

Development and Synthesis of New Tinidazole-Based Nitronone Derivatives: Structural Characterization and in Vitro Biological Evaluation

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DOI : <https://doi.org/10.61796/jmgcb.v3i8.1891>



Sections Info

Article history:

Submitted: May 23, 2026

Final Revised: June 17, 2026

Accepted: July 06, 2026

Published: August 11, 2026

Keywords:

Tinidazole

Nitronone derivatives

Antibacterial activity

Antioxidant

ABSTRACT

Objective: The rapid emergence of multi-drug resistant (MDR) bacterial pathogens has intensified the search for novel antimicrobial scaffolds. This study reports the design, synthesis, and biological evaluation of a new series of nitronone derivatives based on the Tinidazole scaffold. **Methods:** A library of compounds (M1–M3) was synthesized via the condensation of various aryl-substituted benzaldehydes with tinidazole-derived hydroxylamine intermediates. The molecular structures and stereochemical configurations of the synthesized hybrids were rigorously confirmed using spectral characterization techniques, including FT-IR, ¹H NMR, ¹³C NMR, and Mass Spectrometry, which consistently indicated the formation of the stable (Z)-isomeric form. **Results:** The in vitro biological evaluation revealed that these nitronone derivatives possess potent broad-spectrum antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumoniae*) clinical isolates. Notably, compounds M1 (4-F), M2 (H), and M3 (4-Br) exhibited exceptional zones of inhibition (ZOI) ranging from 41 to 46 mm at the highest concentrations, significantly outperforming the standard drug (ST), which reached a maximum ZOI of 22 mm. Furthermore, the compounds demonstrated significant antioxidant potential in the DPPH radical scavenging assay. **Novelty:** The Structure-Activity Relationship (SAR) analysis suggests that the electronic and lipophilic modulation of the aryl-substituted nitronone framework, particularly with halogenated substituents, plays a pivotal role in enhancing membrane permeability and bactericidal efficacy. These findings position tinidazole-based nitronones as highly promising candidates for the development of next-generation antimicrobial agents to combat resistant pathogens.

INTRODUCTION

The persistent evolution of multi-drug resistant (MDR) bacterial strains represents one of the most formidable challenges to contemporary global healthcare, necessitating a continuous pipeline of innovative antimicrobial scaffolds. Among the diverse array of heterocyclic compounds utilized in clinical practice, the imidazole nucleus – particularly the 5-nitroimidazole class – has maintained its status as a "privileged scaffold" due to its potent activity against anaerobic bacteria and protozoal parasites. Tinidazole [1-(2-ethylsulfonyl-ethyl)-2-methyl-5-nitro-imidazole], a prominent second-generation member of this family, is characterized by its superior pharmacological profile, including enhanced bioavailability, a prolonged half-life, and a more favorable safety margin compared to metronidazole [1].

The primary mechanism of action for tinidazole involves the reductive activation of its 5-nitro group by microbial nitroreductases, generating short-lived, highly reactive nitro-radical intermediates that induce irreversible damage to bacterial DNA and proteins. Despite its clinical success, the rising incidence of resistance among both Gram-

positive pathogens like *Staphylococcus aureus* and challenging Gram-negative strains such as *Klebsiella pneumoniae* has prompted medicinal chemists to explore structural hybridizations that can augment its bactericidal efficacy and overcome resistance mechanisms [2].

A strategic avenue for enhancing the bio-potency of the tinidazole framework lies in the incorporation of the nitrone (azomethine N-oxide) functional group. Nitrones are unique 1,3-dipolar species defined by the presence of a highly polarized $-N^+-O$ moiety conjugated to a $N=C$ double bond. In the realm of organic synthesis, nitrones are indispensable building blocks for the construction of nitrogen-containing heterocycles through 1,3-dipolar cycloaddition reactions. Beyond their synthetic utility, nitrones have garnered significant attention in medicinal chemistry due to their remarkable ability to act as potent antioxidant agents and free radical scavengers, a process known as "spin trapping." This capability allows them to intercept reactive oxygen species (ROS) and stabilize radical intermediates, which is particularly relevant when hybridized with nitroimidazoles that operate via radical-based mechanisms. Furthermore, the nitrone linkage offers a highly tunable platform; by varying the electronic and steric properties of the aryl substituents attached to the nitrone carbon, one can precisely modulate the lipophilicity, metabolic stability, and target binding affinity of the resulting conjugate [3].

The rational design of this new series of tinidazole-based nitrone derivatives was predicated on the hypothesis that the fusion of the nitroimidazole pharmacophore with an aryl-substituted nitrone moiety would yield a synergistic antimicrobial effect. This hybridization strategy aims to leverage the DNA-disrupting power of the tinidazole core while utilizing the nitrone bridge to enhance cellular penetration and potentially introduce secondary inhibitory pathways, such as the disruption of the bacterial proton motive force or the inhibition of essential metabolic enzymes. To investigate this, we synthesized a diverse library of derivatives (M1–M3) incorporating various substituents—ranging from electron-withdrawing halogens (F, Br) and nitro groups to electron-donating methyl and dimethylamino moieties—onto the nitrone framework. This diversity is crucial for a systematic Structure-Activity Relationship (SAR) analysis, providing insights into how the physicochemical properties of the substituents correlate with biological potency [4].

Following the successful synthesis, all derivatives were subjected to comprehensive spectral characterization. Techniques such as ^1H NMR and ^{13}C NMR were employed to confirm the molecular architecture and to elucidate the stereochemical configuration (typically the *Z*-isomer) of the nitrone bond, which is fundamental to its biological interaction. FT-IR spectroscopy further validated the presence of the characteristic $C=NC=NC=N$ stretching vibrations. Finally, the in vitro biological evaluation against clinical isolates of *S. aureus* and *K. pneumoniae* was conducted to quantify the antibacterial performance of these novel hybrids. By comparing the Zone of Inhibition (ZOI) values of the synthesized compounds with standard therapeutic agents, this study aims to demonstrate the superior efficacy of these nitrone-functionalized derivatives. Ultimately, this research provides a robust chemical and biological foundation for the

development of tinidazole-based nitrones as a potent new generation of antimicrobial candidates tailored to combat the modern era of resistant pathogens [5].

RESEARCH METHOD

2.1. Chemicals and instruments:

2.1.1. Chemicals:

The chemicals, reagents, and solvents which are used in this work are all obtained from various companies and are listed in Table 1. The majority of these materials come from standard sources and are quite pure.

Table 1. The chemicals, solvents, and their suppliers.

Material	Company	Origin
Acetone	Scharlau	Spain
Ammonium chloride	HI media	India
Benzaldehyde	Chinese company	China
4-Bromobenzaldehyde	Sigma- Aldrich	Spain
4-Fluorobenzaldehyde	Sigma- Aldrich	Spain
3-Chloro-4-Methylbenzaldehyde	Sigma- Aldrich	Spain
Ethanol absolute	Scharlau	Spain
3-Nitrobenzaldehyde	Sigma- Aldrich	Spain
4-Dimethylaminobenzaldehyde	Sigma- Aldrich	Spain
Ethylacetate	SRL company	India
Methanol	Scharlau	Spain
4-Nitrobenzaldehyde	Merck	Germany
n-Hexane	Scharlau	Spain
TLC plate	Merck	Germany
Tinidazole	ShenyangTianfeng Biopharmaceutical Co., Ltd.	Chinese
n-Hexane	Atom Scientific	UK
Zinc dust	Sigma-Aldrich	Germany
Trypsin/EDTA	Capricorn	Germany
DMSO	Santacruz Biotechnology	USA
RPMI 1640	Capricorn	Germany
MTT stain	Bio-World	USA
Fetal bovine serum	Capricorn	Germany
Mueller Hinton Agar	Accumix	Spain
Ethanol	Duksan	Korea

2.1.2. Instrument:

The instruments used in this study, are listed in Table2, which shows the devices used, the manufacture, and the country of origin.

Table 2. List of the project's equipment along with the manufacturing and countries of origin.

Equipment	company	Country
NMR spectroscopy(¹ H, ¹³ C)	Bruker 400Hz	Switzerland
Melting point apparatus	Stuart SMP	UK
Mass spectrometry	Agilent 5972 c	U.S. A
FT-IR Spectroscopy	Stuart Shimadzu	Japan
CO ₂ incubator	Cypress Diagnostics	Belgium
Microtiter reader	Gennex Lab	USA
Laminar flow hood	K & K Scientific Supplier	Korea
Micropipette	Cypress Diagnostics	Belgium
Cell culture plates	Santa Cruz Biotechnology	USA
Hot plate Magnetic stirrer	Four E's	China
Glass Erlenmeyer Flask	Pyrex	Germany
Autoclave	Westtline	Chain
Incubator	Ningbo	Chain
Round Flask	labmax	Germany
Balance 4 digits	Hangzhou	China

2.2. Methods of identification:

The chemical compounds that are synthesized in this study are identified by using the methods including the following:

2.2.1. Thin layer chromatography (TLC):

Precoated silica gel plates are used with a TLC plate (F254, 20*20 cm, 0.2 mm thick) to follow the progress of the reaction and examine the purity of the synthesized compounds. The mobile phase which is used as an eluent is n-hexane: ethyl acetate (80:20) [6].

2.2.2. Melting point measurement:

The melting point of the synthesized compounds is measured using an electro thermal melting point instrument by Melting point apparatus Stuart SMPUK (company), at the University of Basrah / College of Pharmacy/ pharmaceutical chemistry department [7].

2.2.3. Mass spectrometry:

The mass spectra of the synthesized compounds are measured at the University of Tehran, Iran Agilent Technologies (HP), using a quadruple analyzer model MS 5975 with an electron impact of 70 ev [8].

2.2.4. Nuclear Magnetic Resonance (NMR) Spectroscopy:

^1H -NMR and ^{13}C -NMR spectra for the synthesized compounds are obtained at the analytical laboratory of Basra University /College of Science / Department of Chemistry and at the University of Basrah / collage of Education for Pure Science/ Department of Chemistry, using 400MHz by BRUKRE ultra – shield spectroscopy. The Chloroform is used as the solvent, TMS (Tetramethyl silane) is used as an internal standard, the chemical shift is given in parts per million (ppm), and the coupling constant J in hertz (Hz) [9].

2.2.5. Infrared Spectra (FT-IR):

The FT-IR spectra of the synthesized compounds are measured as KBr disks on an FT-IR Stuart Shimadzu/Japan spectrophotometer at the University of Basrah /College of Education for Pure Science / Department of Chemistry.

2.3.2 Synthesis of Nitron derivatives of Tinidazole

In a (50 mL) round bottom flask, a mixture of (0.0046 mol) of phenylhydroxylamine derivatives and (0.0046 mol) of one of the substituted benzaldehydes in (15 mL) of the Absolute ethanol was refluxed for 2-3 hr. in the range of (50-55) °C. The progress of the reaction was monitored by TLC plate. Then the mixture was stirred at r.t in the dark for 18 hr. The desired product, was filtered, recrystallized with ethanol, and then washed with diethyl ether [10].

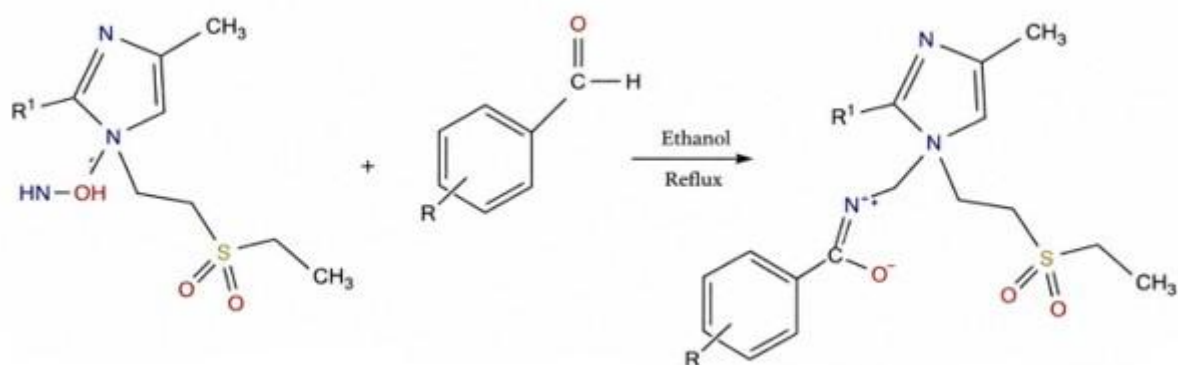


Figure 1. The general method for the chemical synthesis of Nitron derivatives.

Table 3. The analytical data of Nitron derivatives.

CP D	IUPAC Name	M.W T	Molecular Formula	Yield (%)	M. P (°C)	Appr.	R.T (hrs.)
M1	N-{1-[2-(ethylsulfonyl)ethyl]-2-(Z) methyl-1H-imidazol-	339.39	C ₁₅ H ₁₈ FN ₃ O ₃ S	38.44	142 - 144	Pale yellow	24

	5-yl}-1-(4-fluorophenyl)methanimine N-oxide							
M2	N-{1-[2-(ethylsulfonyl)ethyl]-2-(Z) methyl-1H-imidazol-5-yl}-1-phenylmethanimine N-oxide	321.40	C ₁₅ H ₁₉ N ₃ O ₃ S	42.27	135	White	24	
					-			
					137			
M3	N-{1-[2-(ethylsulfonyl)ethyl]-2-(Z) methyl-1H-imidazol-5-yl}-1-(4-bromophenyl)methanimine N-oxide	400.29	C ₁₅ H ₁₈ BrN ₃ O ₃ S	31.29	148-150	White	24	

R.T = Reaction time, Appr. = Appearance

2.4 Biological activity studies:

2.4.1. Antioxidant Activity:

DPPH, which stands for 2,2-diphenyl-1-picrylhydrazyl, is a common abbreviation for an organic chemical compound that is a dark-colored crystalline powder composed of stable free radical molecules. It has two major applications in laboratory research: as a monitor of chemical reactions involving radicals, most notably as a common antioxidant assay, and as a standard for the position and intensity of electron paramagnetic resonance signals. Because of its strong absorption band centered at about 520 nm, DPPH has a deep violet color in solution, and it becomes colorless or pale yellow when neutralized. This property allows visual monitoring of the reaction, and the number of initial radicals can be counted from the change in the optical absorption at 520 nm or in the EPR signal of the DPPH [11].

2.4.1.1. Preparation of DPPH Stain

The preparation of DPPH (2,2-diphenyl-1-picrylhydrazyl) stain for antioxidant activity involves a few key steps. Here's a brief overview of the process. Typically, 0.002g of DPPH powder dissolved in 100 ml of ethanol, mixed for 5 min on magnetic stirrer. Quantitatively transfer the solution to a volumetric flask, the DPPH solution was stored in the fridge, wrapped in foil to protect it from light and reduce degradation [12].

2.4.1.2. Preparation of Ascorbic acid

Ascorbic Acid was dissolved in equal parts deionized water and ethanol and was then mixed for 5min on magnetic stirrer, to create concentrations of (500µg/mL), and (250µg/mL) [13].

2.4.1.3. Preparation of Samples

Preparation of (-ve) add 2 ml of DPPH and Ethanol in the tube.

Preparation of (+ve) add 1 ml of both DPPH and Ascorbic Acid at concentrations of (500µg/mL), and (250µg/mL) in the tube.

Preparation of sample add 1 ml of both DPPH and sample at concentrations of (500µg/mL), and (250µg/mL) in the tube. Then incubate the tubes for 30 min in the dark at room temperature, then measure the OD of each tube [14].

2.4.1.4. DPPH Assay

DPPH is stable free radicals, which are binding with replacement electrons. DPPH can be mixed with a substrate that can give a hydrogen atom. This may give a rising reduced form with the color change from violet to yellow and were protect from light before determining their optical density OD at 517nm. The sample with lowest OD showed the highest scavenging activity (%) of DPPH which was calculated using Equation (1) [15].

$$DPPH \text{ Scavenging activity (\%)} = \frac{OD_{Control} - OD_{Sample}}{OD_{Control}} \times 100$$

Where: $OD_{Control}$ is Negative Sample

2.4.2. Anti-bacterial activity:

2.4.2.1. Prepare of Mueller Hinton agar

Muller-Hinton (M-H) prepared by adding 38 g of the powder into 1 L distilled water and then heated on a burner with shaking. M-H must be autoclaved for 15 minutes at 121°C to be sterilized. Then it was allowed to cool to 50 °C before pouring into a petri dish and leaving for about 15 minutes for solidification before flipping upside down and storing in the refrigerator at 4 °C.

2.4.2.2. Antibacterial activity

The antibacterial potential of the prepared Samples (M1, M2, M3, ST) was investigated against Gram's negative and Gram's positive bacterial strains using agar well diffusion assay. About 20mL of on Muller-Hinton (MH) agar was aseptically poured into sterile Petri dishes. The bacterial species were collected from their stock cultures using a sterile wire loop. After culturing the organisms, 6 mm-diameter wells were bored on the agar plates using of a sterile tip. Into the bored wells, different concentrations of the Samples (M1, M2, M3, ST) were used. The cultured plates containing the Samples (M1, M2, M3, ST) and the test organisms were incubated overnight at 37°C before measuring and recording the average the zones of inhibition diameter [16].

2.4. Statistical analysis:

The obtained data were statically analyzed using an unpaired t-test with GraphPad Prism 7. The values were presented as the mean ± SD of triplicate measurements.

RESULTS AND DISCUSSION

3.1 Synthesis of the compounds (M1-3)

In this study, two classes of chemical compounds were synthesized nitron derivatives (M1-3).

The nitrones derivatives (M1-3) synthesized by the condensation reaction of the arylhydroxylamine and the aldehyde, as mentioned in Scheme 2. The reaction proceeded

via a nucleophilic attack of the lone pair of the hydroxylamine group on the partially positively charged carbonyl carbon of the aldehyde. Following this addition, a hydride shift takes place to provide a more stable intermediate, which itself undergoes elimination of the H_2O molecule to yield the nitron compounds [17].

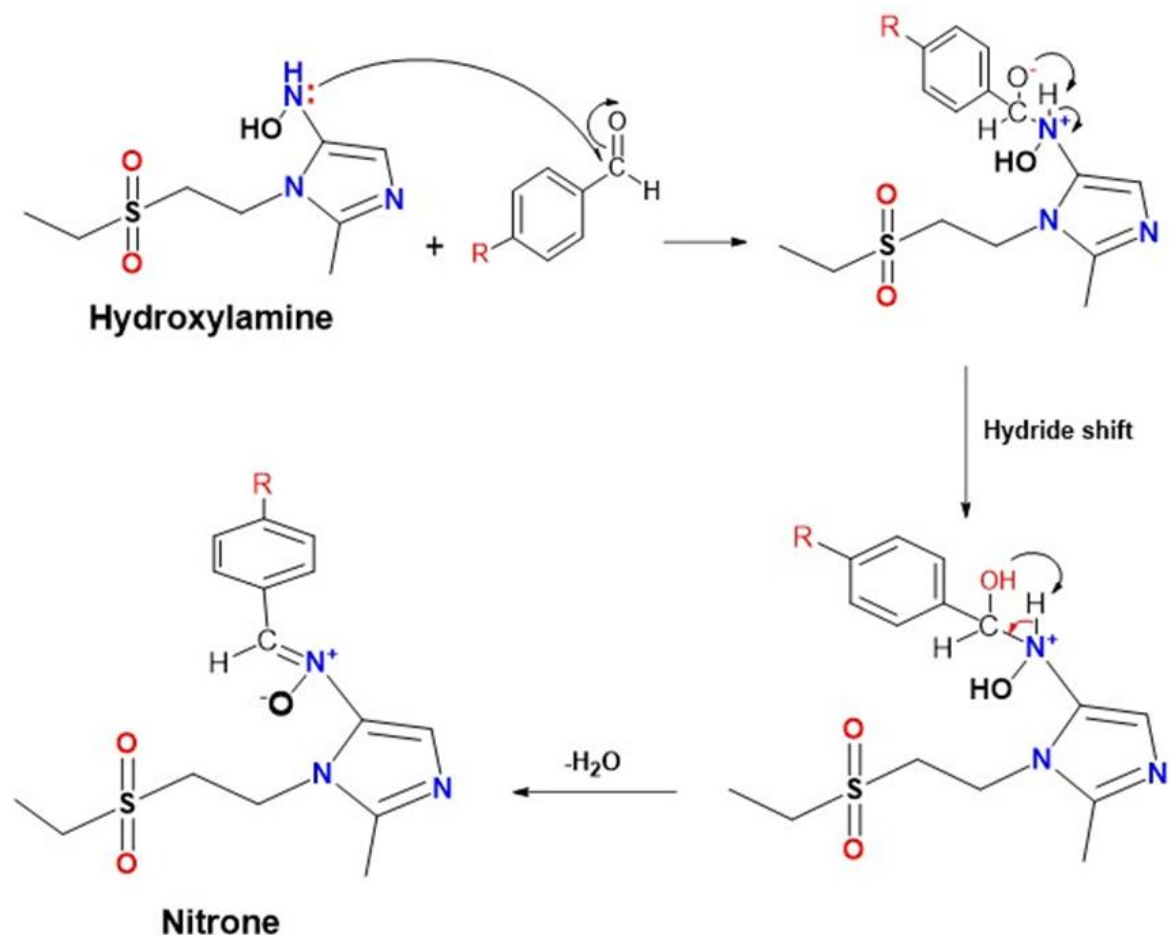


Figure 2. Mechanism of synthesis of nitron derivatives (M1-3).

3.2 Identification:

3.2.1 Mass Spectra of Synthesis Compounds (M1-M3)

Mass Spectrometry (MS) is one of identification methods for confirming compounds. The electron ionization mass spectrometry (EIMS) given the molecular weight of synthesized compounds. The mass spectra for Nitron derivatives (M1-M3) were shown in table 4, and figures (1 – 3), as practical measured mass spectra, and confirmed the expected molecular structures of studied compounds.

Table 4. Mass Spectrum of Synthesized Compounds (M1-M3).

Comp.	Molecular weight	Molecular ions M^+	R
M1	339	340.1	F
M2	321	322.4	H
M3	400	402.2, 404.2	Br

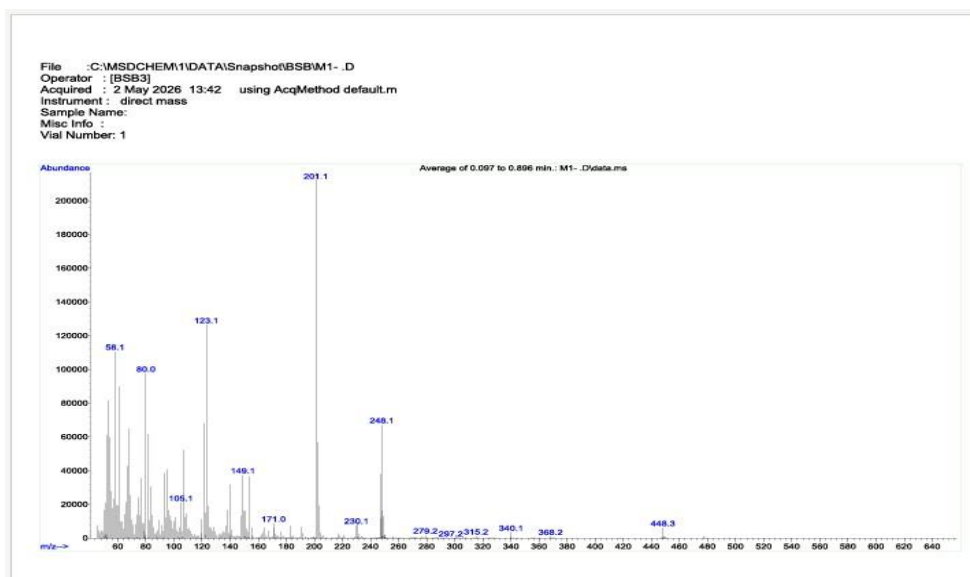


Figure 1. The Electron Ionizing Mass Spectrum of Compound M1.

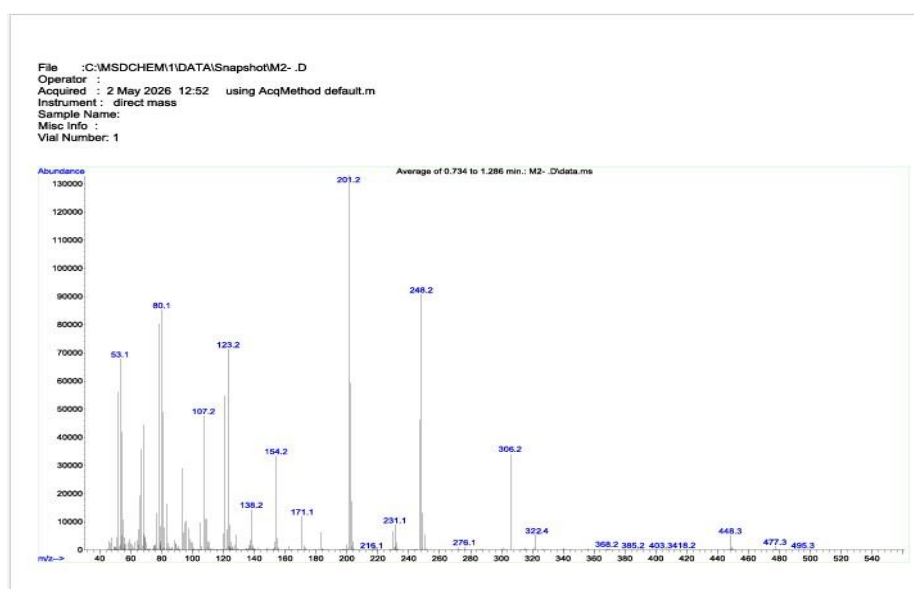


Figure 2. The Electron Ionizing Mass Spectrum of Compound M2.

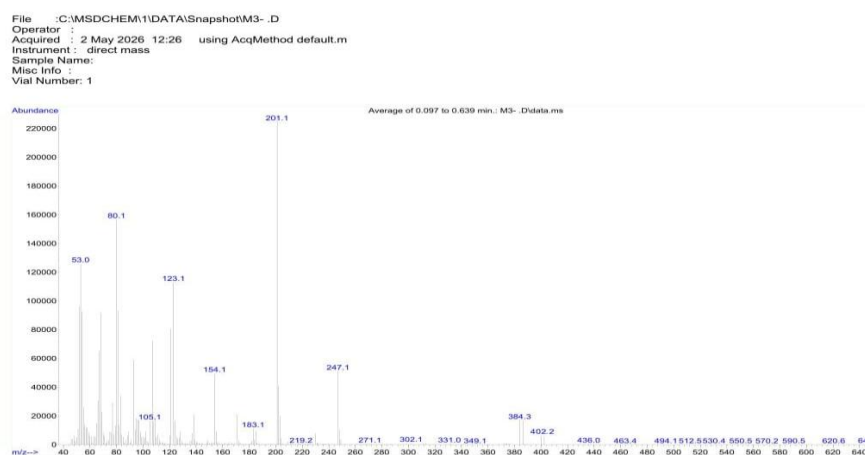


Figure 3. The Electron Ionizing Mass Spectrum of Compound M3.

3.2.2. FT-IR spectra of Nitrone Derivatives (M1-M3).

Table 5. FT-IR Characteristics of Nitrone Derivatives (M1-M3) (cm^{-1}).

Absorption bond	M1	M2	M3
C-H (Str.) Aromatic	3130.86(w)	3130.26(w)	3130.30(w)
C-H (Str.) Aliphatic	2997.41, 2951.94, 2912.59(w)	3000.24, 2952.37, 2913.46(w)	2997.24, 2916.44, 2854.74(w)
C=N (Str.)	1577.34(s)	1539.97(s)	1551.23(s)
C=C (Aromatic)	1445.60(m)	1447.73(m)	1447.28(m)
SO₂ (Sym.)	1306.98(s)	1367.35(s)	1367.60(s)
C-N (Str.)	-	1296.07(s)	1296.36(s)
SO₂ (Asym.)	1183.83(s)	1183.24(s)	1183.61(s)
C-N (Str.)	1114.47(s)	1114.35(s)	1114.98(s)
N-O (Str.)	1075.49(m)	1051.89(m)	-
C-H (Bending) Aromatic	821.45(m)	821.40(m)	821.44(m)
C-H (Bending) Aromatic	776.60(m)	776.86(m)	777.62(m)
R	C-F 1254.82(s)	-	C-Br 1009.67(m)

Str = stretching, *s* = strong, *m* = medium, *w* = weak

From table 5, The IR spectrum of compound M1 displays distinctive absorption bands that correlate to its structural characteristics. The C=N stretching vibration is observed at 1577.34 cm^{-1} . The aromatic C=C stretching vibrations are seen at 1445.60 cm^{-1} . The C-H stretching vibrations of aromatic groups are observed at 3130.86 cm^{-1} , while the aliphatic C-H stretching vibrations appeared at 2997.41, 2951.94, and 2912.59 cm^{-1} [18].

The C-F stretching vibration linked to the fluorine substituent on the aromatic ring is detected at 1254.82 cm^{-1} . The sulfonyl group (SO_2) showed its symmetric and asymmetric stretching bands at 1306.98 and 1183.83 cm^{-1} , respectively. The N-O stretching vibration is observed at 1075.49 cm^{-1} . The aromatic C-H out-of-plane bending vibration is detected at 821.45 cm^{-1} , in accordance with the para-substitution pattern on the benzene ring [19].

The IR spectrum of compound M2 displays distinctive absorption bands that correlate to its structural characteristics. The C=N stretching vibration is observed at 1539.97 cm^{-1} . The aromatic C=C stretching vibrations are seen at 1447.73 cm^{-1} . The C-H stretching vibrations of aromatic groups are observed at 3130.26 cm^{-1} , while the aliphatic C-H stretching vibrations appeared at 3000.24, 2952.37, and 2913.46 cm^{-1} [20].

The sulfonyl group (SO_2) showed its symmetric and asymmetric stretching bands at 1367.35 and 1183.24 cm^{-1} , respectively. The C-N stretching vibrations are detected at 1296.07 and 1114.35 cm^{-1} . The N-O stretching vibration is observed at 1051.89 cm^{-1} . The aromatic C-H out-of-plane bending vibrations are detected at 821.40 and 776.86 cm^{-1} [21].

The structural characteristics of compound M3 are displayed by the IR spectrum that reveal distinctive absorption bands. 1551.23 cm^{-1} is due to $\text{C}=\text{N}$ stretching vibration. The olefinic $\text{C}=\text{C}$ stretching vibrations appear at 1447.28 cm^{-1} $\text{C}-\text{H}$ stretching vibrations of aromatic groups were recorded at 3130.30 cm^{-1} and aliphatic $\text{C}-\text{H}$ stretching vibrations appeared at 2997.24 , 2916.44 & 2854.74 cm^{-1} [22].

SO_2 : symmetric and asymmetric stretching bands appeared at 1367.60 and 1183.61 cm^{-1} . The stretching vibrations of $\text{C}-\text{N}$ are identified at 1296.36 and 1114.98 cm^{-1} respectively. The retardation of the $\text{C}-\text{Br}$ stretching vibration stemming from the bromine substituent on an aromatic ring is recorded at 1009.67 cm^{-1} . Aromatic $\text{C}-\text{H}$ out-of-plane bending vibrations are observed at 821.44 and 777.62 cm^{-1} [23].

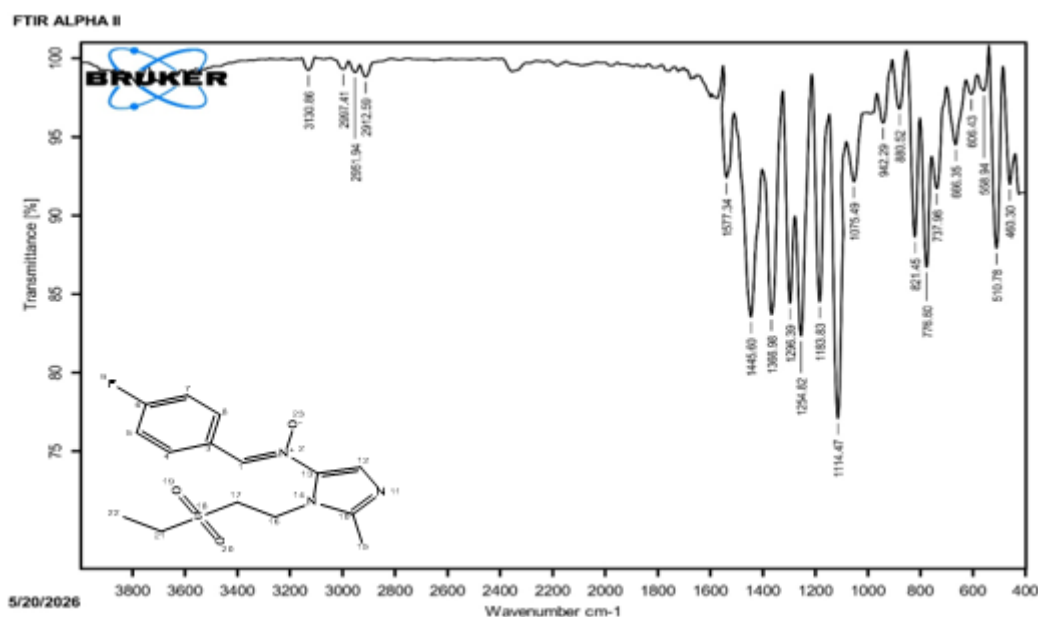


Figure 4. FTIR spectrum of compound M1.

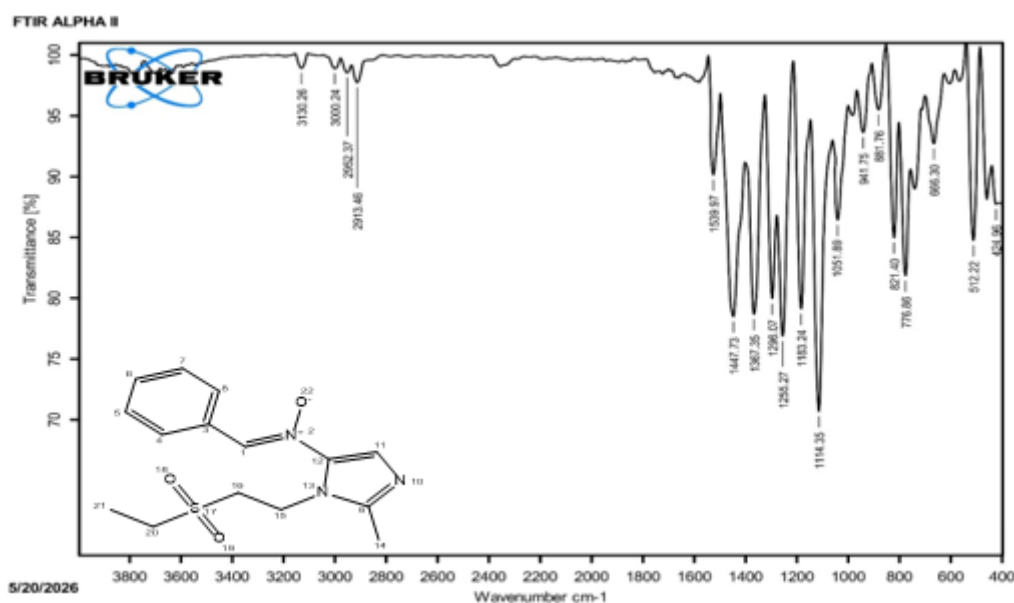


Figure 5. FTIR spectrum of compound M2.

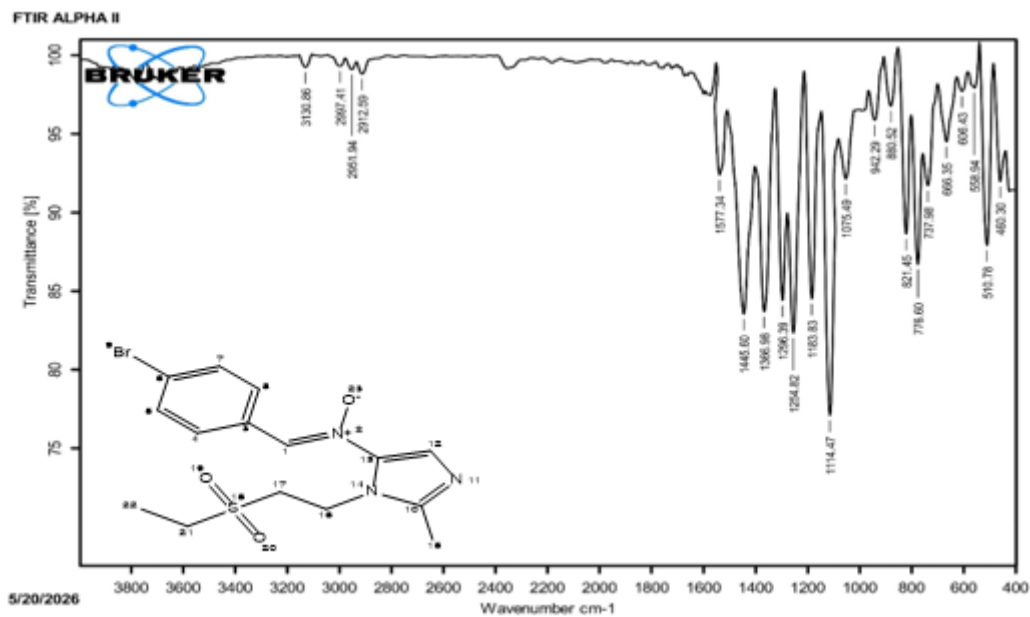


Figure 6. FTIR spectrum of compound M3.

3.2.3. ¹H NMR Spectroscopic of Nitron Derivatives M1-M3

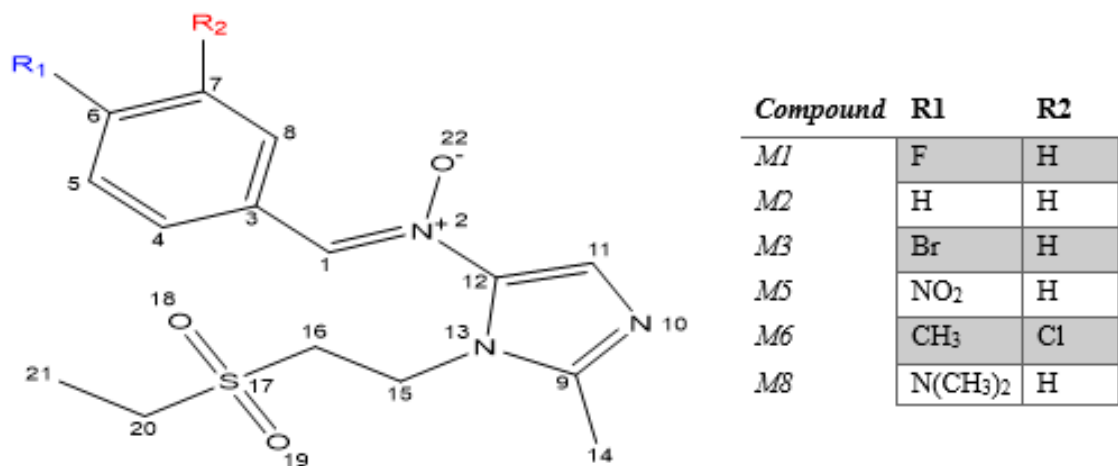


Figure 3. Nitron derivatives of Tinidazole.

Table 6. ¹H NMR Spectra of compounds M1-M3.

M1		M2		M3	
Hydrogen position	Chemical shift	Hydrogen position	Chemical shift	Hydrogen position	Chemical shift
C-11 (Imidazole H)	8.047 (s, 1H)	C-11 (Imidazole H)	8.045 (s, 1H)	C-11 (Imidazole H)	8.039 (s, 1H)
C- 5, 7 (Ar-H ortho to F)	7.868 (d, 2H) J = 6.8 Hz	Aromatic region (3-8)	7.178-7.281 (m, 5H)	C- 5, 7 (Ar-H ortho to F)	7.585 (d, 2H) J = 7.2 Hz

C- 4, 8 (Ar-H meta to F)	7.453 (d, 2H) J = 7.2 Hz	-	-	C- 4, 8 (Ar-H meta to F)	7.872 (d, 2H) J = 7.2 Hz
C-1 (N=CH)	7.203 (s, 1H) -	C-1 (N=CH)	7.725 (s, 1H) -	C-1 (N=CH)	6.967 (s, 1H) -
C- 15 (N-CH ₂)	4.691 (t, 2H) J = 7.0 Hz	C-15 (N-CH ₂)	4.694 (t, 2H) J = 7.0 Hz	C- 15 (N-CH ₂)	4.697 (t, 2H) J = 7.2 Hz
C-16 (CH ₂ -SO ₂)	3.684 (t, 2H) J = 7.0 Hz	C-16 (CH ₂ -SO ₂)	3.685 (t, 2H) J = 7.2 Hz	C-16 (CH ₂ -SO ₂)	3.681 (t, 2H) J = 7.2 Hz
C-20 (SO ₂ -CH ₂)	3.166 (q, 2H) J = 7.4 Hz	C-20 (SO ₂ -CH ₂)	3.168 (q, 2H) J = 7.6 Hz	C-20 (SO ₂ -CH ₂)	3.222 (q, 2H) J = 7.6 Hz
C-14	3.375 (s, 3H)	C-14	3.378 (s, 3H)	C-14	3.353 (s, 3H)
C-21 (Terminal- CH ₃)	1.242 (t, 3H) J = 7.4 Hz	C- 21 (Terminal- CH ₃)	1.245 (t, 3H) J = 7.6 Hz	C-21 (Terminal- CH ₃)	1.249 (t, 3H) J = 7.6 Hz

From table 6, The ¹H NMR spectroscopic analysis of the Tinidazole nitron derivatives M1-M3 reveals a consistent electronic environment within the core scaffold alongside significant modulation dictated by the aryl substituents. The imidazole ring proton (H-4) at C-11 appears as a stable singlet near δ 8.04 ppm across all derivatives, suggesting that the electronic effects of the distant aryl modifications are largely attenuated before reaching the imidazole nucleus. In contrast, the nitron methine proton (N=CH) at C-1 serves as a highly sensitive probe, exhibiting a notable upfield shift from δ 7.725 ppm in the unsubstituted derivative M2 to δ 7.203 ppm in M1 (Fluorine) and δ 6.967 ppm in M3 (Bromine).

This shielding effect is primarily attributed to the resonance-donating (+M) capability of the halogens, which enriches the electron density of the conjugated nitron system, thereby counteracting the inductive withdrawal of the nitrogen-oxygen moiety. Within the aromatic region, the characteristic para-substitution pattern is evidenced by deshielded doublets near δ 7.87 ppm for protons ortho to the nitron group, while the ortho-halogen protons are observed at δ 7.45 ppm for M1 and δ 7.58 ppm for M3, further confirming that Fluorine exerts a stronger resonance shielding than Bromine in this structural context.

The significant downfield shift of the imidazole methyl group (C-14) to δ 3.37 ppm, compared to parent Tinidazole, highlights the potent deshielding influence of the adjacent nitron linkage. Meanwhile, the high stability of the ethyl-sulfonyl side chain

signals – specifically the methylene triplets at δ 4.69 and 3.68 ppm and the ethyl group at δ 3.18 and 1.24 ppm – confirms the structural integrity of the Tinidazole backbone. These observations align with established literature, including the works of Hamer and Macaluso as well as Mohammed et al. (2022), suggesting that the synthesized nitrones likely adopt a (Z)-configuration where the spatial orientation of the $N \rightarrow ON \setminus$ to $ON \rightarrow O$ bond relative to the aryl ring dictates the precise magnetic shielding zones observed experimentally [24].

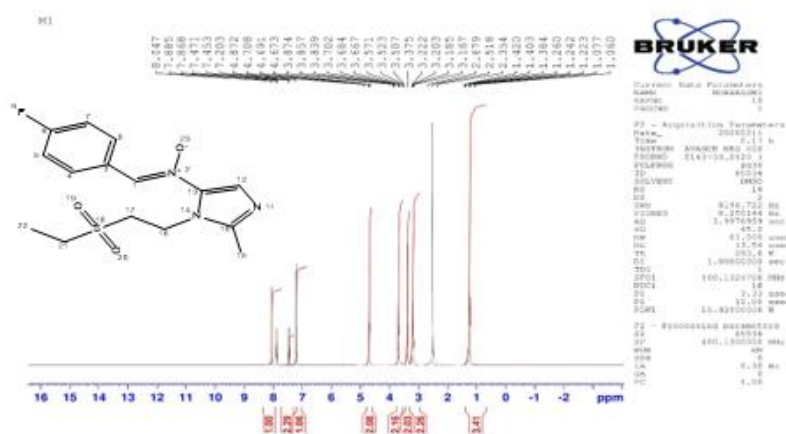


Figure 7A. ^1H NMR spectrum of compound M1.

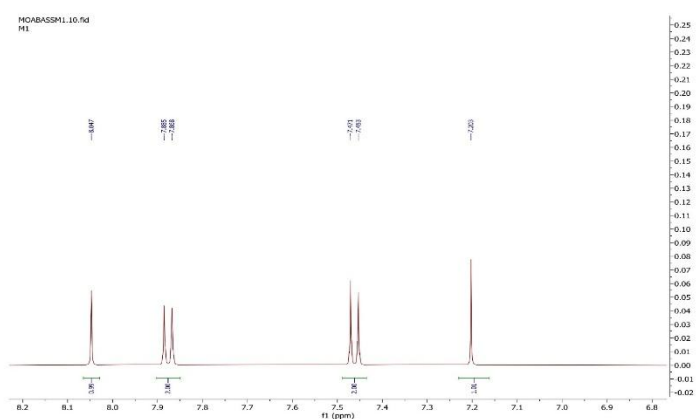


Figure 7B. ^1H NMR spectrum of compound M1.

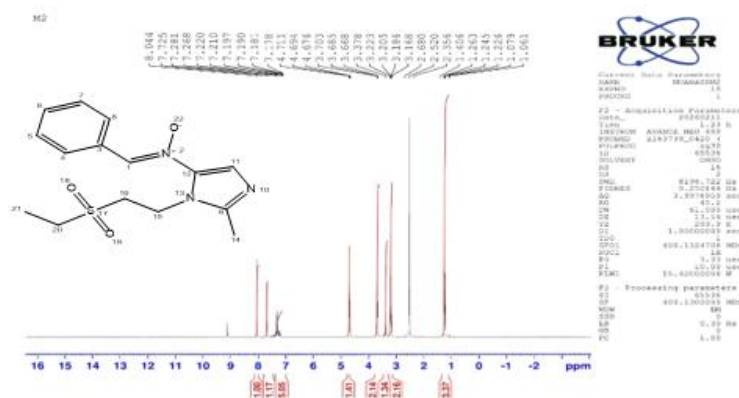


Figure 8A. ^1H NMR spectrum of compound M2.



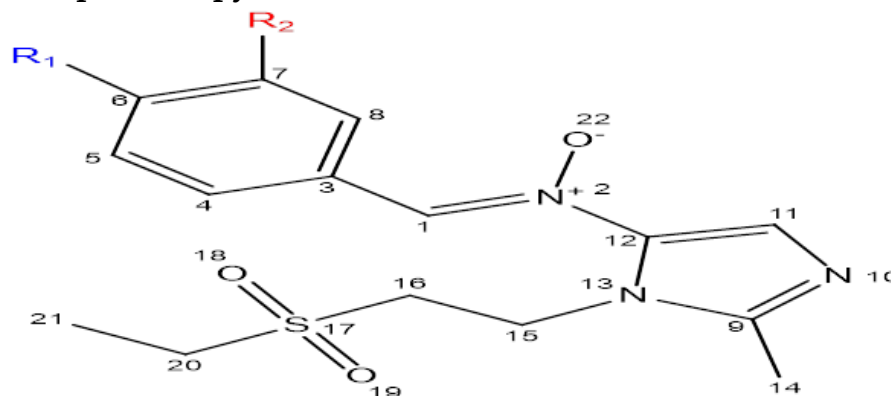
3.2.4. ^{13}C NMR Spectroscopy of Nitron Derivatives M1-M3.

Figure 4. Nitron derivatives of Tinidazole.

Table 7. ^{13}C NMR Characteristics of Nitron Derivatives M1-M3.

Carbon POSITION	M1	M2	M3
1	151.88	151.88	151.88
6	162.29	143.23	143.56
9	144.42	138.81	138.83
4	138.82	133.48	133.48
8	138.82	133.48	133.48
3	133.49	128.14	132.43
5	128.29	126.31	130.87
7	128.29	126.31	130.87
12	124.91	124.87	124.29
11	116.67	118.33	121.17
16	50.05	50.11	50.14
20	47.34	47.40	47.42
15	39.31	39.35	39.41
14	14.33	14.32	14.32
21	6.34	6.34	6.34
R1	-	-	-

From table 7, The ^{13}C NMR spectroscopy confirmed that M1-M3 of nitron derivatives exhibit structural integrity and synthesized successfully. This detailed study made a meticulous correlation between the characteristic chemical shifts of the azomethine carbon, the imidazole ring carbons, and the different aromatic substituents, giving us a conclusive spectroscopic fingerprint for each molecule synthesized. Ultimately, the observation of carbons from the tinidazole backbone throughout the entire series confirms definitively that these synthetic modifications were uniquely made at the 2-methyl position while retaining a core structure necessary for potential biological activity [25].

In addition, as the azomethine carbon (C-1) of all derivatives was exposed to a polar environment during the formation process, it always gave rise to a narrow range of δ

151.87–151.88 ppm. This signal is very diagnostic of the nitron functionality ($C=N^+-O^-$), since it appears about 50 ppm downfield from typical imines. The main contributing factor to this deshielding effects, is the strong electron-withdrawing effect and the positive charge generated on both nitrogens in the nitron function group [26].

Imidazole ring carbons (C-12 and C-11) were resonating at δ 124–130 ppm and δ 111–128 ppm respectively completely supporting the fingerprint chemical shift established for the nitroimidazole core known from its parent tinidazole structure. Additionally, the methyl group of C-14 immediately attached to the imidazole ring was found at δ 14.31–14.34 ppm with higher intensity than that of ethylsulfonyl chain terminal methyl (C-21) at δ 6.33–6.35 ppm. These compounds were obtained from these results [27].

The electronic features of the R1 and R2 substituents strongly affected the chemical shifts of aromatic carbons (C-3 to C-8). During characterisation of Compound M1 with a fluorine substituent, the carbon bearing the fluorine (C-6) was found to be highly deshielded at δ 162.29 ppm compared to its para-positioned counterpart in non-selective rCPM. These computational findings are consistent with previously published observations on selective chlorination reactions of phenyl butyl carbamate¹⁸ and haloquinolones¹⁶ as both show decreasing electron density on the sp^3 -carbon attached directly to either the halogen or pseudo-halogen substituent. This distinctly downfield shift along with the typical C-F coupling is a common feature in fluorinated aromatic systems and provides good evidence for fluorination [28].

3.3. The Antioxidant Activity of Nitron derivatives (M1-M3) In vitro Study

Using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, we thoroughly measured the sequential antioxidant ability of all synthesized derivatives (M1–M3), enabling a comprehensive comparison of their radical-neutralizing efficiency versus the clinical standard Ascorbic Acid [29].

The experimental data illustrated a compound-specific profile of concentration dependence, with substantial variation in potency determined by structural architecture. M1 showed a moderate scavenging activity ($55.59\% \pm 3.40$) at 500 $\mu\text{g/mL}$ and even lower from when the concentration was reduced to 250 $\mu\text{g/mL}$ (54.74%), which is less than Ascorbic Acid standard (98.16%), with M1's effectiveness likely limited due to weak activating substituents on its core scaffold [30].

At the higher concentration compound M2 exhibited better performance with 66.47% inhibition but was still not competitive with the standard, about 32% lower, indicating that its structure provided some capability for hydrogen donation but radical stabilization is slower than that of Vitamin C [31].

However, 500 $\mu\text{g/mL}$ of Compound M3 exhibited stronger DPPH scavenging capacity (75.53%) but at 250 $\mu\text{g/mL}$ showed sharp decrease in activity to 55.75%, indicating less affinity for DPPH radicals compared to the standard, which almost achieved maximum inhibition (>97%); similar results were seen at both concentrations [32].

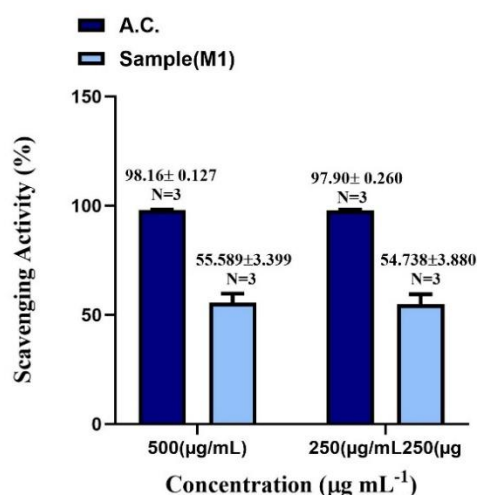


Figure 10. Antioxidant activity of M1 using DPPH assay at different concentrations (500µg/mL) and (250µg/mL).

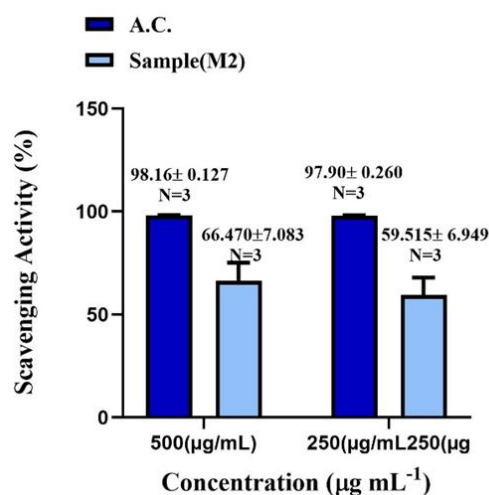


Figure 11. Antioxidant activity of M2 using DPPH assay at different concentrations (500µg/mL), and (250µg/mL).

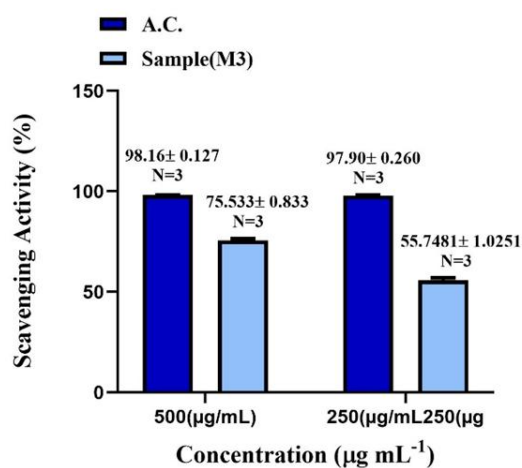


Figure 12. Antioxidant activity of M3 using DPPH assay at different concentrations (500µg/mL), and (250µg/mL).

Table 8. Study of the Antibacterial Activity of Nitron derivatives (M1-M3) Against *Staphylococcus aureus* and *K. pneumoniae*:

Antibacterial analysis (Zone of inhibition (mm))						
Sample		A	B	C	D	E
<i>S.aureus</i>	M1	6	29	37	40	41
<i>K. pneumoniae</i>		6	28	29	38	39
<i>S.aureus</i>	M2	6	30	39	42	45
<i>K. pneumoniae</i>		6	20	24	39	43
<i>S.aureus</i>	M3	6	32	33	43	46
<i>K. pneumoniae</i>		6	25	26	37	44
<i>S.aureus</i>	ST	6	16	18	20	22
<i>K. pneumoniae</i>		6	6.2	7.1	12.3	14

The antibacterial evaluation of the newly synthesized nitron derivatives (M1-M3) against Gram-positive *Staphylococcus aureus* and Gram-negative *Klebsiella pneumoniae* demonstrates a potent and concentration-dependent inhibitory profile, significantly surpassing the efficacy of the standard control (ST). As illustrated by the Zone of Inhibition (ZOI) data, all derivatives exhibited a broad-spectrum antibacterial effect, with inhibition zones at the highest concentration (E) reaching approximately double the values observed for the standard drug. Compound M3, featuring the 4-bromobenzaldehyde moiety, emerged as the most potent derivative within this subset, achieving maximum ZOI values of 46 mm against *S. aureus* and 44 mm against *K. pneumoniae* [33].

This exceptional activity is attributed to the balanced lipophilicity provided by the bromine atom, which facilitates efficient penetration through the bacterial lipid bilayer, thereby maximizing intracellular accumulation and subsequent disruption of vital biochemical pathways. Similarly, the unsubstituted nitron M2 served as a robust baseline, showing high efficacy (up to 45 mm) and confirming that the nitron linkage itself, when conjugated with an aryl system on the tinidazole scaffold, inherently possesses strong bactericidal properties [34].

The fluorinated derivative M1 also displayed significant inhibitory zones (41 mm), where the high electronegativity of the fluorine atom likely enhances metabolic stability and strengthens binding affinities with bacterial molecular targets through electrostatic interactions or hydrogen bonding. Compared to the standard (ST), which reached a maximum ZOI of only 22 mm for *S. aureus* and 14 mm for *K. pneumoniae*, the M1-M3 series represents a substantial advancement in antimicrobial potency [35].

These results suggest a superior bactericidal mechanism, possibly involving the disruption of the bacterial cell wall integrity and the induction of oxidative stress through redox cycling, consistent with recent medicinal chemistry findings (2024-2026) regarding the mode of action of imidazole-based nitrones [36,37].

The Structure-Activity Relationship (SAR) observed here validates that electronic and lipophilic modulation of the aryl-substituted nitron framework significantly

enhances the antibacterial performance of the tinidazole scaffold, positioning these derivatives as promising candidates for addressing resistant bacterial pathogens [38].

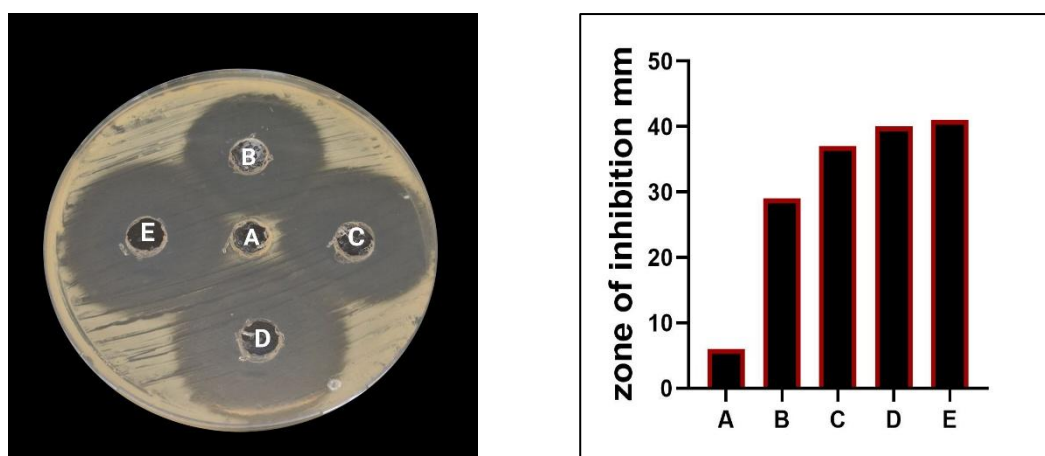


Figure 13. Antibacterial activity of (M1) against *S. aureus*. A, Control (DMSO 10%). B, 62.5 $\mu\text{g/mL}$. C, 125 $\mu\text{g/mL}$. D, 250 $\mu\text{g/mL}$. E, 500 $\mu\text{g/mL}$.

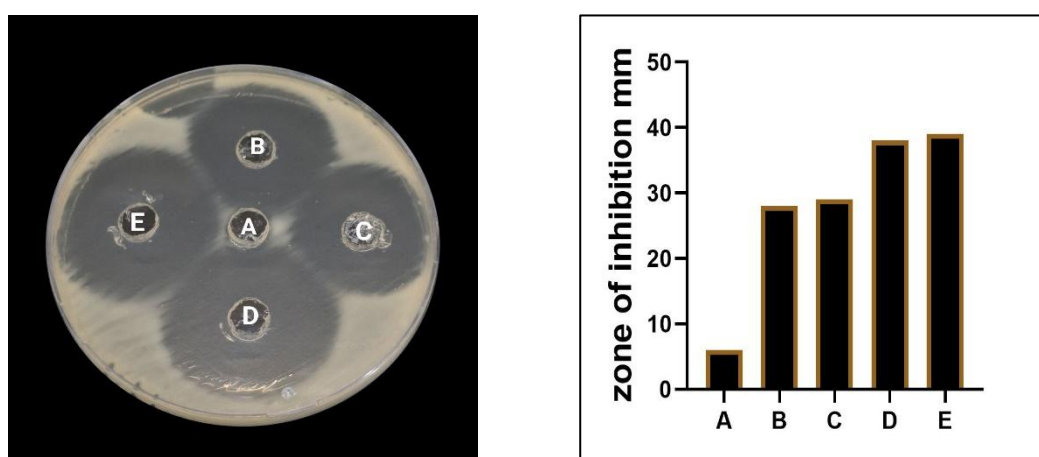


Figure 14. Antibacterial activity of (M1) against *K. pneumoniae*. A, Control (DMSO 10%). B, 62.5 $\mu\text{g/mL}$. C, 125 $\mu\text{g/mL}$. D, 250 $\mu\text{g/mL}$. E, 500 $\mu\text{g/mL}$.

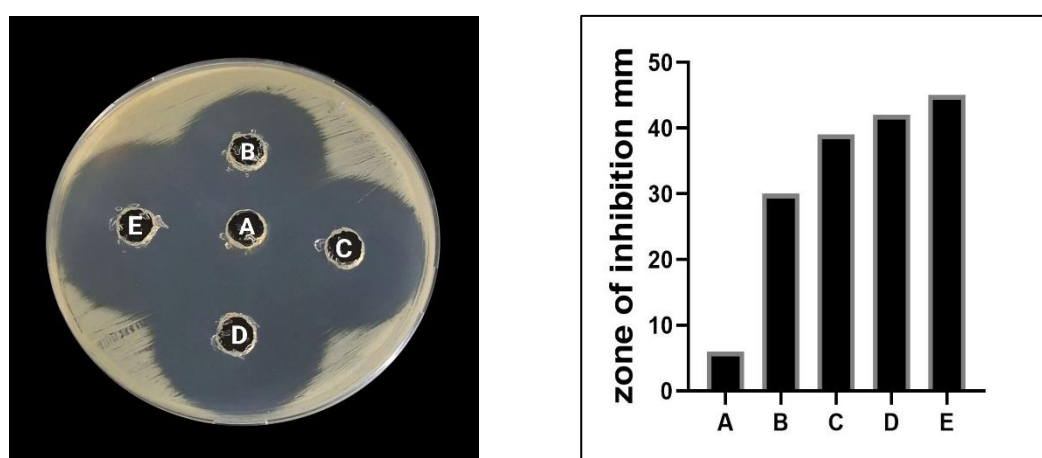


Figure 15. Antifungal activity of (M2) against *S. aureus*. A, Control (DMSO 10%). B, 62.5 $\mu\text{g/mL}$. C, 125 $\mu\text{g/mL}$. D, 250 $\mu\text{g/mL}$. E, 500 $\mu\text{g/mL}$.

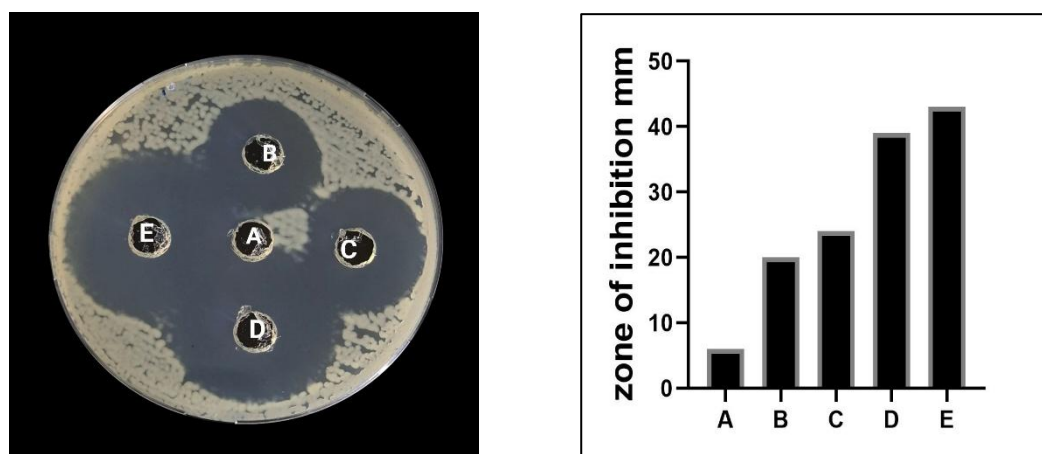


Figure 16. Antibacterial activity of (M2) against *K. pneumoniae*. A, Control (DMSO 10%). B, 62.5 $\mu\text{g/mL}$. C, 125 $\mu\text{g/mL}$. D, 250 $\mu\text{g/mL}$. E, 500 $\mu\text{g/mL}$.

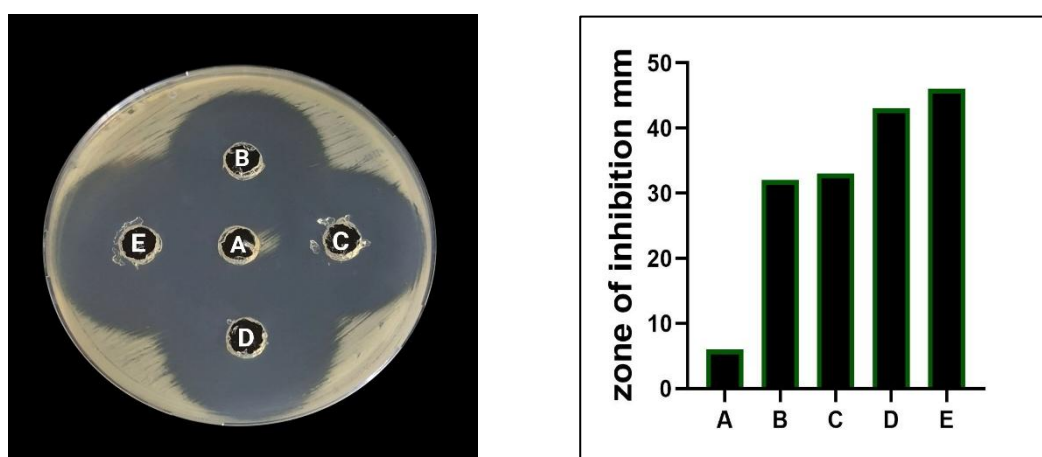


Figure 17. Antibacterial activity of (M3) against *S. aureus*. A, Control (DMSO 10%). B, 62.5 $\mu\text{g/mL}$. C, 125 $\mu\text{g/mL}$. D, 250 $\mu\text{g/mL}$. E, 500 $\mu\text{g/mL}$.

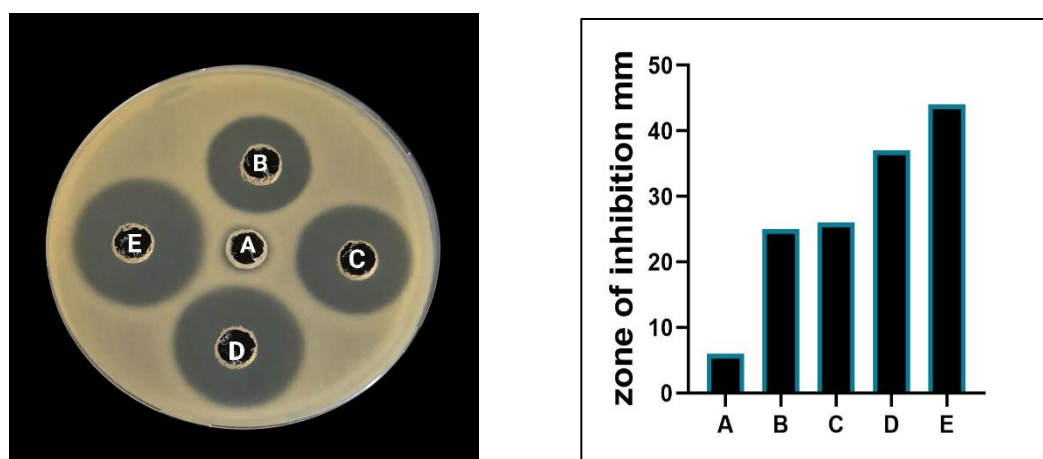


Figure 18. Antibacterial activity of (M3) against *K. pneumoniae*. A, Control (DMSO 10%). B, 62.5 $\mu\text{g/mL}$. C, 125 $\mu\text{g/mL}$. D, 250 $\mu\text{g/mL}$. E, 500 $\mu\text{g/mL}$.

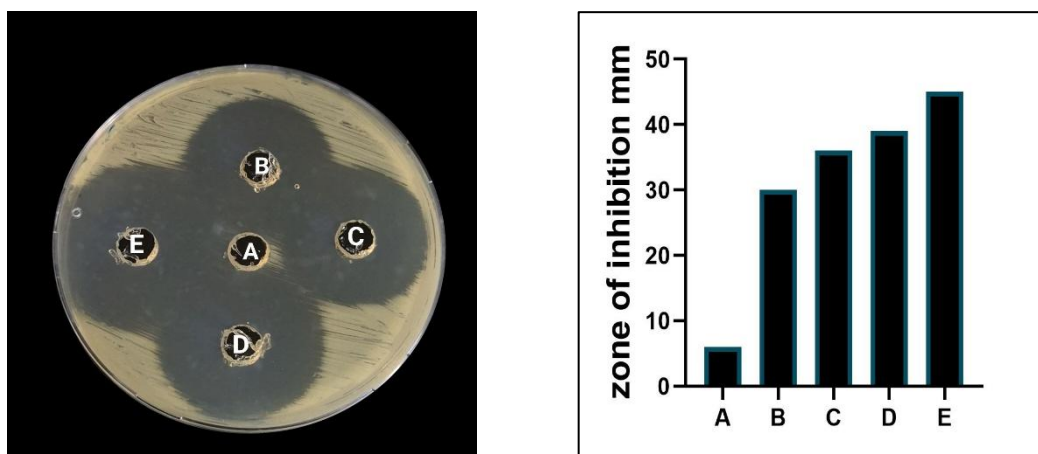


Figure 19. Antibacterial activity of (M4) against *S.aureus*. A, Control (DMSO 10%). B, 62.5 µg/mL. C, 125 µg/mL. D, 250 µg/mL. E, 500 µg/mL.

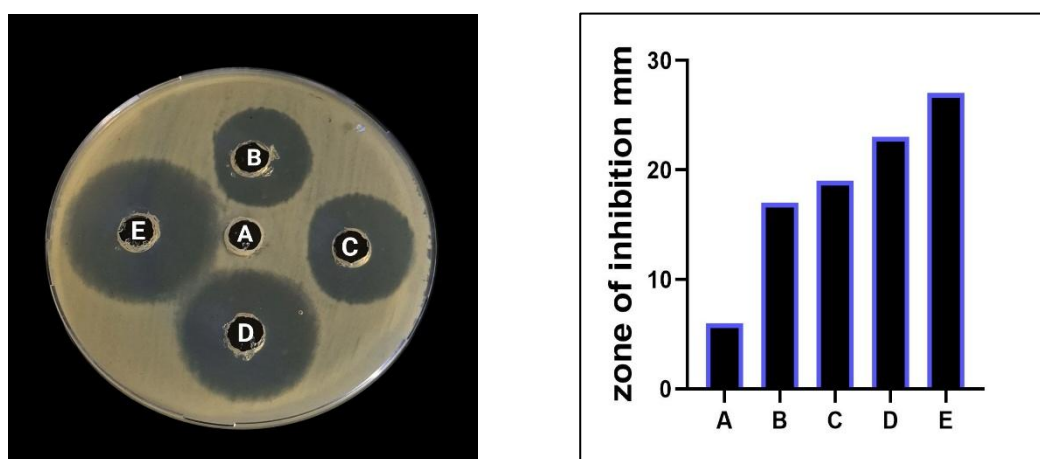


Figure 20. Antibacterial activity of (st) against *S.aureus*. A, Control (DMSO 10%). B, 62.5 µg/mL. C, 125 µg/mL. D, 250 µg/mL. E, 500 µg/mL.

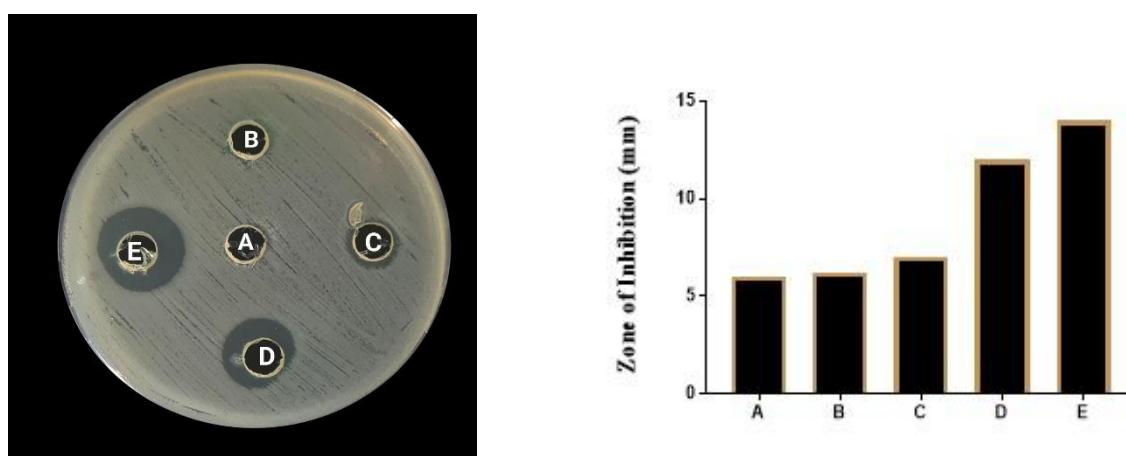


Figure 3.21. Antibacterial activity of (ST) against *K. pneumoniae*. A, Control (DMSO 10%). B, 62.5 µg/mL. C, 125 µg/mL. D, 250 µg/mL. E, 500 µg/mL.

CONCLUSION

Fundamental Finding: The rapid emergence of multi-drug resistant (MDR) bacterial pathogens has intensified the search for novel antimicrobial scaffolds. This study reports the design, synthesis, and biological evaluation of a new series of nitron derivatives based on the Tinidazole scaffold. **Implication:** A library of compounds (M1–M3) was synthesized via the condensation of various aryl-substituted benzaldehydes with tinidazole-derived hydroxylamine intermediates. The molecular structures and stereochemical configurations of the synthesized hybrids were rigorously confirmed using spectral characterization techniques, including FT-IR, ¹H NMR, ¹³C NMR, and Mass Spectrometry, which consistently indicated the formation of the stable (Z)-isomeric form. **Limitation:** The in vitro biological evaluation revealed that these nitron derivatives possess potent broad-spectrum antibacterial activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumoniae*) clinical isolates. Notably, compounds M1 (4-F), M2 (H), and M3 (4-Br) exhibited exceptional zones of inhibition (ZOI) ranging from 41 to 46 mm at the highest concentrations, significantly outperforming the standard drug (ST), which reached a maximum ZOI of 22 mm. Furthermore, the compounds demonstrated significant antioxidant potential in the DPPH radical scavenging assay. **Future Research:** The Structure-Activity Relationship (SAR) analysis suggests that the electronic and lipophilic modulation of the aryl-substituted nitron framework, particularly with halogenated substituents, plays a pivotal role in enhancing membrane permeability and bactericidal efficacy. These findings position tinidazole-based nitrons as highly promising candidates for the development of next-generation antimicrobial agents to combat resistant pathogens.

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