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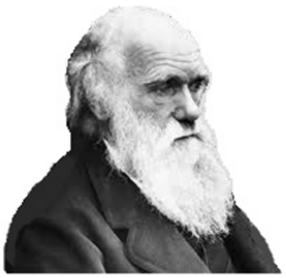

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Synthesis, Characterization, In-Vitro Antimicrobial Evaluation and Antioxidant Studies of Some Isoxazoline Derivatives

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ABSTRACT

The tremendous rise in development of resistance to antimicrobials has created an alarming situation for researchers and clinicians. In this regard, an attempt has been made to develop a series of azole-based derivatives. The presented study consists of the design and synthesis of some newer derivatives by incorporating the isoxazole nucleus in the pharmacophore. These are also characterized physicochemically and by spectral means (IR and microanalysis). Moreover, the antioxidant activity of these derivatives was assessed using DPPH radical scavenging methods. Finally, all of the newly isolated compounds were tested for their antimicrobial activities. Herein, antimicrobial screening using the agar disc diffusion method revealed that the majority of the derivatives are most active.

***Keywords*:** Chalcone, isoxazole, synthesis, antibacterial, antifungal, antioxidant activity

1. INTRODUCTION

One of the major objectives of organic and medicinal chemistry is the design, synthesis and production of molecules, which are having highly therapeutic interest. On the other hand, because of the resistance of pathogenic bacteria towards the available anti-biotics is rapidly becoming a worldwide undigestive problem, in the same way fungal infections continue to increase rapidly because of the increased number of immune-compromised patients. So in view of the above discussions it will be necessary to design a new class of molecules to deal with

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resistant bacteria and fungi to have become one of the most important areas of antimicrobial research today. Antibacterial and antifungal activities of the azoles are most widely studied and some of them are in clinical practice as antimicrobial agents. The design of noble chemical entities like chalcone and isoxazole derivatives could lead to the availability of better drugs for the treatment of various diseases. Isoxazoles are unsaturated aromatic heterocyclic compounds that contain a ring with three carbon atoms and one oxygen atom. The isoxazole behavior can be modified by the effects of substituent at position 1 and one nitrogen atom at position 2 [1]. Isoxazoles exhibit a broad spectrum of pharmacological and biological activities which include anti-HIV [2], GABA antagonist [3], anti-cancer [4], antinociceptive [5], antithrombotic [6], antifungal [7], antibacterial [8], dopamine D4 receptors antagonist [9], and immunomodulatory [10]. The chalcones are reactive intermediates in the synthesis of isoxazole that exhibit a broad range of biological activities [11-14].

In view of the above observations it was a thought to be of interest to design and synthesize a new class of isoxazole derivatives. So, in this present communication we report a facile synthesis of diverse 3-(4-substitutedphenyl)-4,5-dihydro-5-p-tolylisoxazole derivatives and their antimicrobial and antioxidant activities against various organisms. The results revealed that the newly synthesized derivatives exhibited significant biological activities.

2. EXPERIMENTAL

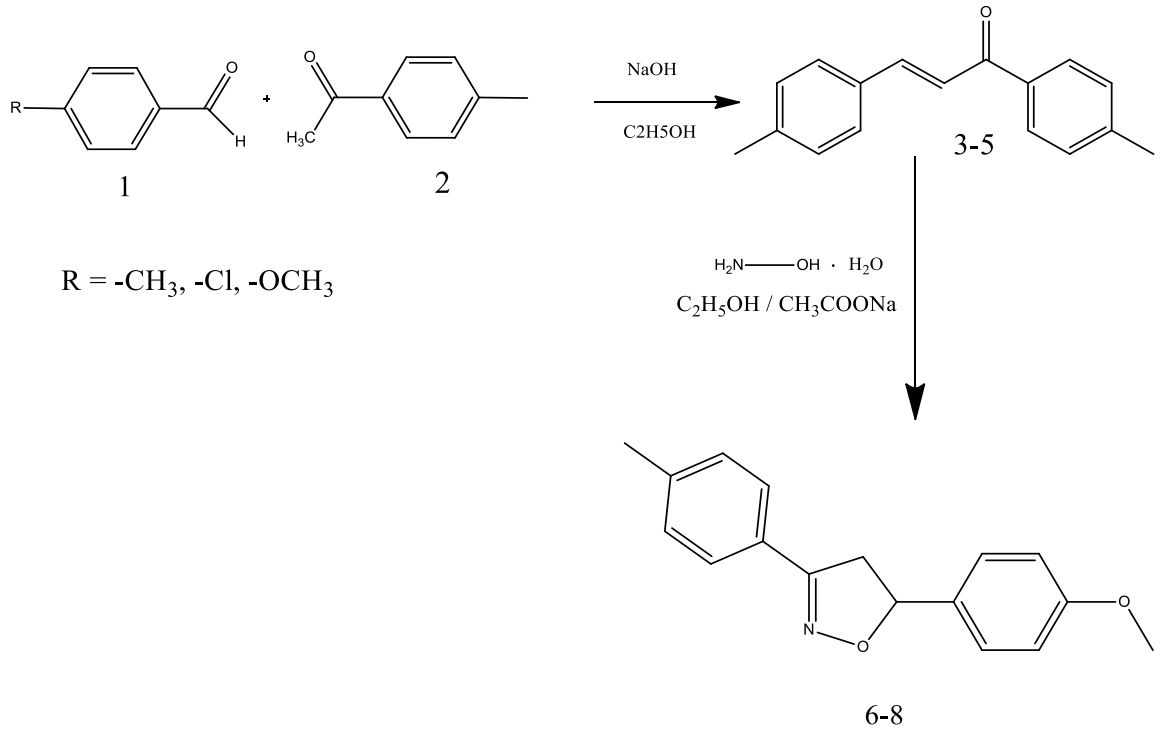
All chemicals were purchased from commercial suppliers, and used without further purification. All solvents used for reaction were freshly distilled from proper dehydrating agents. Melting points were determined in open capillaries on a Gallenkamp Melting Point Apparatus and are uncorrected. The purity of the compounds was checked by a 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. 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 [15]. 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 were also prepared to give a concentration of 1000 µg/mL in a sterilized distilled water. The pH of all the test solutions and control was maintained in between 2 to 3 by using concenytated 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 the 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



Scheme 1.

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 concentrations (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 a higher free radical scavenging activity (expressed as % inhibition). The capability to scavenge the DPPH radical was calculated using the following equation (**Scheme 1**).

The formula used for %inhibition is as follows:

%inhibition = (Blank OD – Sample OD/Blank OD) × 100
Control is the absorbance of the methanol in DPPH alone.
Test means the absorbance in the presence of sample.

4. 1. Synthesis of (E)-3-(4-substitutedphenyl)-1-p-tolylprop-2-en-1-one (3-5)

A solution of 4-substitutedbenzaldehyde (1mmol) and 4-methylacetophenone (1mmol), sodium hydroxide (0.5 g) and 10 mL of ethanol were shaken occasionally for 1 hour. After the

completion of the reaction, the mixture was cooled at room temperature. The resulting precipitate was filtered and washed with a cold water. The product appeared as a pale yellow solid. Then this was recrystallised using ethanol to obtain pale yellow glittering solid.

4. 2. Synthesis of 3-(4-substitutedphenyl)-4,5-dihydro-5-p-tolylisoxazole (6-8)

The chalcone derived from 4-methylacetophenone and 4-substitutedbenzaldehyde was refluxed with hydroxylamine hydrochloride (0.2 mmol) and 2 g sodium acetate in ethanol (10 mL) for 8 h. The completion of the reaction was monitored by TLC. The reaction mixture was cooled, and poured into ice water. The precipitate was filtered, dried (Scheme 1).

5. RESULTS AND DISCUSSION

5. 1. Analysis of IR Spectrum of synthesized chalcone and isoxazoline compound

Table 1. Antibacterial activity of chalcone and isoxazoline derivatives

Sample No.	Compound	Zone of inhibition (mm)			
		B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
1	3	8	7	7	6
2	4	7	6	7	7
3	5	7	6	8	7
4	6	6	6	6	8
5	7	7	6	8	7
6	8	8	-	11	6
	Standard	25	-	8	8
	Control	-	-	-	-

The IR Spectrum of compound (3-5) shows the CO s-cis stretching frequency appeared at 1566-1594 cm^{-1} and CO s-trans stretching frequency appeared at 1565-1589 cm^{-1}. CHip stretching frequencies appeared at 1166-1179 cm^{-1} and CHop stretching frequency was observed at 734-772 cm^{-1}. CH=CH op stretching frequency was observed at 1030-1088 cm^{-1}. C=Cop stretching frequency was observed at 668-673 cm^{-1}. The IR Spectrum of compound 6-8, in which the C=N stretching frequency appeared at 1597 cm^{-1}, Aromatic (CH) stretching frequencies appeared at 3026-3050 cm^{-1}. N-N stretching frequency appeared at 1114-1118 cm^{-1}. The synthesized chalcone and isoxa viz., *Bacillus subtilis* and *Staphylococcus aureus* and two Gram-negative bacteria viz., *Escherichia coli* and *Pseudomonas aeruginosa* were done by

using the disc diffusion method. Streptomycin was used as a reference standard for comparing the results. The antibacterial activities of the hetrocyclic derivatives are shown in **Table 1**. Table shows that heterocyclic derivatives of **(3)** to **(8)** possess a significant activity almost equipotent with the standard Streptomycin against both, Gram +ve and Gram –ve pathogenic organisms. Thus the substituents place a vital role in imparting enhanced antibacterial activity to the compounds. The screening results indicate that compounds **(3)** were found to be more active against *S. aureus.* Compounds **(3)** and **(8)** were found to be highly active against *B. subtilis*. All other compounds were found to be moderately active against *B. subtilis.* Compounds **(5)**, **(7)**, and **(8)** were found to be more active against *E. coli.* All other compounds were found to be from moderate to less active against *E. coli.*

Compound **(6)** was found to be highly active against *P. aeruginosa*, where as all other compounds, was found to be less active against *P. aeruginosa*. A filter paper disc method was employed for the *in vitro* study of antifungal effects against *Aspergillusflavus*, *Aspergillusniger*, *Penicillim chryogenum*, *Fusarium oxysporum*, and *Trigodermaveride*. The results of this evaluation were compared with Amphotericin –B as a reference standard. The antifungal activities of the isoxazoline derivatives are shown in **Table 2**.

Table 2. Antifungal activity of chalcone and isoxazoline derivatives

DISC DIFFUSION METHOD		**ANTIFUNGAL ACTIVITY**				
		ZONE OF INHIBITION (mm)				
S.No	Comp. No	*Aspergillus flavus*	*Aspergillus niger*	*Penicillim chryogenum*	*Trigoderma veride*	*Fusarium oxysporum*
1	3	13	13	12	12	14
2	4	-	8	-	11	11
3	5	12	-	11	10	-
4	6	13	-	-	11	12
5	7	12	8	12	-	12
6	8	20	-	22	24	20
Amphotericin-B		14	12	12	13	14
Control		-	-	-	-	-

Table 2 shows that isoxazoline and chalcone derivatives of **(3)** to **(8)** posses a significant activity, almost equipotent with the standard Amphotericin –B. Thus the substituents play a vital role in imparting enhanced antifungal activity to the compounds. However, the majority of the compounds like **(3)**, **(7)**, and **(8)** showed the activity almost equal to that of the standard.

The screening results indicate that compounds **(3)**, **(6)**, and **(8)** were found to be active against *Aspergillus flavus*. Compounds **(5)** and **(7)** were found to be moderately active active against *Aspergillus flavus*, Compound **(3)** was found to be active against *Aspergillus niger* .Compounds **(4)** and **(7)** were found to be moderately active against *Aspergillus niger*. Compound (**8)** was found to be active against *Trigoderma veride*. Compounds (**3)**, **(6)**, and **(7)** were found to be moderately active against *Trigoderma veride.*

Table 3. Antioxidant activity of Isoxazoline derivatives

Sample No.	Compounds	Antioxidant activity (%) DPPH
1	Ascorpic acid	97.67 ±0.58
2	3	51.00 ±1.00
3	4	46.67 ±2.52
4	5	57.00 ±2.65
5	6	65.00 ±2.65
6	7	89.67 ±2.08
7	8	44.39 ±1.25

All the synthesized compounds **(3)** to (**8)** were evaluated for their in-vitro Antioxidant activity by DPPH method. The result of this study is collected in **Table 3**. The following observations were made within the series, Compounds **(7)** and **(6)** showed maximum oxygen scavenging activity which is comparable to ascorbic acid. Compounds **(3)** and **(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

Chalcones were prepared from substituted 4-methylacetophenone and substituted benzaldehyde and condensed with hydroxylamine hydrochloride in ethanol to get the corresponding isoxazoline. The compounds were synthesized and characterized by TLC, melting points, and IR spectra. All the synthesized compounds were screened for their antibacterial activities. The *in vitro* antibacterial activity was checked against two Gram positive microorganisms (*S. aureus* and *B. subtilis)* and two Gram negative microorganisms (*E. coli* and *P. aureginosa*). Some of the tested compounds exhibited promising antibacterial activities. It is concluded that the compounds against *Pseudomonas aeruginosa*, show a very good activity. Compounds against *Bacillus subtilis*, *Escherichia coli*, and *Staphylococcus*

aureus show a moderate activity compared with other compounds. The rest of the compounds against rest of the organisms have a lower activity.

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