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SYNTHESIS AND BIOLOGICAL EVALUATION OF HYDRAZONES DERIVED FROM 2, 4-DINITROPHENYLHYDRAZINE

Ibrahim Usman Kutama[*1], Fatima J. Suleiman[1], Ibrahim I. Fagge[1], Aminu Ahmed[1]

[1*]Department of Chemistry, Faculty of Science, Kano University of Science and Technology, Wudil, PMB 3244, Kano-Nigeria.

ABSTRACT

Three hydrazone derivatives were synthesized by condensation reaction between 2, 4-dinitrophenylhydrazine (2, 4-DNPH) and some carbonyl compounds. The hydrazones obtained from aldehydes: acetaldehyde and benzaldehyde were found to have higher % yields (65% and 74% respectively) than that obtained from acetone (60%). Structures of the compounds were confirmed by spectral analysis. Measured λmax for all the three hydrazone derivatives fall within the literature values. Antimicrobial screening of all the three compounds showed high activity against *Salmonella typhi and Streptococcus faecalis* with average zone of inhibition of 25mm. The compounds showed activity against *Escherichia coli* only at higher concentrations of 100µg/ml and 50µg/ml but become inactive at lower concentrations.

KEYWORDS

Hydrazones, 2, 4-dinitrophenylhydrazine (2, 4-DNPH), Spectral analysis, λmax, Antimicrobial screening, *Salmonella typhi*, *Streptococcus faecalis* and *Escherichia coli*.

Author for Correspondence:

Ibrahim Usman Kutama,
Department of Chemistry,
Faculty of Science,
Kano University of Science and Technology,
Wudil, PMB 3244, Kano-Nigeria.

Email: iukutama@hotmail.com

INTRODUCTION

Hydrazones are a class of organic compounds which possess the structure $R_1R_2C=NNH_2$ and are readily obtained by condensation reactions between aldehydes or ketones with appropriate hydrazine[1]. Hydrazones have been reported to possess, among other, antimicrobial, anticonvulsant, analgesic, anti-inflammatory, antiplatelet, anti-tubercular and anti-tumoral activities[2]. Because of this several synthesis and biological evaluation of hydrazones have been reported and many reviews of such works have been published[2-7]. Padmini *et al* for example reported that

series of Hydrazone derivatives synthesized from analogues of pyrazole with 1, 3, 4-substitution were evaluated for anti-angiogenic and anti-tumor properties and showed some promising results[4]. In another work a number of hydrazone derivatives were synthesized and screened for antimicrobial properties by Sevim *et al*. The hydrazones ethyl 2-arylhydrazono-3-oxobutyrates showed significant activity against *Staphylococcus aureus* whereas others had no remarkable activity on this strain. A particular derivative was found to be more active than the others against *Mycobacterium fortuitum* at a MIC value of 32µg/ml[5]. Sevim *et al*. Also reported that the antiplatelet activity of novel tricyclic acylhydrazone derivatives was evaluated by their ability to inhibit platelet aggregation of rabbit platelet-rich plasma induced by platelet-activating factor (PAF) at 50 nm. Benzylidene- 4-bromobenzylidene 3-hydroxy-8-methyl-6-phenylpyrazolo [3, 4b] thieno-[2, 3-d] pyridine-2-carbohydrazide were evaluated at 10µml, presenting, respectively, 10.4 and 13.6% of inhibition of the PAF-induced platelet aggregation[5].

In a continuation of the search for the medicinal value of these important compounds, two hydrazone derivatives were synthesized by condensation reaction between 2, 4-dinitrophenylhydrazine and the aldehydes acetaldehyde and benzaldehyde. The third hydrazone was obtained by the same reaction with 2, 4-dinitrophenylhydrazine and acetone. All the three hydrazone derivatives were carefully screened against the disease causing pathogens *Salmonellatyphi*, *Streptococcus faecalis* and *Escherichia coli*. The results of the research work are hereby presented.

Experimental

All solvents were obtained dry from a Grubbs dry solvent system and glassware was flame dried and cooled under vacuum before use. ^{1}H and ^{13}C NMR spectra were measured using $CDCl_3$ as solvent on a Bruker 250MHz machine. Chemical shifts for carbon and hydrogen are given on the δ scale relative to TMS (tetramethylsilane, δ = 0 ppm). ^{13}C NMR spectra were recorded using the JMOD method. IR spectra were recorded on a Perkin-Elmer 1600 FT-IR machine using 0.5mm NaCl cells.

λmax were found from measurements of absorptions at various wavelengths on Model 721 spectrophotometer. Melting point was carried out at room temperature using Gillen Kamp melting point apparatus. Bioactivity was carried out using standard agar (Mueller-Hinton agar and Nutrient Broth).

General Procedure for the synthesis of the Hydrazones (Kumaran *et al.* 2013)[2]

The carbonyl compound (1 equivalent) was dissolved in 20ml of ethanol, 2, 4-DNPH (1 equivalent) was added to the solution and shaken. The reaction mixture was stirred well, warmed on a water bath at 50ºC for 20 minutes and stored in the refrigerator for 3 hrs. The crystals were filtered, washed with cold water, dried and weighed.

1-(2, 4-dinitrophenyl)-1, 2-diazabut-2-ene: $C_8H_8N_4O_4$ (compound C-1)

From the general procedure above, starting from 0.56cm^3 (0.01mol) of acetaldehyde and 1.98g (0.01mol) of 2,4-DNPH, the title compound was obtained as reddish orange crystals (1.46g, 65%), melting point 160-162ºC, FT-IR 1629cm^{-1} (C=N), 3085cm^{-1} (=C-H) and λ_{max} = 350nm.^{1}H NMR (250 MHz; $CDCl_3$) δ_H0.91 (3H, d, C*H*$_3$), 7.75 (1H, s, N-N-*H*), 7.25 (1H, q, N=C(C*H*$_3$)-H), 7.00-8.33 (2H, m, Ar-H), 8.87 (1H, s, Ar-H); ^{13}C NMR (100 MHz; $CDCl_3$) δ_C 9.80 (*C*H$_3$), 117.0 (Ar*C*H), 119.5 (Ar*C*H), 130.0 (Ar*C*H), 136.0 (Ar*C*-NO$_2$), 139.0 (Ar*C*-NO$_2$), 148.0 (Ar*C*NH-N), 154.7 (N=*C*).

1-(2, 4-dinitrophenyl)-3-phenyl-1, 2-diazapropene: $C_{13}H_{10}N_4O_4$ (compound C-2)

Using the general procedure above, starting from 1.02cm^3 (0.01mol) benzaldehyde and 1.98g (0.01mol) of 2, 4-DNPH, the title compound was obtained as light orange crystals (2.11g, 74%), melting point 235-237ºC, FT-IR 1614cm^{-1} (C=N), 3100cm^{-1} (=C-H) and λ_{max} = 350nm.^{1}H NMR (250 MHz; $CDCl_3$) δ_H7.65 (1H, s, N-N-*H*), 7.27 (1H, s, N=C(Ph)-H), 7.00-7.60 (5H, m, Ar-H), 7.90-8.43 (2H, m, Ar-H), 8.87 (1H, s, Ar-H);^{13}C NMR (100 MHz; $CDCl_3$) δ_C 116.8(Ar*C*H), 119.5 (Ar*C*H), 128.7 (2 × Ar*C*H), 129.1 (2 × Ar*C*H), 130.5 (Ar*C*H), 130.8 (Ar*C*H), 131.4 (Ar*C*), 136.0 (Ar*C*-

NO_2), 139.0 (Ar*C*-NO_2), 148.0 (Ar*C*NH-N), 154.7 (N=*C*).

1-(2, 4-dinitrophenyl)-3-methyl-1, 2-diazabut-2-ene: $C_9H_{10}N_4O_4$ (compound C-3)

Using the general procedure above, starting from 0.74cm^3 (0.01mol) acetone and 1.98g (0.01mol) of 2,4-DNPH, the title compound was obtained as orange crystals (1.42g, 60%), melting point 125-127°C, FT-IR, 1614cm^{-1} (C=N), 3085cm^{-1} (=C-H) and λ_{max} = 350 nm.^{1}H NMR (250 MHz; $CDCl_3$) δ_H 0.90 (6H, s, 2 × C*H*$_3$), 7.15 (1H, s, N-N-*H*), 7.00-8.33 (2H, m, Ar-H), 8.87 (1H, s, Ar-H);^{13}C NMR (100 MHz; $CDCl_3$) δ_C9.80 (*C*H_3), 12.1 (*C*H_3), 117.0 (Ar*C*H), 119.5 (Ar*C*H), 130.5 (Ar*C*H), 136.0 (Ar*C*-NO_2), 139.0 (Ar*C*-NO_2), 148.0 (Ar*C*NH-N), 155.6 (N=*C*).

Antimicrobial Test

The antimicrobial test was carried out by an adapted agar (Mueller-Hinton agar and Nutrient Broth). The antimicrobial activity of the synthesized compounds was tested against *Salmonella typhi*, *Streptococcus faecalis* and *Escherichia coli* microbial strains according to the following procedure:

Preparation of the Medium

The nutrient agar medium was prepared by dissolving 9.0g of agar in 500ml of distilled water in a conical flask and swirled to dissolve. The solution was sterilized in an autoclave at 121°C for 15min. It was then poured aseptically into petri dishes, allowed to solidify and set for the analysis.

Preparation of the Hydrazone samples

The concentrations of the hydrazone compounds were prepared by serial dilution. 0.5g of each compound was dissolved in 0.5ml of dimethyl sulfoxide (DMSO) to yield a concentration of 1.0g/ml equivalent to 10^6µg/ml as stock solution. From the stock solution, 0.1ml was transferred into a sterile bijou bottle containing 0.9ml of DMSO thus giving a concentration of 10^5µg/ml. From this solution 0.1ml was transferred into another sterile bijou bottle containing 0.9ml of DMSO which gave a concentration of 10^4µg/ml and this was further diluted to 100µg/ml, 50µg/ml and 12.5µg/ml.

Preparation of culture medium and inoculation

Cultures of *Salmonella typhi*, *Streptococcus faecalis* and *Escherichia coli* were obtained from Bayero University, Department of microbiology. Pure isolates were obtained by sub-culturing unto fresh nutrient agar plates. The freshly grown microbial cultures were appropriately diluted in test tubes containing sterile normal saline solution to march McFarland standard described by Cheesebrough M. (2000)[8]. The McFarland standard was prepared by mixing 0.6ml of 1% (W/V) dehydrated barium chloride solution with 99.4ml of 1% (V/V) sulphuric acid solution and was labeled as the standard inoculums. The standard inoculums were then evenly smeared onto the prepared nutrient agar plates. After smearing, plates were dried for 15min and wells were punched using sterile cork borers. Once wells were formed, they were filled with concentrations of the sample compounds. Commercially available ciprofloxacin (500mg) was used as positive control in this study. Plates were inoculated for 24hours at 37°C to allow extracts to diffuse through the agar media to form a zone of inhibition. The diameters of the zone of inhibition for different extracts against the different bacteria were measured in millimeter for further analysis. An agar well (6mm) showing no zone of inhibition was considered as no antimicrobial activity.

Determination of Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration of the hydrazone samples were prepared by serial dilution using distilled water to obtain concentrations of 10µg/ml, 8µg/ml, 6µg/ml and 4µg/ml. Equal volume (2ml) of the hydrazone sample and Nutrient broth were mixed. Specifically 0.1ml of standardized inoculation (3.3x106 CFU/ml) was added to each of the test tubes above. The tubes were inoculated aerobically at 37°C for 24hours. Tubes containing broth and thehydrazone samples without inoculation served as positive control while tubes containing broth and inoculation served as negative control. The tubes were observed after 24hours of incubation to determine minimum inhibitory concentration; that is the lowest concentration that showed no evidence of growth[9].

RESULTS AND DISCUSSION

The general method for the synthesis of the hydrazone derivatives is represented in Scheme 1. All the three compounds were obtained as coloured crystals in reasonable yields (60 - 74%) having the indicated melting point ranges (Table No.1).

Furthermore the structures of the hydrazones were established by spectroscopic analysis. Their FT-IR spectra showed signals around 1629-1614cm^{-1} characteristic of C=N stretching vibrations of hydrazone with complete absence of C=O signals around 1700-1740cm^{-1} which were observed in the starting carbonyl compounds. All the ^{1}H and ^{13}C NMR spectra agreed with the structures as indicated under Experimental section.

The antimicrobial activity of the compounds showed very good activity against *salmonella typhi* and *streptococcus faecalis* (Table No.2). Ciprofloxacin was used as a reference drug. Generally the activity increases with the increasing concentrations achieving the highest activity at 100µg/ml having an average inhibition zone of 25mm. What is worthy of noting is that when the concentrations of the compounds were significantly dropped from 100µg/ml to 50µg/ml, there was no significant change in the activity in both test organisms. Compound C-3 showed similar zone of inhibition with the test drug against *Streptococcus faecalis* at 100µg/ml. At concentrations of >100µg/ml the same results were obtained showing that maximum activity is reached at 100µg/ml concentration.

The results of screening the three compounds against *E. coli* on the other hand showed activity at only 100µg/ml and 50µg/ml concentrations. Compound C-2 showed the highest activity at 100µg/ml having a zone of inhibition of 22mm which slightly drops to 20mm at 50µg/ml. The other two compounds have lower activity at the two concentrations. This result shows that all the compounds have good antimicrobial activity almost similar to that of the test drug ciprofloxacin. None of the compounds was found to have higher activity than the test drug. All the compounds have MIC of 4µg/ml against the tested organisms (Table No.3).

Table No.1: Physical properties of the hydrazone derivatives

S.No	Compounds	Colour	% Yield	Melting Point (°C)
1	C-1	Reddish orange	65%	160-162
2	C-2	light orange	74%	235-237
3	C-3	Orange	60%	125-127

Table No.2: Result of the antimicrobial activity of the synthesized hydrazone derivatives

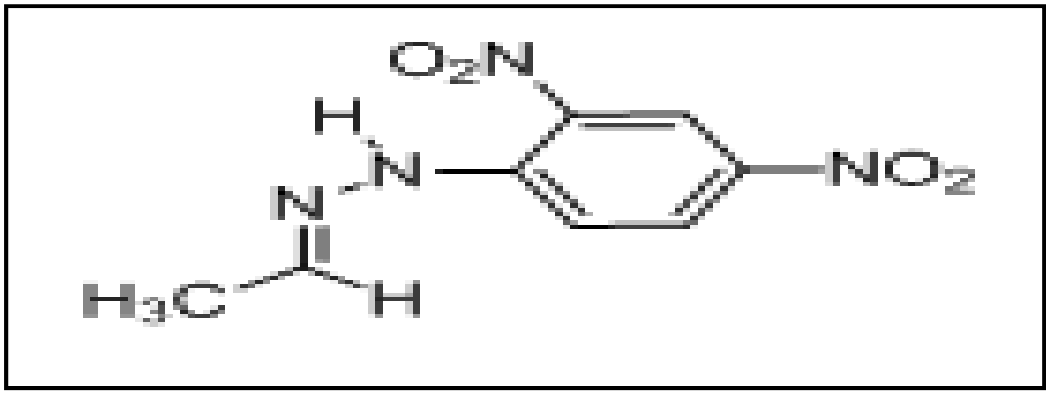
S.No	Product	Organism	Concentration / Diameter of Zone of Inhibition (mm)				
			100µg/ml	50µg/ml	25µg/ml	12.5µg/ml	Control
1	C-1	*Salmonella typhi*	20	17	15	10	30
		Streptococcus faecalis	25	20	15	10	30
		Escherichia coli	12	10	00	00	30
2	C-2	*Salmonella typhi*	25	15	12	10	30
		Streptococcus faecalis	25	20	17	10	30
		Escherichia coli	22	20	00	00	30
3	C-3	*Salmonella typhi*	25	15	12	10	30
		Streptococcus faecalis	30	22	15	11	30
		Escherichia coli	15	10	00	00	30

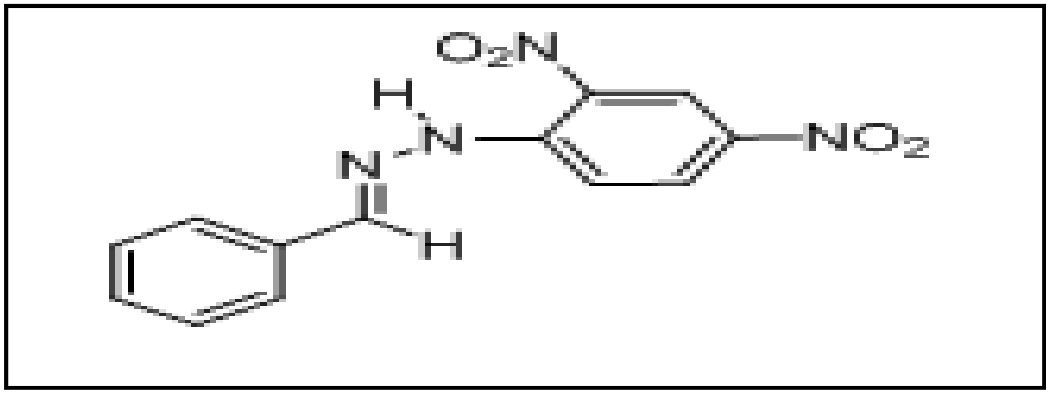
Inactive (inhibition zone <6mm)

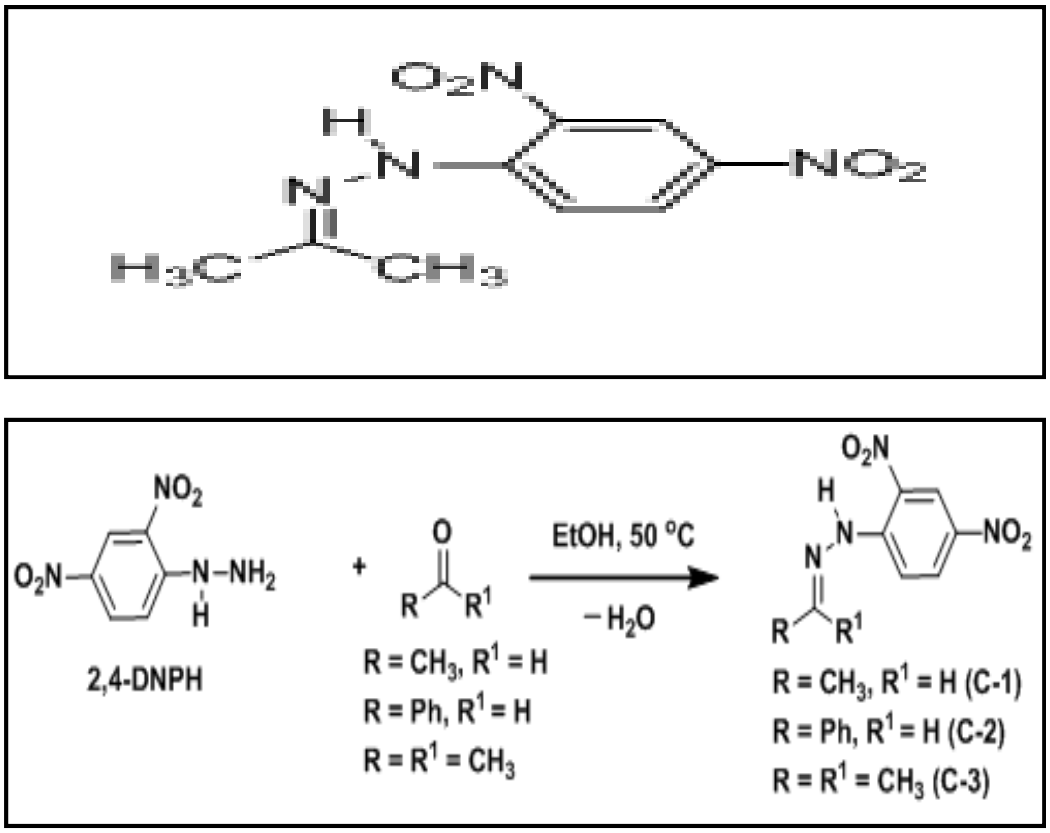
Table No.3: Minimum Inhibition Count (MIC) of the synthesized hydrazone derivatives against *salmonella*, *S. faecalis* and *E. coli*

S.No	Compounds	*Salmonella*				*Streptococcus faecalis*				*Escherichia coli*				
		10ug/ml	8ug/ml	6ug/ml	4ug/ml	10ug/ml	8ug/ml	6ug/ml	4ug/ml	10ug/ml	8ug/ml	6ug/ml	4ug/ml	10ug/ml
1	C-1	-	-	-	+	-	-	-	+	-	-	-	-	-
2	C-2	-	-	-	+	-	-	-	+	-	-	-	-	-
3	C-3	-	-	-	+	-	-	-	+	-	-	-	-	-

+ = Growth; - = No growth







Scheme No.1: Synthesis of the hydrazone derivatives

CONCLUSION

In conclusion, two hydrazone derivatives from aldehydes and one from ketone were readily synthesized in good to excellent yields (60-74%) using literature procedure.[2] The assigned structures of the compounds were supported by FT-IR, ^{1}H NMR and ^{13}C NMR spectra. When the compounds were screened for activity against three disease causing pathogens *Salmonella typhi*, *Streptococcus faecalis, Escherichia coli* using Mueller-Hinton agar &Nutrient Broth method, they were found to have good activity with the test drug at 100 µg/ml and 50 µg/ml concentrations. All the compounds did not show activity against *E. coli* at concentrations of ≤ 25 µg/ml. This study shows that the hydrazone compounds can potentially be developed as antibiotics. Similar work on azo compounds is in progress.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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