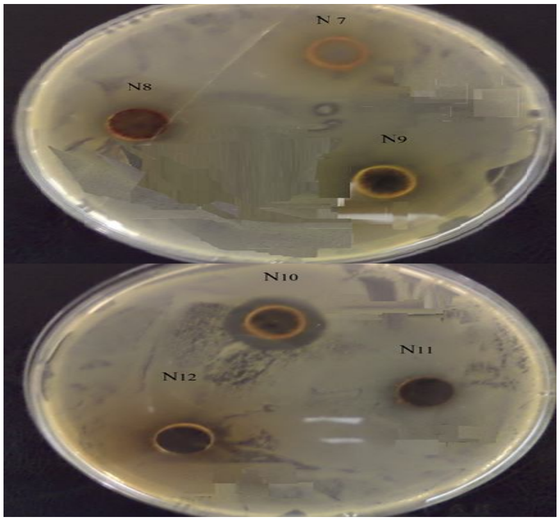


Fig. (4): Antibacterial activity of compounds [N_7, N_8, N_9, N_{10}, N_{11} and N_{12}] against *Escherichia coli*

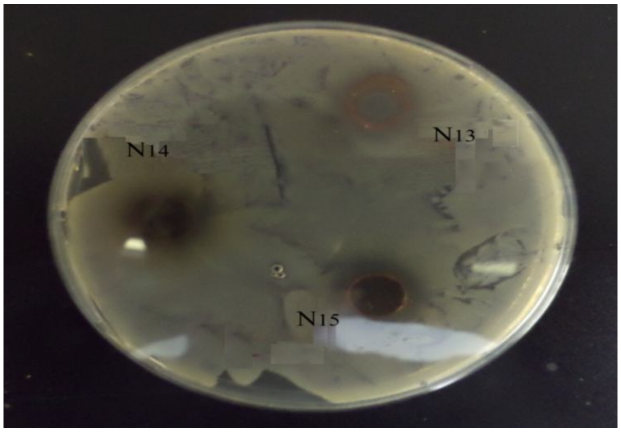


Fig. (5): Antibacterial activity of compounds [N_{13}, N_{14} and N_{15}] against *Escherichia coli*

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