

Original Research Article

Synthesis, Antibacterial and Antioxidant Activity Studies of 2, 4-Dinitrophenyl Hydrazone Derivatives of 4-Methoxyphenyl Propenone Chalcones

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Abstract: Hydrazones are an important class of biologically active molecules and could be synthesized from various methods. Similarly 2,4-dinitrophenyl hydrazine derivatives of 4-methoxyphenyl propenone chalcones are equally important hydrazones that were synthesized by the coupling of 2,4-dinitrophenyl and the 4-methoxyphenyl propenone chalcones counterparts. The coupling reaction resulted in making non - reactive 4-methoxyphenyl propenone chalcones becoming more reactive by exhibiting antibacterial, antifungal and anti-oxidant activity.

Keywords: Hydrazones, Chalcones, antibacterial, antifungal, anti-oxidant.

INTRODUCTION

Emerging bacterial resistance causes wide spread problem for the treatment of various infections, hence the search for new antimicrobials is a non - ending task and this has led to the continuous exploration and exploitation of natural products and synthetic compounds. It is to this backdrop that the researchers have decided to explore the chemistry of chalcones and its derivatives.

The study of chalcones has continued to attract attention both in academia and industry owing to its vast potential and broad spectrum of biological and pharmacological activities. Literature search reveals that chalcones biological/pharmacological activities includes oxidative, amoebicidal, anti-tubercular, anti-trichomonal, anti-inflammatory, anti-malarial, anti-microbial, anti-fungal, anti-cancer etc [1,2]. Chalcones are referred as natural biocides and exhibit an array of pharmacological activities. They occur abundantly in edible plants [3] and are responsible for plant defensive mechanism to counteract reactive oxygen species in order to survive and prevent molecular damage and damage by insects and herbivores[4,5]. Chalcones are known to exist as *trans* and *cis* isomers having aromatic rings that are connected by a three carbon α, β -unsaturated carbonyl system. In most cases the *trans* isomer are reported to be more stable from the thermodynamic perspective, which makes it the predominant configuration among chalcones. The unstable *cis* isomer is as a result of steric hindrance between the carbonyl group and the A ring [6]. Matos, Vazquez, Uriarte, and Santana [7], reported that chalcones are flexible molecules that exist in various conformations and their properties depend on appropriate ring substitution. Chalcones have proper electron pulling and electron pushing functional groups connected to the benzene ring(s) and can be fluorescent because of the conjugated system which makes them a potential chemical probe for mechanistic investigations.

Hydrazones are compounds that constitute an important class of biologically active drug molecules which has continued to attract the attention of medicinal chemists owing to their wide range of pharmacological properties. These compounds are being synthesized as drugs by many researchers in order to combat diseases with minimal toxicity and

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maximal effects. This have now provided a therapeutic pathway to develop new effective biologically active compounds; hydrazones. A number of hydrazone derivatives have been reported to exert good biological activities [1, 2].

Hydrazones possess an azomethine $-NHN=CH$ group which are considered as derivatives of aldehydes and ketones (chalcones) in which the oxygen atom has been replaced by the $=NNH_2$ group. Hydrazones are of wide interest because of their diverse biological applications such as anticonvulsant, antidepressant, analgesic, anti-inflammatory, antiplatelet, antimalarial, antimicrobial, antimycobacterial, anticancer, vasodilator, antiviral, anti-HIV, anthelmintic, antidiabetic, and trypanocidal activities [8 - 19].

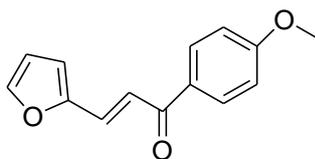
In Ere, Dode and Usifoh [20] it was recorded that a group of 4-methoxyphenyl propenone chalcones did not exhibit any antibacterial activity. However, Fadare *et al.* [5] in their work "1-Indanone chalcones and their 2, 4-dinitrophenyl hydrazone derivatives reported that the hydrazone derivatives had antibacterial activity. It was based on this backdrop that the group of 4-methoxyphenyl propenone chalcones that were reported not to have any antibacterial activity was coupled with 2, 4-dinitrophenyl hydrazine and the resulting hydrazones were then tested for antibacterial, antifungal and anti-oxidant activity.

MATERIALS AND METHODS

Evaluation of Compounds: Structures and purity of compounds were confirmed by 1H -NMR using JOEL Lambda 400 spectrometer, an internal standard of TMS was used. Thin layer chromatography (TLC) (E. Merck Kieselgel 60 F254) was used to check purity of compounds. Melting point (uncorrected) was determined using Gallenkamp melting point machine.

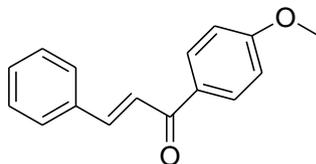
Synthesis of Chalcones

General synthesis of 4-methoxy phenyl propenone chalcones were carried out using modified Claisen-Schmidt condensation reaction.



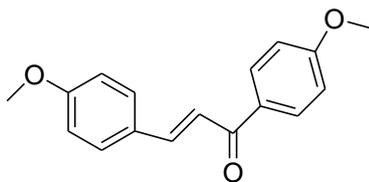
3-Furan-2-yl-1-(4-methoxy-phenyl)-propenone (A)

The compound (A) was synthesized by mixing furfural (5.0 g, 0.05 mol) and p-methoxyacetophenone (7.8 g, 0.05 mol) in ice cold ethanol (20 ml) in a round bottom flask equipped with magnetic stirrer for 2 hours. The temperature was monitored such that the reaction flask remained below 10 °C. Ice cold 10 % Potassium hydroxide (20 ml) was added and stirred for a further 2 hours. The reaction mixture was then allowed to stand on the reaction bench at room temperature for 72 hours. To the resultant mixture was added cold distilled water (50 ml) and then 10 % acetic acid (30 ml) to acidify using litmus paper to determine the acidity. The resultant crude precipitate was collected by filtration, then washed with cold water and dried. It was then recrystallized from ethanol, collected by filtration and dried *in vacuo* in a desiccator over silica to yield the compound A. Yield: 7.98 g, 70 %; mp 68 – 70 °C; Colour: Yellow; UV: λ_{max} 260, 500, 640 nm; IR: 2922.59, 1956.71, 1607.88, 1168.99 $V\text{ cm}^{-1}$; 1H -NMR: [400 MHz, $CDCl_3$], δ_H : 3.86 (3H, d, J = 2.8 Hz).



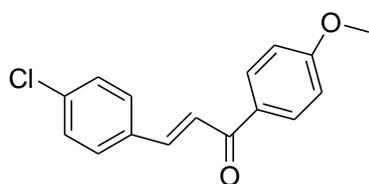
1-(4-Methoxy-phenyl)-3-phenyl-propenone (B)

The compound (B) was synthesized by mixing p-anisaldehyde (5.0 g, 0.04 mol) and acetophenone (4.41 g, 0.04 mol) in ice cold ethanol (20 ml) in a round bottom flask equipped with magnetic stirrer for 2 hours. It was treated as the reaction above. Yield: 7.93 g, 90.6 %; mp 56 – 59 °C; Colour: Pale Yellow; UV: λ_{max} 480, 580, 640, 780 nm; IR: 3734.82, 3648.69, 2923.10, 1653.21, 1601.38 $V\text{ cm}^{-1}$; 1H -NMR: [400 MHz, $CDCl_3$], δ_H : ^{13}C -NMR,



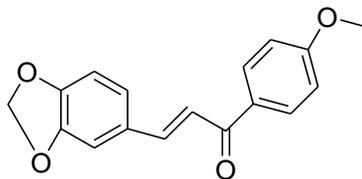
1,3-Bis-(4-methoxy-phenyl)-propenone (C)

The compound (C) was synthesized by mixing p-anisaldehyde (5.0 g, 0.04 mol) and p-methoxyacetophenone (5.51 g, 0.04 mol) in ice cold ethanol (20 ml) in a round bottom flask equipped with magnetic stirrer for 2 hours. Treated as in (A) above. Yield: 9.12 g, 85 %; mp 68 – 70 °C; Colour: Yellow; (Found C, 76.01; H, 6.01 %; $C_{17}H_{16}O_3$ requires C, 76.10; H, 6.01 %); UV: λ_{max} 260, 500, 640 nm; IR: 2922.59, 1956.71, 1607.88, 1168.99 $V\text{ cm}^{-1}$; $^1\text{H-NMR}$: [400 MHz, CDCl_3], δ (ppm): 3.75 (3H, s, O- CH_3), 3.84 (3H, s, O- CH_3), 6.77 -7.05 (2X 2Ar-H, dd), 7.66 -7.68 (2Ar-H, d), 7.57(1H, d, =CH), 7.71 (1H, d, =CH), 7.75-7.93 (2Xar-H, d); $^{13}\text{C-NMR}$ [100 MHz, MeOD] δ_c 47.8, 48.0, 54.6, 113.6, 114.1, 119.1, 127.7, 130.1, 130.6, 130.9, 144.1, 162.0, 163.9, 189.6; m/z 268.15 (M^+ , 100 %)



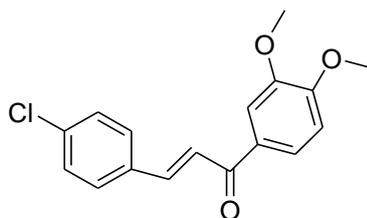
3-(4-Chloro-phenyl)-1-(4-methoxy-phenyl)-propenone (D)

The compound (D) was synthesized by mixing p-chlorobenzaldehyde (5.0 g, 0.04 mol) and p-methoxyacetophenone (5.33 g, 0.04 mol) in ice cold ethanol (20 ml) in a round bottom flask equipped with magnetic stirrer for 2 hours. It was then allowed to stand at room temperature and treated as in A above. Yield: 9.42 g, 97.1 %; mp 113-115 °C; Colour: Cream; (Found C 70.56, H 5.02, Cl 13.00, O 11.42; $C_{16}H_{13}ClO_2$ requires C 70.46, H 4.80, Cl 13.00, O 11.73) UV: λ_{max} 380, 500, 640 nm; IR: 2922.46, 2359.77, 1654.73, 1602.89, 1178.64 $V\text{ cm}^{-1}$;



3-Benzo[1,3]dioxol-5-yl-1-(4-methoxy-phenyl)-propenone (E)

The compound (E) was synthesized by mixing piperonal (5.0 g, 0.03 mol) and p-methoxyacetophenone (5.0 g, 0.03 mol) in ice cold ethanol (20 ml) in a round bottom flask equipped with magnetic stirrer for 2 hours and was treated as A above. Yield: 9.25 g, 98.4 %; mp 117-120 °C; Colour: Pale-Yellow; (Found C, 72.3; H 5.02 %; $C_{17}H_{14}O_4$ requires C, 72.33; H 5.00 %), UV: λ_{max} 400, 500, 640, 780 nm; IR: 2922.59, 1956.71, 1607.88, 1168.99 $V\text{ cm}^{-1}$; GC-MS (m/z) 282.11 (M^+ , 100 %), 267.09 (14 %), 135.04 (40 %), 77 (25 %); $^1\text{H-NMR}$: [400 MHz, MeOD], δ (ppm): 3.02 (3H, s O- CH_3), 6.76 (1H, d, $J = 19.6\text{ Hz}$, =CH), 7.6-7.09 (m-H, $J = 3.0, 15.5\text{ Hz}$, 7 X Ar-H), 8.02 (1H, d, $J = 19.6\text{ Hz}$, =CH); $^{13}\text{C-NMR}$ [MeOD, 100 MHz] δ_c 38.8, 44.9, 46.9, 47.3, 47.6, 48.2, 110.8, 111.7, 115.9, 122.4, 127.9, 128.3, 130.4,



3-(4-Chloro-phenyl)-1-(3,4-dimethoxy-phenyl)-propenone (F)

The compound (F) was synthesized by mixing p-chlorobenzaldehyde (5.0 g, 0.04 mol) and dimethoxyacetophenone (6.37 g, 0.04 mol) in ice cold ethanol (20 ml) in a round bottom flask equipped with magnetic stirrer for 2 hours and then treated as sample A above. Yield: 10.21 g, 95.2 %; mp 109 -111 °C; Colour: Cream; UV: λ_{max} 500, 580 640 nm; IR: 2923.48, 1668.59, 1576.67 $V\text{ cm}^{-1}$; m/z 302.07 (M^+ , 5 %), 180 (43 %), 165 (100 %).

Synthesis of hydrazones

The scheme and table below shows the synthesis of hydrazones.

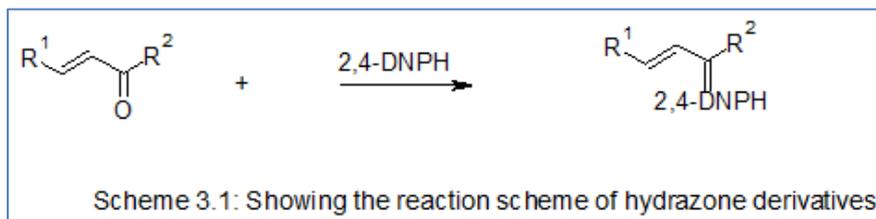
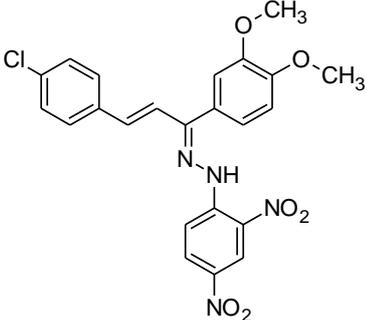
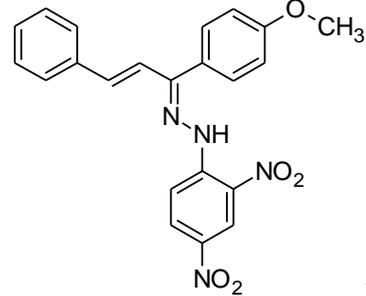
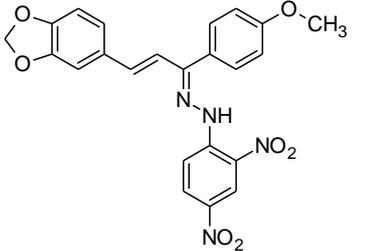
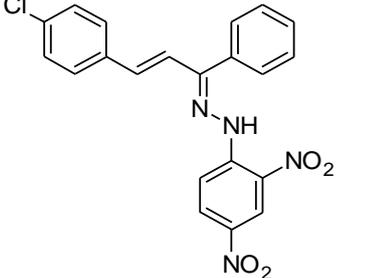


Table-1: Showing reagents used and corresponding hydrazone products.

R ₁	R ₂	Structure and name of compound	Physical properties/Code
Furan	4-Methoxy phenyl	 (E)-1-(2,4-dinitrophenyl)-2-((E)-3-(furan-2-yl)-1-(4-methoxyphenyl)allylidene)hydrazine	Yield:(4.8 g, 89 %); Color: Dark brown; mp 143-144 °C; R _f : 0.57; UV(Chloroform) λ_{max} :300,500; IR(KBr) $V_{max/cm^{-1}}$: 1619 (C=N), 1303 (NO ₂), 2040.21 (C=C), 1111.44 (N-N), 3686.66 (N-H), 1479.55 (C-H). ¹ H-NMR (CDCl ₃) δ (ppm): 6.34 (s 1H 1.6Hz), 7.29 (d J=6 Hz Ar-H). Calculated C (58.82 %), H (3.95 %), O (23.51 %). Found C (58.77 %), H (3.91 %), O (23.50 %).
Phenyl	Benzo[d][1,3]dioxole		Yield:(3.4 g, 66.6 %); Color: Reddish precipitate; mp 189 - 191 °C; R _f : 0.64; UV(Chloroform) λ_{max} : 200, 250, 300,400 nm; IR (KBr) $V_{max/cm^{-1}}$: 1661 (C=N), 1545 (-Ar), 3367 (NH), 1380.34 (NO ₂), 1147.61 (N-N), 1712 (C=C), 1452 (C-H). ¹ H-NMR (CDCl ₃) δ (ppm): 1.17 (1H 1.2Hz), 4.07 (1H 2.3Hz), 6.63 (Ar-12H) Calculated C (61.11 %), H (3.73 %), O (22.20 %). Found C (61.06 %), H (3.75 %), O (22.50 %).
4-Chlorophenyl	4-Methoxy phenyl		. Yield: (7 g, 84.3 %); Color: Reddish precipitate; R _f : 0.73; mp 146 - 147 °C, UV(Chloroform) λ_{max} : 300,500; IR (KBr) $V_{max/cm^{-1}}$: 1662.18 (C=N), 1546.62 (Ar), 2087 (C=C), 3760.26 (NH), 1463.62 (C-H), 654.87 (C-Cl), 1376.13 (NO ₂). ¹ H-NMR (CDCl ₃) δ (ppm): 117.56 (s 1H 2.3Hz), 130.83 (d 9.1Hz Ar-14H). Calculated C (53.35 %), H (3.78 %), C-Cl (7.83 %), O (17.67). Found C (61.54 %), H (3.70 %), Cl (7.72 %), O (17.66 %).
4-Methoxy phenyl	4-Methoxy phenyl		Uc. Yield:(4.4 g, 88 %); Color: Brown precipitate; R _f : 0.64; mp 143-145 (°C); UV (Chloroform) λ_{max} : 300, 500 ; IR (KBr) $V_{max/cm^{-1}}$: 2380 (C=N), 1550.28 (R-Ar), 1795 (C=C), 3225.22 (NH), 1455.78 (C-H), 1376.51 (NO ₂), 1153.47 (N-N). ¹ H-NMR (CDCl ₃) δ (ppm): 1.68 (2H 1.2Hz), 3.91 (s 1H 3.2Hz). Calculated C (61.60 %), H (4.50 %), O (21.41 %). Found C (61.54 %), H (4.46 %), O (21.40 %).

4-Chloro phenyl	3,4-dimethoxy phenyl	 <p>(E)-1-((E)-3-(4-chlorophenyl)-1-(3,4-dimethoxyphenyl)allylidene)-2-(2,4-dinitrophenyl)hydrazine</p>	Tc. Yield:(6 g, 95.2 %); Color: Reddish precipitate, R_f : 0.56; mp 173-175 ($^{\circ}$ C); UV(Chloroform) λ_{max} : 220, 270, 370; IR (KBr) V_{max}/cm^{-1} : 1728.54 (C=N), 1542.62 (-Ar), 1375.16 (NO ₂), 1662.91 (C=C), 1459.18 (C-H), 3315.02 (N-H), 1100.42 (N-N), 652.28 (C-Cl). Calculated C (57.21 %), H (3.97 %), Cl (7.34 %), O (19.88 %), Found C (57.15 %), H (3.93 %), Cl (7.24 %), O (19.88 %).
Phenyl	4-Methoxy phenyl	 <p>(E)-1-(2,4-dinitrophenyl)-2-((E)-1-(4-methoxyphenyl)-3-phenylallylidene)hydrazine</p>	Rc. Yield (5.4 g, 77.1 %); Color Reddish precipitate; R_f : 0.7, mp 148-150 ($^{\circ}$ C); UV (Chloroform) λ_{max} : 280, 480. IR (KBr) V_{max}/cm^{-1} : 1729.96 (C=N), 1604.70 (-Ar), 1503.32 (NO ₂), 1801.79 (C=C), 3261.02 (NH), 1432.15 (C-H), 1118.01 (N-N). ¹ H-NMR (CDCl ₃) δ (ppm): 3.96 (1H 1.8Hz), 6.64 (d 6.2Hz Ar-13H). Calculated C (63.15 %), H (4.34 %), O (19.12 %). Found C (63.09 %), H (4.30 %), O (19.12 %).
Benzo[d]1,3-dioxole	4-methoxy phenyl	 <p>(E)-1-((E)-3-(benzo[d][1,3]dioxol-5-yl)-1-(4-methoxyphenyl)allylidene)-2-(2,4-dinitrophenyl)hydrazine</p>	Xc. Yield: (5 g, 76.9 %); Color: Brown precipitate; R_f : 0.61; mp 150-152 ($^{\circ}$ C); UV (Chloroform) λ_{max} : 210, 380; IR (KBr) V_{max}/cm^{-1} : 1830.17 (C=N), 1601.55 (-Ar), 1497.49 (NO ₂), 1445.51 (C-H), 2044.47 (C=C), 3278.22 (NH). Calculated C (59.74 %), H (3.92 %), O (24.22 %). Found C (59.68 %), H (3.89 %), O (24.22 %).
4-Chloro phenyl	Phenyl	 <p>(E)-1-((E)-3-(4-chlorophenyl)-1-phenylallylidene)-2-(2,4-dinitrophenyl)hydrazine</p>	Oc. Yield:(6.5 g, 74.7 %); Color: Reddish precipitate, R_f : 0.68; mp 176-178 ($^{\circ}$ C); UV(Chloroform) λ_{max} : 300, 500; IR (KBr) V_{max}/cm^{-1} : 1827.61 (C=N), 1501.15 (-Ar), 3278.06 (NH), 1593.49 (C=C), 770.27 (C-Cl), 1422.94 (N-O), 1105.04 (N-N), 1307.41 (C-H). ¹ H-NMR (CDCl ₃) δ (ppm): 3.91 (s 1H 2.4Hz), 6.51 (m 5Hz Ar-10H). Calculated C (59.65 %), H (3.58 %), Cl (8.38 %), O (15.14 %). Found C (59.59 %), H (3.54 %), Cl (8.27 %), O (15.13 %).

Antibacterial Activity

The test organisms were clinical isolates obtained from the Department of Medical Microbiology, College of Health Sciences, Niger Delta University Wilberforce Island Amassoma Bayelsa State Nigeria. *Bacillus subtilis* (Gram positive) *Escherichia coli* (Gram negative), *Staphylococcus aureus* (Gram positive), *Pseudomonas* species (Gram negative), *Proteus* species (Gram negative) and *Candida albican* were used. These organisms were stored in a Cryopreservation medium prior to the experiment.

Preparation of standard of synthesized compound

The synthesized compounds (100 mg) were weighed and dissolved in DMSO 10 ml (10 mg/ml). McCartney bottles were sterilized and labeled accordingly. The various respective concentrations of the synthesized compounds were prepared and transferred into the bottles.

Evaluation of antimicrobial activity

Agar dilution method using Mueller Hintonagar (Merck, Germany) medium was used to evaluate the antimicrobial effects of the dimethylsulfoxide (DMSO) solvent. Mueller Hintonagar medium was prepared sterile, cooled to 45 °C and was poured on the sterile plates and allowed to set. Media plates were then seeded with the test isolates, holes were bored in the culture medium with the aid of a cork borer of 11 mm in diameter by sterilizing the cork borer with a spirit lamp after each hole was bored. Molten agar was used after to seal the bottom of the holes to prevent leakage. The prepared compounds and standard antimicrobial disc of known concentrations were placed in culture medium using a pipette and disc dispenser. Plates were then incubated inverted at 37 °C for 18-24 hrs and the diameter of freezone was measured exactly by using a ruler in millimeters.

Evaluation of antimicrobial minimum inhibitory concentration (MIC)

Serial dilutions were made from the original 10 mg/ml concentration to 5 mg/ml, 2.5 mg/ml and 1 mg/ml of the various hydrazones in DMSO at 0.4 ml drop and the minimum inhibitory concentration was determined.

Antioxidant Activity

The coupled products were dissolved in DMSO at various concentrations of 0.01 g/10 ml, 0.03 g/10 ml, 0.05 g/10 ml and 0.07 g/10 ml. Ascorbic acid was used as a reference standard. 0.006 g of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was freshly prepared in ethanol (20 ml). 500 µl of DPPH was added to each test tube containing various concentrations of the hydrazones and standard solution, and was shaking vigorously. The mixture was then left in the dark for 30 minutes. The control was carried out without the addition of DPPH and the hydrazones and the absorbance were taken at 516 nm in UV-Visible spectrophotometer. The scavenging activity was determined as a percentage of the DPPH free radical activity with the formula % RSA = (DPPH - COMPOUND)/DPPH.

RESULTS AND DISCUSSION

Table-2: Zone of inhibition (ZOI) of each synthesized compound at 10 mg/ml

Sample	Zone of inhibition (mm)					
	Isolate					
	<i>Bacillus subtilis</i>	<i>Echerichia coli</i>	<i>Candida albican</i>	<i>Proteus species</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas species</i>
Bc	--	--	--	--	--	22
Pc	20	--	--	17	20	20
Wc	22	--	--	--	--	11
Uc	14	--	--	--	--	11
Tc	--	--	--	--	20	16
Rc	--	--	--	--	--	20
Xc	--	--	--	--	--	21
Oc	15	19	--	10	--	18
AML	20	--	--	--	22	26
CIPRO	32	28	--	30	31	28
ERY	--	17	--	--	10	24
DMSO	--	--	--	--	--	--

Key: -- No zone of inhibition; AML Amoxicillin; CIPRO Ciprofloxacin; ERY Erythromycin; DMSO Dimethylsulfoxide

Table-3

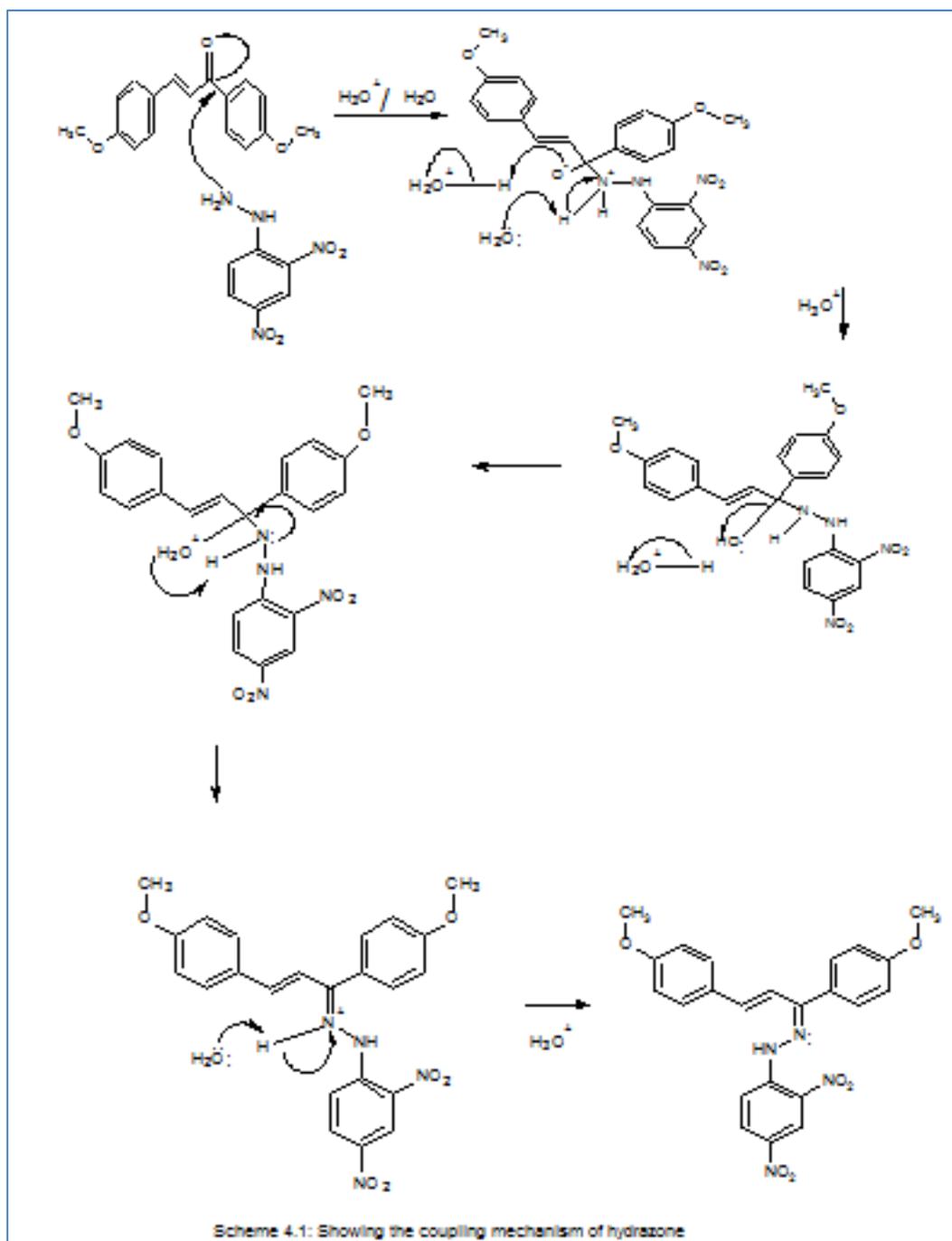
Samples	Minimum inhibitory concentrations (MICs)						
	Isolates						
	Concentration (mg/ml)	<i>Bacillus subtilis</i>	<i>Echerichia coli</i>	<i>Candida albican</i>	<i>Proteus spp</i>	<i>Staphylococcus aureus</i>	<i>Pseudomonas aeruginosa</i>
Bc	5	--	--	--	--	--	+
	2.5	--	--	--	--	--	+
	1	--	--	--	--	--	+
Pc	5	+	--	--	--	+	+
	2.5	+	--	--	--	+	+
	1	+	--	--	--	+	+
Wc	5	+	--	--	+	--	+
	2.5	+	--	--	+	--	+
	1	+	--	--	+	--	+
Uc	5	+	--	--	--	--	+
	2.5	+	--	--	--	--	+
	1	+	--	--	--	--	+
Tc	5	--	--	--	--	+	+
	2.5	--	--	--	--	+	+
	1	--	--	--	--	--	+
Rc	5	--	--	--	--	--	+
	2.5	--	--	--	--	--	+
	1	--	--	--	--	--	+
Xc	5	--	--	--	--	--	+
	2.5	--	--	--	--	--	+
	1	--	--	--	--	--	+
Oc	5	+	+	--	+	--	+
	2.5	--	+	--	+	--	+
	1	--	+	--	+	--	+
AML	disc (10 μ g)	+	+	--	+	+	+
CIPRO	disc (10 μ g)	+	+	--	+	+	+
ERY	disc (10 μ g)	+	+	--	+	--	+

Key: -- No growth; + Growth

Table-4: Antioxidant activity of synthesized compounds by DPPH method.

Compounds	Scavenging Effect (% @ μ g/mL)			
	1 μ g/ml	3 μ g/ml	5 μ g/ml	7 μ g/ml
Bc	73.35 \pm 0.016	73.34 \pm 0.016	76.43 \pm 0.016	78.35 \pm 0.016
Pc	40.79 \pm 0.087	64.35 \pm 0.087	66.12 \pm 0.087	68.48 \pm 0.087
Wc	-17.52 \pm 0.033	-12.51 \pm 0.033	-10.01 \pm 0.033	-5.89 \pm 0.033
Uc	62.44 \pm 0.038	67.45 \pm 0.038	54.49 \pm 0.038	65.25 \pm 0.038
Tc	87.03 \pm 0.007	87.92 \pm 0.007	87.33 \pm 0.007	89.39 \pm 0.007
Rc	62.16 \pm 0.049	64.65 \pm 0.049	70.69 \pm 0.049	76.14 \pm 0.049
Xc	74.22 \pm 0.062	77.76 \pm 0.062	90.27 \pm 0.062	92.78 \pm 0.062
Oc	63.91 \pm 0.042	68.77 \pm 0.042	73.93 \pm 0.042	78.20 \pm 0.042
Ascorbic acid	88.80 \pm 0.018	88.51 \pm 0.018	92.63 \pm 0.018	93.81 \pm 0.018

The chalcones were synthesized following Claisen-Schmidt condensation reaction of aromatic aldehyde (Benzaldehyde) with an appropriate acetophenone in the presence of aqueous alkali at low temperature. The corresponding hydrazones were achieved when the chalcones were coupled with 2,4-DNPH in ethanol using concentrated sulfuric acid as a catalyst. The reaction mechanism is perceived to have followed the scheme as shown below.



The coupling with 2,4-DNPH was relatively quick, lasting just about 20 minutes maximum as confirmed by TLC and the yields were good, between (66.6 – 95.2 %).

The various hydrazones obtained gave melting points ranging from 144 to 191 °C and it was observed that they all gave bright colors ranging from yellow to red. The λ_{max} for each of the synthesized hydrazones was determined and the values are shown in the spectra table. Compound Pc has λ_{max} at 300, 450 nm and the corresponding chalcone moiety recorded its λ_{max} at 500, 580 nm indicating a hypsochromic shift. This shift could be attributed to the auxochromes attached to the imine conjugate as a result of the 2, 4-DNPH coupling.

From the IR spectra, the hydrazones recorded absorption at 1475.55 cm^{-1} and 1560 cm^{-1} relating to compound Bc, which indicates the presence of aromatic and imine functional groups. The imine ($-\text{HC}=\text{N}$) functional group was absent in the chalcone moiety and it shows a strong peak at 1640 - 1680 cm^{-1} in the corresponding hydrazone product which indicates that the coupling was successful. The aromatic rings of (1Z)-1-[(2E)-3-(4-chlorophenyl)-1-(4-

methoxyphenyl)prop-2-en-1-ylidene]-2-(2,4-dinitrophenyl)hydrazine[Wc] shows a sharp peak of medium intensity at 1546.63 cm^{-1} pointing towards the presence of aromatic compounds and these may just affirm that coupling took place.

$^1\text{H-NMR}$ of the hydrazones was recorded using deuterated chloroform with TMS (tetramethylsilane) as internal standard. The chemical shifts are expressed as δ values (ppm). The $^1\text{H-NMR}$ spectra of hydrazones shows a cluster of signals around the aromatic region 6.60 – 8.40 ppm and this may signify the presence of three aromatic rings as the total number of proton was found to be nineteen (19) in (1Z)-1-[(2E)-3-(4-chlorophenyl)-1-phenylprop-2-en-1-ylidene]-2-(2,4-dinitrophenyl)hydrazine [Oc]. (2Z)-1-(2,4-dinitrophenyl)-2-[(2E)-1-(furan-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-ylidene]hydrazine [Bc] on the other hand showed a highly deshielded methyl peak at 1.4 ppm indicating the presence of methoxy group.

From the spectra analysis of the $^{13}\text{C NMR}$, peaks were observed at 40 – 52 ppm. The peak observed between 120 – 154 ppm showed twenty three (23) numbers of carbons indicates the presence of aromatic carbon in compound Bc. The presence of methoxy group indicates that a methyl carbon is attached to an electronegative element which could cause a shielding effect in compound Wc.

The antimicrobial activities of the hydrazones were carried out using the agar-well diffusion method against two strains of Gram-positive, three Gram-negative bacteria and one *Candida albican*. Antimicrobial susceptibility of the eight (8) compounds was tested by determining the zone of inhibition and the minimum inhibitory concentrations, whose values are summarized in Tables above. The standard drugs used were Amoxicillin, Ciprofloxacin and Erythromycin, and the activity of all the coupled hydrazones were less in comparison to the standard drugs. The hydrazone compounds (2Z)-1-(2,4-dinitrophenyl)-2-[(2E)-1-(furan-2-yl)-3-(4-methoxyphenyl)prop-2-en-1-ylidene]hydrazine[Bc] and (1Z)-1-[(2E)-3-(4-chlorophenyl)-1-(4-methoxyphenyl)prop-2-en-1-ylidene]-2-(2,4-dinitrophenyl)hydrazine[Wc] having furan and 4-chlorophenyl-1-4-methoxyphenyl in their respective hydrazone framework were found to be the most active against Gram-negative *Pseudomonas* species and Gram-positive *Bacillus subtilis* bacterial strains. Compound Xc having 3-(benzol[d][1,3]dioxol-5-yl)-1-(4-methoxyphenyl) and Pc having -3-(1,3-benzodioxol-5-yl) in their hydrazone structures were next in potency against Gram-negative *Pseudomonas* species and Gram-positive *Bacillus subtilis* strains of bacteria.

Compound Tc and Oc with chlorine substituent in their hydrazone moiety also have a lesser susceptibility on Gram-positive *Staphylococcus aureus* and Gram-negative *Echerichia coli* and *Proteus spp* bacterial strains. None was susceptible to the *Candida albican*.

Minimum inhibitory concentration (MIC) was further carried out in agar diffusion medium at concentrations of 5 mg/ml, 2.5 mg/ml and 1 mg/ml, and compound Tc has a minimum inhibitory activity on *Staphylococcus aureus* at a concentration of 5 mg/ml. These indicates that when chalcones are coupled with 2,4-DNPH to form hydrazones, antibacterial activity is certain. This is contrary to Ere, Dode and Usifoh [17] report which states that the synthesized 4-methoxyphenylpropenone (chalcones) does not have antibacterial activity.

There are numerous antioxidant assays in literature with different methodologies but the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity was used. The antioxidant activities of the hydrazones were evaluated using DPPH radical scavenging method. Ascorbic acid was used as a positive control. The DPPH radical scavenging activity was carried out at concentration of 0.01 g/10 ml and the results were reported in three replicates. The absorbance which was taken at 516 nm showed a significant relationship between percentage concentration/inhibitions of hydrazones. The DPPH radical scavenging activity was expressed as percentage (%) of inhibition and results are tabulated in Table 4.4. Among the derived hydrazones, compound Xc gave (92.78 %) while Tc and Bc gave (89.39 %) and (78.35 %) respectively which exhibited maximum DPPH free radical scavenging activity followed by compound Oc, Pc, and Rc which also gave (78.20%), (68.48 %), (76.14 %) respectively, and these compounds have lesser radical scavenging activity when compared with the reference standard ascorbic acid which gave (93.81 %). The lowest scavenging activity was observed in compounds Uc and WC which gave (65.25%) and (-5.89%). The investigation of antioxidant screening revealed that some of the tested compounds show moderate to good antioxidant activity. This could be attributed to the positioning of the benzene rings in the structures of the various chalcones conferring some antioxidant activity based on resonance stability. The antioxidant activity could also be due to the activated benzene ring making it to scavenge more free radicals than inactivated rings in the chalcones.

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