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Synthesis, Characterization and Biological Studies of Some New Dinitrone Compounds

Batool S. Haddad

Department of Chemistry, College of Science, University of Basrah, Basrah, Iraq

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ABSTRACT

Nitrone compounds are known to possess antioxidant, anticancer and antibacterial activities. In the present study, different dinitrone derivatives were synthesized by the reaction of substituted benzil with *N*-(2-hydroxylethylamine) and characterized by elemental analysis, UV, IR, ^{1}H-NMR and ^{13}C-NMR. The synthesized compounds showed good activity against *Staphylococcus aureus* and *Escherichia coli*. N_4 showed the highest antimicrobial activity among all the synthesized compounds, and hence, Minimum Inhibitory Concentration (MIC) was determined for this compound against the gram-positive and gram-negative bacteria. When these compounds were tested for their Median Lethal Dose (LD_{50}), it was noted that they were all moderately toxic.

***Keywords*:** Antibacterial, Dinitrone. LD_{50}, (MIC), *N*-(2-hydroxylethylamine).

Introduction

Nitrones are a very important class of compounds due to their ability as an intermediate compound in organic synthesis. There is a great deal of interest in nitrone compounds because they are considered as building blocks in natural and biologically active compounds.[1] Nitrone is used as a molecular weight regulator during the polymerization process.[2,3] There are many ways to synthesize nitrone, but the most common of these are due to their ease, It is a method of condensation between carbonyl compounds and *N*-monosubstituted hydroxylamine.[4,5] This reaction has another advantage, which is the high yeild of the product.[6,7] Some nitrone compounds have been used in therapeutic areas, especially in the treatment of diseases,[8] resulting from oxidative processes caused by free radicals in the biological system. [9, 10] Some nitrone compounds have shown great efficacy as anti-fungal and anti-bacterial. [11-14] Due to the ability of nitrone to scavenge free radicals, in addition to its ability to improve the functions of the immune system, it has been used in the field of cancer prevention.[15-17] Based on the literature on the biological activity of the nitrone, new derivatives of Dinitrone were synthesized and their efficacy as antibacterials against two types of bacteria, namely *Esherishia coli* and *Staphylococcus aureas*, were studied. The minimum inhibitory concentration of one of the synthesized compounds (N_4) was studied, Which showed higher activity than the other synthesized compounds agains Gram negative *Escherichia coli* and Gram-positive *Staphylococcus aureas*. The toxicity of the synthetic compounds was studied and the LD_{50} was determined based on the Klassen and Doull toxicity scale.

Materials and Methods

The compounds were synthesized based on the literature. Substituted benzil compounds were synthesized by the condensation reaction of substituted benzaldehyde.

*Corresponding author. E mail: batool.mahdi@uobasrah.edu.iq
Tel: +9647826990908

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Nitrone compounds were synthesized by the condensation of benzil with *N*-(2-hydroxyethylhydroxylamine).
The Gallenkamp instrument was used to determine the melting points of compounds. The FT-IR 8400S SHIMADZU (Japan) was used as a KBr disk in recording infrared spectroscopy analyses. The instrument SPECTROSCAN80D, a UV spectrophotometer was used to record the UV-visible spectra (10^{-4} M/ethanol). The Bruker model device using ultra-shield 300 MHz was used to record ^{1}H-NMR and ^{13}C NMR spectra in DMSO and δ units downfield from internal reference Me_4Si.The EuroVector EA3000A was used for CHN analysis

General Procedure of Preparation substituted Benzil
(1.33 gm.; 0.036 mol) of KCN in water (20 ml) was added to a solution of (2.46 gm.; 0.03 mole) of substituted benzaldehyde in ethanol (35 ml)[18] . Reflux have been used for half an hour, as the benzoin was obtained, it was washed with (1: 1- ethanol: H_2O). After that (14 ml) of concentrated HNO_3 was added to (0.2 gm.; 0.09 mol) of benzoin. The reaction mixture was heated with continuous stirring for eleven minutes at 50 ^{o} C. (60 ml) of H_2O was added. After cooling the product, benzil was obtained, as shown in scheme (1). The product was filtered and recrystallized from methanol.

General Procedure of Synthesis Dinitrone
(0.01 mole) of benzil and anhydrous $CaCl_2$ (15 gm) was disolved in absolute ethanol 50 ml. The solution was stirred at 50 ^{o}C for half an hour[19] , and then a solution of (0.02 mole) of *N*-(2-hydroxyethylhydroxylamine dissolved in absolute ethanol 50 ml was added. Three drops of glecial acetic acid was added as a catalist. The solution was refluxed, and at the end of the reaction, the solution was cooled and the product was filtered and then recrystallized from absolute ethanol, as shown in scheme (1). The mechanism of reaction explain in scheme (2). All reactions in this study were followed by

Thin-layer chromatography (*TLC*)
All the synthesized compounds were diagnosed by spectroscopic methods, and the results of the analysis confirmed the validity of the expected chemical composition of the synthesized compounds. The physical properties are shown in Table (1).

biological Experiments
Antibacterial activity
Primary Screening
Primary screening active of dinitrone compounds was tested according to Jorme method against two becterial species gram positive,[20]

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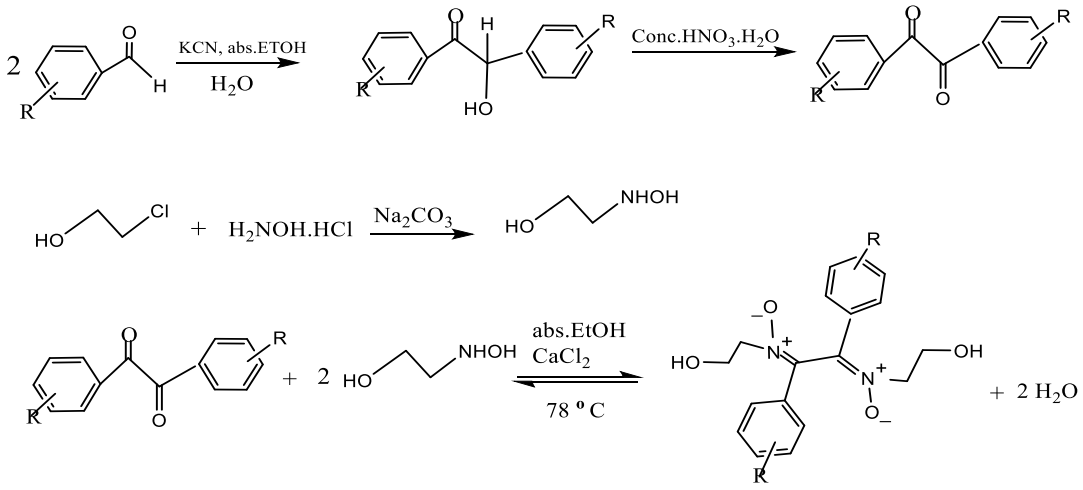
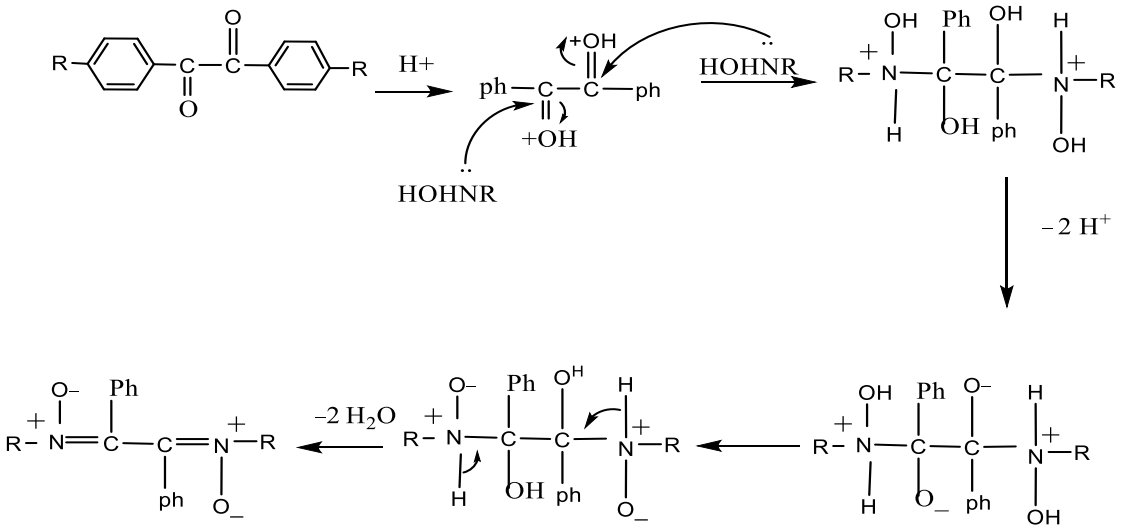


N_1 (R = H), N_2 (R= 4-OH-3-OMe), N_3 (R= 3-NO_2), N_4 (R= 2-OH).

Scheme (1): Reactions synthesis steps of dinitrone derivatives.



Scheme (2): Mechanism formation of dinitrone compounds

Staphylococcus aureus (NCTC 6571) and Gram negative *Escherichia coli* (NCTC 5933). The two isolated were strecked on nutried agar plate, to obtain 24 hours aged colonies. Then one colony from each isolates was cultured on 5 ml nutried broth andincubated at 37 ^{o}C for 6 hours to obtain a growth with concentration of (10 ^{6} cell /ml). 0.1 ml of this growth was spreaded on Muller Hinton agar by wing L- shape rod and petri dishes left to dry after that discs impregnated with synthetic compounds which disolved in dichloro ethanol with concentration of (500µg / 0.1 ml) was layed on the surface of cultured media and incubated for 24 hours at 37 ^{o} C. Biological activityfor each compound was determined by measuring the diameter of inhibition zone (millimeters) around the disc, as shown in Table (2).

Determination of Minimum Inhibitory Concentration (MIC)
The minimum inhibitory concentration (MIC) of compound (N_4), that most active compound against bacteria, was determined according to Jorme method [20]. The compound (N_4) exhibited the strong activity aganst bacteria. Concentration (0.4-100 µg / 0.1 ml) of the studying compound was prepared by dissolving with dichloro ethane. The MIC was determind as described previously (primary screening), as shown in Table (3).

Determination of Median Lethal Dose (LD_{50})
Toxicity, study in term of LD_{50} of some new active dinitrone compounds, was determined in Swiss albino mice BALB/c, weighting

Table 1: Physical properities of dinitrones

Compounds	M.p ^{O} C	Time of reaction	Yield(%)
N_1	163-165	12	64
N_2	121-123	13	51
N_3	214-216	6	61
N_4	158-160	8	60

Table 2: Inhibition zones (mm) of the synthesized dinitrone derivatives against – standard microorganisms

Compounds (conc. 500µg / 0.1 ml) (-)	E scherichia coli	Staphylococcus aureus (+)
N_1	4	4
N_2	10	11
N_3	13	3
N_4	26	13

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about 25 gm, one month old and were housed under controlled conditions [21]. For each compound was used 9 groups, each consisting of 4 mice. The control group was given 0.5 ml of olive oil by oral administration and using a special oral tube, (Stomach tube). Othere groups given, in the same way, a graduated dose (0.25, 0.5, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00 g / kg) of synthesized dinitrone compound (N_1, N_2, N_3, N_4) and monitored for 72 hours, as shown in Tables (4, 5).

Results and Discussion

(N_1) C, C'-Bis(phenyl-N-2-hydroxyethyl nitrone).
Yellow powder; Yield: 64%; M.p. 163-165° C; UV (nm): 381-395 (n→π*) of (C=N→O), 223-274 and 312-352 (π→π*) of (aromatic); IR (ν cm^{-1}): 1590(ν C=N), 1569 (ν C=C), 1281 (ν N-O), 3110(ν CH aromatic), 2896-2877 (ν CH aliphatic), 3315(ν OH),; ^{1}H NMR (DMSO, 300 MHz; δ ppm) δ: 7.72(m,5H, ph), 2.22 (t, 2H, CH_2, J = 6.0 Hz), 4.46 (s, OH); ^{13}C NMR (DMSO, 300 MHz; δ ppm): 121.45, 122.45, 125.52, 126.65, 128.00, 161.36, 161.54; Anal. for $C_{18}H_{20}N_2O_4$: C 65.85, H 6.09, N 8.53. Found: C 65.67, H 6.20, N 8.36.

(N_2) C, C'-Bis(4-hydroxy3-methoxyphenyl-N-2-hydroxyethyl nitrone).
Orange powder; Yield: 51 %; M. p. 121-123 ° C; UV (nm): 387-399 (n→π* nm) (C=N→O), 315-364 and 250-285 (π→π* nm) (aromatic); FTIR (ν cm^{-1}): 1532 (ν C=N), 1413 (ν C=C), 1196 (ν N-O), 1324 (ν C-N), 3114 (ν CH aromatic), 2890-2874 (ν CH aliphatic), 3311(ν OH); ^{1}H NMR (DMSO, 300 MHz; δ ppm): 1.9 (s, OCH_3), 7.76 (m, 4H, ph), 2.20 (t, 2H, CH_2, J = 6.0 Hz), 4.13 (s, OH), 9.50 (s, OH); ^{13}C NMR (DMSO, 300 MHz; δ ppm): 117.87, 119.64, 120.45, 122.12, 124.51, 127.53, 129.10, 13295, 141.76, 164.22; Anal. for $C_{20}H_{24}N_2O_8$: C 57.14, H 5.71, N 6.66. Found: C 57.21, H 5.69, N 6.42.

(N_3) C, C'-Bis(3-nitrophenyl-N-2-hydroxyethyl nitrone)
Yellow powder; Yields: 61 %; M.p. 214-216 ° C; UV(nm): 387-391(n→π*) of (C=N→O), 229-280 and 323-377 (π→π*) of (aromatic); IR (ν cm^{-1}): 1601 (ν C=N), 1589 (ν C=C), 1293 (ν N-O), [(s) 1389 - (as) 1510 (ν NO_2)], 3108 (ν CH aromatic), 2849-2855 (ν CH aliphatic), 3312(ν OH),; ^{1}H NMR (DMSO, 300 MHz; δ ppm) δ: 7.99 (m,4H, ph), 2.23 (t, 2H, CH_2, J = 6.0 Hz), 4.21 (s, OH); ^{13}C NMR (DMSO, 300 MHz; δ ppm): 122.34, 125.12, 127.42, 129.45, 131.62, 132.05, 143.71, 160.17, 160.53; Anal. for $C_{18}H_{18}N_4O_8$: C 51.67, H 4.30, N 13.39. Found: C 51.62, H 4.21, N 13.41.

(N_4) C, C'-Bis(2-hydroxyphenyl-N-2-hydroxyethyl nitrone)
Yellow powder; Yields: 60 %; M.p. 158-160 ° C; UV (nm): 388-397 (n→π*) of (C=N→O), 331-382 and 224-279 (π→π*) of (aromatic); IR (ν cm^{-1}): 1591 (ν C=N), 1566 (ν C=C), 1273 (ν N-O), 3101 (ν CH aromatic), 2889-2865 (ν CH aliphatic), 3317(ν OH),; ^{1}H NMR (DMSO, 300 MHz; δ ppm) δ: 6.99 (m,4H, ph), 2.13 (t, 2H, CH_2, J = 6.0 Hz), 4.18 (s, OH), 9.3 (s, OH-ph); ^{13}C NMR (DMSO, 300 MHz; δ ppm): 121.86, 123.10, 125.47, 126.15, 126.04, 130.00, 131.61, 159.70, 163.13; Anal. for $C_{18}H_{20}N_2O_6$: C 27.77, H5.55, N 7.77. Found: C27.53, H 5. 74, N 7.59.

The reaction of *N*-(2-hydroxylethyl)amine with carbonyl derivatives (benzil) gave new dinitrone compounds as shown in (Scheme 1). Ultraviolet rays were used in the study of the synthesized dinitrone compounds. The concentration of $1*10^{-4}$ was used in measuring the UV spectra. The spectra showed the presence of two bands, one of which represents the electron transitions of the type π to π * in the aromatic ring and the other represents the electronic transitions of the type n to π * in the nitrone group (CH = N → O). Infrared spectra of the synthesized compounds showed peaks representing the streching vibration of the following groups: hydroxyl, (CH) aliphatic and aromatic, (N-O) in the nitrone group, (C = C) and (C = N). The spectra confirmed the appearance of the nitrone peak and the disappearance of the streching vibration of the carbonyl bond, which was in the region (1701-1679) cm^{-1}. The nuclear resonance spectra of the proton showed the presence of multiple signals representing the aromatic ring protons. Also, two multiple signals appeared, representing the two groups CH_2. The spectra also showed the appearance of a signal representing the protein in the hydroxyl group. The spectra of ^{13}C prove the correctness of the proposed structures of dinitrones. Element analysis was used to diagnose the synthesized compounds, and the practical results matched the theoretical results.

Table 3: Inhibition zones (mm) of minimum inhibitory concentration (MIC) of compounds (N_4) against – standard microorganisms

Bacteria	µg / 0.1 ml of compound N_4							
	100	50	25	15	5	1.0	0.5	0.4
***Staphylococcus aureus* (+)**	8.5	7.5	3.0	25	2	1.2	1.2	-
***Escherichia coli* (-)**	15.0	10.0	6.0	2.7	2.5	1.5	10	-

Table 4: The number and percentage of mortalities of mice and LD_{50} values of compounds (N_1, N_2 and N_3)

Group number	Dose (mg/kg)	Mortality			Total No. of mortality	Mortality (%)
		First day	Second day	Third day		
Control	0	0	0	0	0	0
1	0.25	0	0	0	0	0
2	0.5	0	0	0	0	0
3	0.75	0	0	0	0	0
4	1.0	0	0	0	0	0
5	1.25	0	0	0	0	0
6	1.5	1	0	0	1	25
7	1.75	2	0	0	2	50
8	2.0	3	0	0	3	75

Table 5: The number and percentage of mortalities of mice and LD_{50} values of compound (N_4)

Group number	Dose (mg/kg)	Mortality			Total No. of mortality	Mortality (%)
		First day	Second day	Third day		
Control	0	0	0	0	0	0
1	0.25	0	0	0	0	0
2	0.5	0	0	0	0	0
3	0.75	0	0	0	0	0
4	1.0	0	0	0	0	0
5	1.25	1	0	0	1	25
6	1.5	2	0	0	2	50
7	1.75	3	0	0	3	75
8	2.0	4	0	0	4	100

Conclusion

Four dinitrone compounds have been synthesized in this study. The antibacterial activity of the synthesized compounds showed that all synthesized compounds exhibited a good activity against Gram negative *Escherichia coli* as compared with Gram positive *Staphylococcus aureus*. Nitrone (N_4) compound, exhibited a very good activity against Gram negative *Escherichia coli* as compared with other synthesized compounds as shown in. The toxicity of the synthesized compounds, (N_1, N_2, N_3 and N_4) showed a moderately toxic in the range of graded doses.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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