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Synthesis, Antimicrobial and Antioxidant Activities of Some Naphthalene - Containing Hydrazone Derivatives

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ABSTRACT

The inevitable consequence of the widespread use of antimicrobial agents has been the emergence of antibiotic resistant pathogens. This necessitates an ever-increasing search for new drugs. In an effort to develop antimicrobial agents, a series of hydrazone 2-6-derivatives were synthesized. These were characterized on the basis of physical and spectroscopic data, and were evaluated for their antimicrobial activity against various bacterial and fungal strains using the disc diffusion method and nutrient agar media. The antioxidant activities of the products were also evaluated. Our data showed that many derivatives have promising activities as antioxidant agents.

Keywords: antimicrobial activities, antioxidant activities, antibiotic, naphthalene, hydrazone

1. INTRODUCTION

A hydrazone is a class of compounds with the structure $R_1R_2C=NNH_2$ [1]. They are related to ketones and aldehydes by the replacement of the oxygen with the NNH_2 functional group. They are formed usually by the action of hydrazine on ketones or aldehydes [2-4]. Hydrazones are mainly synthesized by refluxing the appropriate quantity of substituted hydrazines/hydrazides with ketones and aldehydes in appropriate solvents like tetrahydrofuran, butanol, methanol, glacial acetic acid, ethanol, ethanol-glacial acetic acid etc. Hydrazones can also be synthesized by using coupling of aryldiazonium salts with active hydrogen compounds.

Hydrazone compounds are not only intermediates, but they are also very effective organic compounds. They can be used as intermediates to synthesize coupling products by using the active hydrogen of $-\text{CONHN}=\text{CH}-$ azomethine group. Various effective compounds for example: iproniazide and isocarboxazide. They are synthesized by reduction of hydrazidehydrazones. Iproniazide just like isoniazide is used as antitubercular drug [5].

The fast resistance of bacteria against antibiotics has become a widespread medical problem. Treatment options for these infections are often limited, especially in debilitated and immune compromised patients. The dramatically rising incidence of multi-drug resistant microbial infections in the past few decades has become a serious health care problem. The search for new antimicrobial agents will consequently always remain as an important and challenging task for medicinal chemists.

Hydrazones are a versatile class of compounds which present a wide range of biological applications as antimicrobial [6], antitubercular [7], anticonvulsant [8], anti-inflammatory [9], cytotoxic [10] and vasodilator [11] agents. Moreover, 9-acridanone-hydrazones proved to be active against *Schistosoma mansoni* [12] and quinoxaline-N-acylhydrazones showed trypanocidal activity [13]. In addition, methyl pyrazinyl ketone isonicotinoyl hydrazones have been synthesized in an attempt to develop novel chelators with high affinity for iron for the treatment of iron overload disease. [14]

Hydrazones also proved to be useful as sensitive analytical reagents for the determination of trace amounts of metal ion [15, 16]. Metal complexes with hydrazones have potential applications as catalysts [17], luminescent probes [18], and molecular sensors [19]. Moreover, metal complexes with hydrazones present antimicrobial [20,21] DNA-binding and cytotoxic activities [22]. It has also been shown that metal complexes with hydrazones can be potent inhibitors of cell growth and DNA syntheses [23].

In view of the above observations it was a thought of interest to design and synthesize a new class of hydrazone derivatives. So, in this present communication we report a facile synthesis of diverse 2-(substituted benzylidene)-1-(1-naphthalene-6-yl-ethylidene)hydrazine derivatives and their antimicrobial and antioxidant activities against various organisms. The results revealed that the newly synthesized derivatives exhibited significant biological activities.

2. EXPERIMENTAL

All chemicals were purchased from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from proper dehydrating agents. Melting points were determined in open capillaries on a Gallenkamp Melting Point Apparatus and are uncorrected.

The purity of the compounds was checked by thin layer chromatography (TLC) (silica gel H, n-hexaneacetone 3:1). The IR spectra were performed on a Shimadzu FTIR 8101 spectrometer in potassium bromide (KBr) pellets and the wave numbers were given in cm^{-1} . Compounds 3-5 and 6-8 were tested for their *in vitro* antimicrobial properties against the Gram-positive bacteria *Bacillus subtilis* (ATCC6633), *Streptococcus pyogenes* (ATCC19655), Methicillin-resistant *Staphylococcus aureus* (ATCC 43300), the Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), using conventional agar disc diffusion method [24].

Ampicillin was the reference drug for antibacterial activity. The observed data on the antimicrobial testing are presented in **Tables 1** and **2**. Compounds 3-5 and 6-8 were assessed for antioxidant activity using 1,1-biphenyl-2-picrylhydrazyl (DPPH) radical scavenging method [25]. The observed data on the antioxidant activity are given in **Table 3**.

3. ANTIMICROBIAL ACTIVITY

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml Analar grade) to give a concentration of 1000 µg/mL. Streptomycin for bacteria and Amphotericin –B for fungal solutions was also prepared to give a concentration of 1000 µg/ml in sterilized distilled water. The pH of all the test solutions and control was maintained in between 2 to 3 by using conc. HCl. All the compounds were tested at dose levels of 1000 µg and DMSO used as a control. The solutions of each test compound, control and reference standard were added separately in the cups and the plates were kept undisturbed for at least 2 hours in a refrigerator to allow diffusion of the solution properly into nutrient agar medium. Petri dishes were subsequently incubated at 37±1 °C for 24 hours. After incubation, the diameter of zone of inhibition surrounding each of the cups was measured with the help of an antibiotic zone reader.

4. ANTIOXIDANT ACTIVITY

0.1 mM solution of DPPH in methanol was prepared and 1.0 mL of this solution was added to 3.0 mL of test solution in methanol at different concentration (1-16 µg/mL). Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding sample. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity (expressed as %inhibition). The capability to scavenge the DPPH radical was calculated using the following equation:

The formula used for %inhibition is as follows:

- %inhibition = $(\text{Blank OD} - \text{Sample OD} / \text{Blank OD}) \times 100$
- Control is the absorbance of the methanol in DPPH alone.
- Test means the absorbance in the presence of sample

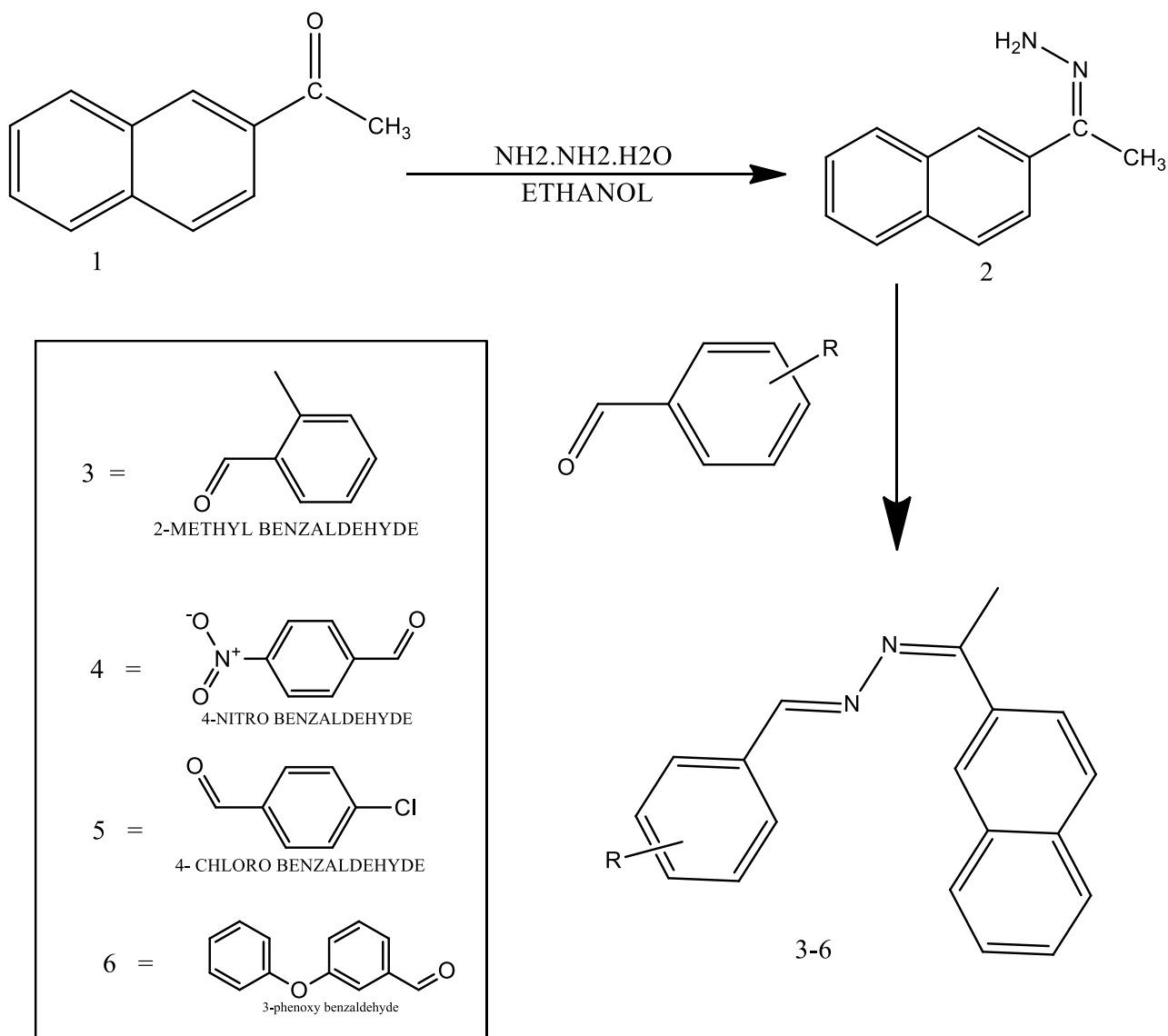
4. 1. Preparation of 1-(naphthalene-6-yl)ethylidene)hydrazine (2)

2 g of 2-acetyl naphthalene is dissolved in 10 mL of ethanol and 10 mL of hydrazine hydrate is added drop by drop with stirring at room temperature, the product is within 6-10 minutes and immediate colour change was observed. The completion of the reaction is monitored by TLC. The reaction mixture is poured into crushed ice, filtered and then dried over anhydrous CaCl₂, to give bright yellow colour. The dried product is recrystallized from ethanol, to give 95% yield with high purity (**Scheme 1**).

4. 2. Preparation of 2-(substitutedbenzylidene)-1-(1-naphthalene-6-yl-ethylidene) hydrazine (3-6)

About 1.84 g (1 mol) of (z)-1-(naphthalene-6-yl)ethylidene)hydrazine was dissolved in 10 mL of ethanol. To this 1 mol of substituted benzaldehyde slowly added with stirring at room temperature, the product is within 6-10 minutes and immediate colour change was absorbed.

After completion of the reaction monitored by TLC, the reaction mixture is poured into crushed ice, filtered and then dried over anhydrous CaCl_2 , to give bright yellow colour. The dried product recrystallized from ethanol, to give 95% yield with high purity (Scheme 1).



Scheme 1

5. RESULTS AND DISCUSSION

All the arylazines derivatives were characterized by a comparison of the melting point with the literature data, and by the IR spectra.

Table 1. Physical data of unsymmetrical hydrazone derivatives (2-6)

Arylazine	Molecular formula	Molecular weight	Yield %	Melting point (°C)
2	C ₁₂ H ₁₂ N ₂	184.2	95	96
3	C ₂₀ H ₁₈ N ₂	286.37	95	110
4	C ₁₉ H ₁₅ N ₃ O ₂	317.34	95	155
5	C ₁₉ H ₁₅ Cl N ₂	306.09	95	146
6	C ₂₅ H ₂₀ N ₂ O	364.44	95	104

If the C=N stretching frequency appears at 1585-1598 cm⁻¹, aromatic C-H stretching appears at 3051-3062 cm⁻¹, an aliphatic C-H stretching appears at 2912-2997 cm⁻¹, IR values are in agreement with the theoretical values of hydrazones.

The synthesized hydrazone derivatives were screened for the antibacterial activity against three Gram-positive bacteria viz., *Streptococcus pyogenes*, *Bacillus subtilis* and *Staphylococcus aureus* and two Gram-negative bacteria viz., *Escherichia coli* and *Pseudomonas aeruginosa* by using the cup plate method. Streptomycin was used as reference standard for comparing the results. The antibacterial activity of the Schiff base derivatives are shown **Table 2**. Thus the substituent's place a vital role in imparting enhanced antibacterial activity to the compounds. The screening results indicate that compounds **(3)**, **(5)** and **(6)** were found to be active against *S. aureus*. Compounds **(2)** and **(4)** were found to moderately active be active against *S. aureus*, Compounds **(3)**, **(5)** and **(6)** were found to be active against *B. subtilis*. Compounds **(2)** and **(4)** were found to be moderately active against *B. subtilis*. Compound **(2)**, **(3)** and **(5)** was found to active against *E. coli*. Compounds **(6)** and **(4)** were found to be moderately active against *E. coli*, Compound **(4)**, **(5)** and **(6)** were found to be active against *S. pyogenes*. Compounds **(2)**, **(4)**, **(5)** and **(6)** were found to moderately active be active against *P. aeruginosa*.

An agar well diffusion method was employed for the *in-vitro* study of antifungal effects against *Aspergillus flavus*, *Aspergillus niger*, *Penicillium chrysogenum*, *Trichoderma viride* and *Fusarium oxysporum*. The results of this evaluation were compared with Amphotericin – B as reference standard. The antifungal activity of the hydrazone derivatives are shown **Table 3**. Thus the substituents place a vital role in imparting enhanced antifungal activity to the compounds. Compounds **(2)**, **(5)**, **(6)** were found to moderately active be active against *Aspergillus flavus*, whereas all other compounds were found to be inactive against *Aspergillus flavus*. Compounds **(2)**, **(3)**, **(6)** were found to be active against *Aspergillus niger*. Compounds **(4)**, **(5)** were found to be moderately active against *Aspergillus niger*, where as all other compounds were found to be less active against *Aspergillus niger*. Compound **(4)**, were found to active against *Penicillium chrysogenum*. Compounds **(6)** were found to be moderately active against *Penicillium chrysogenum*. Compounds **(3)**, **(4)** and **(6)** were found to be moderately active against *Trichoderma viride*. Compound **(5)**, was found to be active against *Fusarium oxysporum*. Compounds **(3)**, **(6)** were found to be moderately active against *Fusarium oxysporum*.

Table 2. The antibacterial activities of schiff base derivatives by disc diffusion method

S. No.	Bacteria	Standard Antibiotic Disk*	Zone of inhibition (mm)					
			2	3	4	5	6	Control [DMSO]
1	<i>Bacillus subtilis</i>	28	-	9	12	11	10	-
2	<i>Escherichia coli</i>	25	9	-	9	12	13	-
3	<i>Pseudomonas aeruginosa</i>	29	-	10	14	9	9	-
4	<i>Staphylococcus aureus</i>	28	12	10	14	12	-	-
5	<i>Streptococcus pyogenes</i>	29	09	11	13	16	-	

Table 3. The antifungal activities of schiff base derivatives by disc diffusion method (1-5)

S. No.	Fungi	Amphotericin - B	Zone of inhibition in diameter (mm)				
			2	3	4	5	6
1	<i>Aspergillus flavus</i>	20	11	-	-	8	11
2	<i>Aspergillus niger</i>	21	19	17	12	14	16
3	<i>Penicillium chrysogenum</i>	21	-	-	23	-	14
4	<i>Trichoderma viride</i>	20	-	08	08	11	06
5	<i>Fusarium oxysporum</i>	24	08	16	-	26	14

Table 4. Antioxidant activity of hydrazone derivatives

S. No.	Antioxidant activity (%) DPPH
Ascorpic acid	95.80±0.88
2	63.08±0.52
3	41.25±0.36
4	58.66±1.02
5	51.23±0.39
6	60.12±1.89

All the synthesized compounds (2) to (6) were evaluated for their in-vitro Antioxidant activity by **DPPH method**. The result of this study is collected in Table 4. The following observations were made within the series, Compounds (2), (6) and (4) showed maximum oxygen scavenging activity which is comparable to ascorbic acid. Compounds (5) exhibited moderate oxygen scavenging activity as compared to ascorbic acid, where as all other compounds were exhibited minimum antioxidant activity. However none of the compounds exhibited greater activity with respect to standard ascorbic acid.

6. CONCLUSION

The study has proved that the synthesized heterocyclic hydrazone analogues have some antibacterial, antioxidant and fungal effects. The results, based on the potentially active hydrazone skeleton, have pointed out the importance of the positions of the phenolic substitution groups in the ring for obtaining antibacterial, antioxidant and antifungal activity. All the compounds exhibited a potent inhibitory activity against bacterial, antioxidant and fungal activity. They may provide a template to design new combinations of antibiotics and non-antibiotics to treatment of infections.

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