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# Design and Synthesis of Some New Phenylhydrazone Derivatives for Antimicrobial Evaluation

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## Article

# Design and Synthesis of Some New Phenylhydrazone Derivatives for Antimicrobial Evaluation

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**Abstract:** Antimicrobial chemotherapeutic failure as a result of pathogenic resistance stain is great concern across the globe, there is need to search for an effective antimicrobial agent from synthetic sources to wipe microbial resistant in clinical practice. The phenylhydrazone derivatives were scientifically found to have wide application in area of drug discovery due to their anticancer, anti-tubercular, anti-bacterial and anti-fungal activities. Five (5) novel (E)-Substituted-N-(phenylhydrazones) derivatives were obtained by condensation reaction through one step reaction, here are the five compounds, HS1 (E)-1-(1-(4-bromophenyl)ethylidene)-2-(2,4-dinitrophenyl)hydrazine), HS2 (E)-1-(1-(4-bromophenyl)ethylidene)-2-(4-nitrophenyl)hydrazine), HS3 (E)-1-(4-nitrophenyl)-2-(1-(3-nitrophenyl)ethylidene)hydrazine), HS4 (E)-1-(2,4-dinitrophenyl)-2-(1-(3-nitrophenyl)ethylidene)hydrazine), and HS5 (E)-1-(1-(3-nitrophenyl)ethylidene)-2-phenylhydrazine) and their structural elucidation were established on the basis of FTIR, 1D and 2D NMR spectra and the Insilco prediction of physicochemical properties found within the lipinski's rule of five and the newly synthesized compounds were evaluated for antimicrobial assessment via an in-vitro test model using inhibition zone technique, MIC, MBC and MFC.

**Keywords:** hydrazine–hydrazone; antibacterial activity; antifungal activity; zone of inhibition; MIC; MBC; MFC,

## 1. Introduction

Infectious diseases caused by pathogenic agents have been a major problem to humans which account for the death of millions of people annually worldwide (Reygaert, 2018). Some of these diseases threaten the health of millions people especially where no cure or vaccine exists (Ade et al., 2020)). Recently, infectious disease caused by corona virus (covid-19) became one of the top cause of death across the globe (Facts, 2015).

Antimicrobial resistance (AMR) possess urgent public health problem and economic challenge across the world (WHO, 2023). AMR occurs when pathogens change over time and no longer respond to antimicrobial drugs making the infection hard to treat (WHO, 2023). Misuse and overdose of antimicrobial drugs in humans result to antimicrobial drug resistance (Alberta et al., 2020).

Hydrazone are class of organic compounds formed by replacement of oxygen of carbonyl compound to carbon nitrogen double (R-CH=N-), (Li et al., 2021). Hydrazone Schiff base compounds and their complexes have also drawn great attention last two decades due to their high level pharmaceutical potentials such as, antimicrobial, antibacterial, antifungal, anti-inflammatory etc. (Nesterkina et al., 2020).

## 2. Materials and Methods

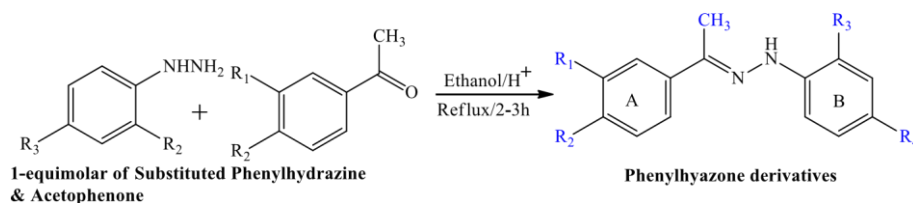
Most of the glassware used for this experiments were of Pyrex product and the glassware were cleaned and rinsed with organic solvent and dried in an oven before use to avoid contamination of the products. Some of these equipment were sourced from the Department of Pharmaceutical and Medicinal Chemistry and pharmaceutical microbiology, Ahmadu Bello University (ABU), Zaria.

### 2.10. Reagents, Solvents and Standard Drug

The reagents were purchased from Sigma-Aldrich, Germany. All the solvents and reagents used for this research are of analytical grade and used without further purification such as, Phenylhydrazine, 4-bromoacetophenone, 4-nitrophenylhydrazine, Sodium hydroxide (20%), substituted Acetophenone, Hydrazine hydrate, 2,4-diphenylhydrazine, glacial acetic acid, distilled water, and some organic solvents such as methanol (Merck), ethanol absolute (Merck), n-hexane, ethyl acetate (EtOAc), chloroform (CHCl<sub>3</sub>), and dichloromethane (DCM).

### 2.20. Chemistry

In this current research (E)-1-phenyl-2-(1-phenylethylidene) hydrazone derivatives (1-5) were obtained by following the synthetic protocols as mentioned in Scheme.1 below where an equimolar of substituted both acetophenone and phenylhydrazone were dissolved in 100mL round bottle flask contained (30ml) of absolute ethanol with few drops glacial acetic acid as catalyst, under refluxed for 3hrs at 65°C (*scheme 1*) (Arshad et al., 2019). The progress of the reactions monitored by thin layer chromatography (TLC) and the reaction mixture was filtered off using vacuum machine filtration, and dried to obtained the desire product (Moussa et al., 2020), (Fen et al., 2023). The purity of the compounds was checked by TLC using the solvent ratio of hexane: ethyl acetate (7:3) and their structures were confirm using spectral data (IR, <sup>1</sup>H NMR) and elemental analysis.



Scheme 1. Synthesis of Phenylhydrazone derivatives HS1-HS5

Cpd	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	R <sub>4</sub>
HS1	H	Br	NO <sub>2</sub>	NO <sub>2</sub>
HS2	H	Br	H	NO <sub>2</sub>
HS3	NO <sub>2</sub>	H	H	NO <sub>2</sub>
HS4	NO <sub>2</sub>	H	NO <sub>2</sub>	NO <sub>2</sub>
HS5	NO <sub>2</sub>	H	H	H

## 3.0. Antimicrobial Activity

### 3.1. Antimicrobial Evaluation of the Synthesized Compounds

Synthesized compounds were screened for antimicrobial evaluation by MIC (Minimum Inhibitory Concentration) method as follows:

### 3.2. Source of Test Organisms

The clinical strains obtained from Ahmadu Bello University Teaching Hospital (ABUTH) Shika and pure cultures of *Staphylococcus aureus* (SA), *Escherichia coli* (EC), *Streptococcus pneumoniae* (SP), *Bacillus subtilis* (BS), *Salmonella typhi* (ST), *Aspergillus niger* (AN) and *Pseudomonas aeruginosa* (PS) from Department of Pharmaceutical microbiology Laboratory, Faculty of Pharmaceutical Sciences, at Ahmadu Bello University (ABU) in Zaria.

### 3.3. Procedure Preparation of Inoculum:

Three Gram positive (*S.aureus*, *S.pneumonia* and *Bacillus subtilis*), three Gram negative (*E. coli*, *P. aeruginosa* and *S. typhi*) and one fungal (*A.niger*) cultures were taken for the study where the microbial cultures were periodically sub-cultured in Nutrient broth for bacterial cultures while potato dextrose broth for fungal cultures.

For the study the cultures were grown overnight in the respective medium (Agrawal & Jeyabalan, 2017).

### 3.4. Preparation of Test Sample:

All the glassware used for the experiments were sterilized using (AUTOCLAVE) and dry off at 60 °C for 30min in an oven before used (Al-otibi et al., 2021). Different concentrations of the five (5) test samples were dissolved in dimethyl sulphoxide (DMSO). Various concentrations of the test solutions were made in DMSO, such as 50 µg/mg, 25 µg/mg, 12.5 µg/mg and 6.25 µg/mg for zone of inhibition and 12.5µg/mg, 6.25µg/mg, 3.125µg/mg and 1.5625µg/mg were subsequently were used for determination of MIC

### 3.5. Standard References

The antibiotic Ciprofloxacin was used as standard reference in the case of gram negative and gram positive bacteria where Terbinafine was used as standard reference in the case of fungi (Elhady et al., 2017).

### 3.6. Determination of Antimicrobial Activity (Zone of Inhibition)

The synthesized compound were evaluated in testing antibacterial activity and antifungal by measuring the zone of inhibition for each bacterial culture by agar well diffusion method, where the bacterial strains were cultured on the Mueller–Hinton Agar (MHA) and fungus strains on the potato dextrose agar (PDA) media plate (Al-otibi et al., 2021).

A sterile cork borer of 6mm diameter was used to make wells on the medium and 1ml of the synthetic compound was dropped into the well and the experiment was repeated in triplicate and the plates were then incubated at 37°C for 24h. After incubation period, the diameters with no growth of inoculated microorganisms (inhibition zones) around the well were measured with transparent ruler in millimeter, averaged and the mean values were tabulated (Thi et al., 2015). The reference drugs ciprofloxacin and Terbinafine used as control for antibacterial and antifungal were completely inhibited the growth of the test organism

### 3.7. Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) is the least concentration of an antimicrobial agent that would inhibit the visible growth of the pathogen after 24h (bacteria) and 48h (fungi) incubation (You et al., 2021) (Ade et al., 2020).

MIC values of the synthesized compounds were determined using agar dilution technique and where the four concentration of different five samples, twenty eight test tubes for each compounds were sterilized and labelled appropriately. 2ml of sterile Mueller hinton broth (MHB) for bacterial and potato dextrose broth (PDA-broth) for fungal were added to each test tubes followed by dilution

of 1 ml from my synthetic sample and was inoculated with the test microorganism and one tube was set as a control with 2 ml of medium and 1ml of 0.5 Mc Farland standard solution where all the inoculums and All tested tubes were incubated at 37°C for 24hrs (antibacterial) and 30°C (antifungal) for 3 days. The test tubes were then checked after the incubation period for growth of microorganisms, where the presence of turbidity indicates the growth while those resemble to control indicate the inhibitory effect of the test compound against the tested microbes this method was developed using (Agrawal & Jeyabalan, 2017).

4.0. Results and Discussion

Final Product obtained were given below in figure 1-5

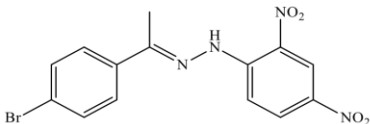


Fig.1 ((E)-1-(1-(4-bromophenyl)ethylidene)-2-(2,4-dinitrophenyl)hydrazine)

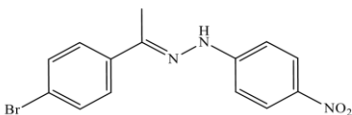


Fig.2 ((E)-1-(1-(4-bromophenyl)ethylidene)-2-(4-nitrophenyl)hydrazine)

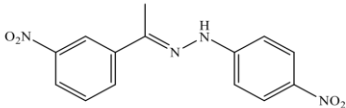


Fig.3 ((E)-1-(1-(4-nitrophenyl)ethylidene)-2-(1-(3-nitrophenyl)ethylidene)hydrazine)

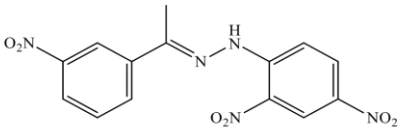


Fig.4 ((E)-1-(1-(2,4-dinitrophenyl)ethylidene)-2-(1-(3-nitrophenyl)ethylidene)hydrazine)

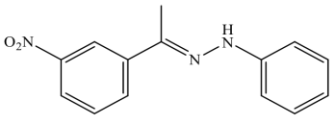


Fig.5 ((E)-1-(1-(3-nitrophenyl)ethylidene)-2-phenylhydrazine)

Table 1. Zone of inhibition using (Agar well diffusion method).

Cpd Code	Conc. (µg/ml)	Inhibition zone represented by (mm)						
		Gram-positive bacteria				Gram-negative bacteria		Fungi
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>S.pneumonia</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>A. niger</i>
HS <sub>1</sub>	50	20	22	NA	18	20	12	15
	25	18	18	NA	15	18	NA	13
	12.5	16	NA	NA	NA	15	NA	12
	6.25	16	NA	NA	NA	NA	NA	NA
HS <sub>2</sub>	50	19	15	15	15	18	17	25
	25	17	13	12	12	15	13	20
	12.5	15	12	11	11	13	12	17
	6.25	14	11	NA	NA	NA	NA	15
HS <sub>3</sub>	50	17	17	20	17	22	14	20
	25	15	18	18	13	20	13	18
	12.5	14	15	15	12	19	12	15
	6.25	12	NA	NA	NA	18	NA	NA
HS <sub>4</sub>	50	19	20	15	15	20	15	20
	25	16	17	NA	13	19	R	17
	12.5	15	15	NA	12	16	R	15
	6.25	13	12	NA	NA	NA	NA	12
HS <sub>5</sub>	50	20	11	18	22	20	22	18

	25	17	NA	15	19	19	18	16
	12.5	15	NA	12	16	16	16	13
	6.25	NA	NA	NA	NA	NA	NA	NA
CF	10	-	-	-	-	-	-	-
TN	30	-	-	-	-	-	-	-

Meaning; Positive (+) resistant / growth or Negative (-) inhibition / no growth and NA mean no activity CF: Ciprofloxacin & TN: Terbinafine.

**Table 2.** Minimal inhibitory concentrations (MIC) (mg/ml) of hydrazones Derivatives Against seven Pathogenic Microbes.

	Test Name	Gram-positive bacteria				Gram- negative bacteria		Fungi
		<i>S. aureus</i>	<i>B. subtilis</i>	<i>S.pneumonia</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	<i>A. niger</i>
HS <sub>1</sub>	MIC	12.5	3.125	NA	1.56	12.5	3.125	12.5
HS <sub>2</sub>	MIC	12.5	12.5	6.25	12.5	12.5	6.25	12.5
HS <sub>3</sub>	MIC	6.25	3.125	3.125	1.56	6.25	6.25	12.5
HS <sub>4</sub>	MIC	12.5	3.125	6.25	3.125	6.25	6.25	12.5
HS <sub>5</sub>	MIC	3.125	6.25	12.5	6.25	12.5	3.125	6.25

NA mean no activity.

**Table 3.** Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) (mg/ml) of synthetic hydrazones Derivatives Against seven isolate.

Cpd.	Test Name	Gram-positive bacteria				Gram- negative bacteria		
		Fungi						
Code		<i>S. aureus</i>	<i>B. subtilis</i>	<i>S.pneumonia</i>	<i>E. coli</i>	<i>S. typhi</i>	<i>P. aeruginosa</i>	
		<i>A. niger</i>						
HS1	MBC/MFC	+	6.25	NA	3.125	+	6.25	+
HS2	MBC/MFC	12.5	3.25	6.25	12.5	12.5	12.5	+
HS3	MBC/MFC	+	12.5	+	+	+	12.5	12.5
HS4	MBC/MFC	+	6.25	12.5	3.125	12.5	12.5	+
HS5	MBC/MFC	6.25	12.5	+	12.5	+	6.25	12.5

Meaning; Bacteriostatic (+) inhibition = No MBC, Fungal static (+) inhibition = No MFC and NA mean no activity.

**Table 4.** Phenylhydrazone compounds and their characteristics. .

Code	Mol. Formula	Mol.Wt. g/mol	Color	M.P. (°C)	R <sub>f</sub> Value	Yield (%)
HS1	C <sub>14</sub> H <sub>11</sub> BrN <sub>4</sub> O <sub>4</sub>	379.17	Pink	213-215	0.91	40.00
HS2	C <sub>14</sub> H <sub>12</sub> BrN <sub>3</sub> O <sub>2</sub>	334.17	Brown	220-222	0.85	67.00
HS3	C <sub>14</sub> H <sub>12</sub> N <sub>4</sub> O <sub>4</sub>	300.27	Sandy-brown	213-215	0.83	67.00
HS4	C <sub>14</sub> H <sub>11</sub> N <sub>5</sub> O <sub>6</sub>	345.27	Yellow	210-212	0.87	89.00
HS5	C <sub>14</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>	255.27	Reddish Brown	110-112	0.89	81.00

HS= hydrazone, Mol=molecular, Wt= weight, M.P= melting point, g/mol= gram per mole.

## 5.0. Conclusion



Five (5) new compounds of (E)-Substituted-N-(phenylhydrazones) derivatives were synthesized and obtained by cyclization reaction with an excellent yield of (40–89%) and all compounds were structurally characterized and confirmed by spectral analysis.

The compounds were screen out for their antimicrobial activity and found to have shown promising antimicrobial activity in vitro compared to reference standard drugs.

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## Abbreviations:

❖ **MIC:** Minimal inhibitory concentration, **ZOI;** Zone of inhibition, **MBC;** Minimum Bactericidal concentration, **MFC;** Minimum Fungicidal concentration, **FTIR;** Fourier transform infrared, **NMR;** nuclear magnetic resonance, **1D;** one-dimensional, **2D;** two dimensional and **HS1-5:** synthetic hydrazone (symbolic code number)

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