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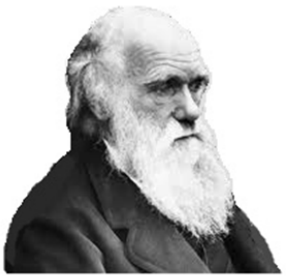

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In silico screening, synthesis, in vitro evaluations antibacterial and DPPH scavenging activity of some 1,3,5-trisubstituted 2-pyrazoline derivatives as dihydrofolate reductase inhibitors

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ABSTRACT

In this work, synthesis of three pyrazoline derivatives (6-8) is described. (E)-1,3-(phenylsubstituted)-prop-2-en-1-one (3-5) is prepared by the reaction of substituted benzaldehyde with 4-methylacetophenone, whereas condensation cyclization of the same chalcones (3-5) with phenylhydrazine hydrate in ethanol yielded 6-8. The structures of the title compounds (6-8) were characterized by chemical reactions, elemental analysis, and spectral methods such as IR spectra. The synthesized chalcone and pyrazolines were evaluated for in-vitro antibacterial and antioxidant activities against standard. The zone of inhibition for some of the newly synthesized compounds showed notable antibacterial activity against selected bacterial strains when compared with ampicillin. Significant antioxidant activities were also shown by chalcone and pyrazolines.

***Keywords*:** Synthesis, chalcone, pyrazoline, antibacterial, antioxidant

1. INTRODUCTION

Resistance gained by microorganism to conventional antimicrobial agents has lead to the development of new antimicrobial agents against pathogenic microbes. Chalcones and their derivatives proved to be an important molecular scaffold for the search of new pharmaceutically active molecules. In the past and recent years various physical and chemical studies are made on heterocylic ring system having pyrazoline unit. The pyrazoline

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derivatives serve synthetic intermediate for the prepation of various alkaloids. Among the various derivatives of chalcones, synthesis of pyrazolines has gained major attention due to their promising biological activities such as anticancer, antimicrobial, antidepressant, immunosuppressive, anti-inflammatory, etc. [1-3]. Many studies revealed that incorporation of pyrazole moiety into various heterocyclic ring systems gives worthwhile molecules from the biological point of view [4-6] Several marketed drugs such ascelecoxib [7] and rimonabant [8] contains pyrazole as their core molecular unit [9-10]. In this study, different pyrazolines [6-8] were synthesized via cyclization of substituted chalcone intermediates in the presence of phenyl hydrazine. The structures of the pyrazoline derivatives were confirmed by spectral analysis. The compounds were screened for their in vitro antibacterial activity using gram-positive bacteria and gram negative bacteria. Compounds were also screened for their antifungal activity. Several derivatives of pyrazoline produced good to moderate activities against number of bacteria and fungus. to their wide range of 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 [11-17].

Ampicillin was the reference drug for antibacterial activity. The observed data on the antimicrobial testing are presented in Table 1. Compounds 3-5 and 6-8 were assessed for antioxidant activity using 1,1-biphenyl-2-picrylhydrazyl (DPPH) radical scavenging method [18]. The observed data on the antioxidant activity are given in Table 2.

3. ANTIBACTERIAL ACTIVITY

Each test compound (5 mg) was dissolved in dimethyl sulfoxide (5 ml Analar grade) to give a concentration of 1000 µg/ml. Ampicillin solution 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. 1. 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 = (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. 1. Synthesis of ((E)-1,3-(substitutedphenyl)prop-2-en-1-one

A solution of 4-substitutedbenzaldehyde (1 mmol) and 4-methylacetophenone (1 mmol), 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 cold water. The product appeared as pale yellow solid. Then this was recrystallised using ethanol to obtain pale yellow glittering solid melting at 328-334 °C, Scheme 1.

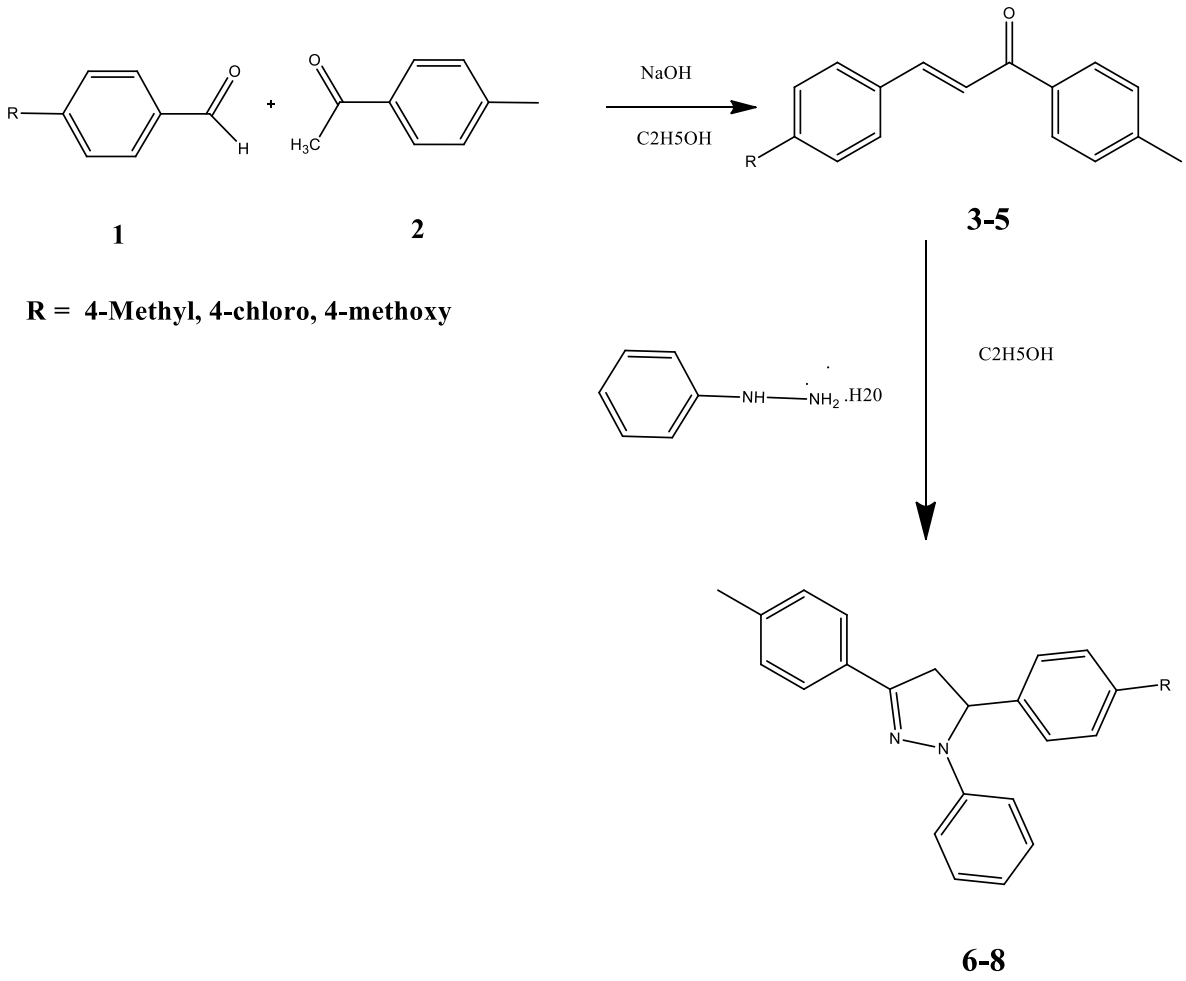
4. 1. 2. Synthesis of 4,5-dihydro-1-substitutedphenyl-3,5-dip-tolyl-1H-pyrazole

The chalcone derived from 4-methylacetophenone and 4-substitutedbenzaldehyde was refluxed with phenyl hydrazine hydrate (0.2 mmol) and 2 g sodium acetate in ethanol (10 mL) for 8h. The completion of the reaction was monitored by TLC. The reaction mixture was cooled, and poured into ice water. The precipitate was filtered, dried. Yield 85%, m.p 145 °C

5. RESULTS AND DISCUSSION

The IR Spectrum of compound (3-5) shows the $CO_{s\text{-}cis}$ stretching frequency appear at 1566-1594 cm-^{1} and $CO_{s\text{-}trans}$ stretching frequency appear at 1565-1589 cm^{-1}. CH_{ip} stretching frequencies appear at 1166-1179 cm^{-1} and CH_{op} stretching frequency observed at 734-772 cm^{-1}. $CH{=}CH_{op}$ stretching frequency observed at 1030-1088 cm-^{1}. $C{=}C_{op}$ stretching frequency observed at 668-673 cm^{-1}.

The IR Spectrum of compound (6-8) shows the C=N stretching frequency appear at 1597 cm^{-1} Aromatic (CH) stretching frequencies appear at 3026-3050 cm^{-1} and stretching frequency observed at 1385-1381 cm^{-1}. N-N stretching frequency appear at 1114-1123 cm^{-1}. The synthesized pyrazoline derivatives were screened for the antibacterial activity against three Gram-positive bacteria viz., *Bacillus subtilis* and *Staphylococcus aureus* and two Gram-negative bacteria viz., *Escherichia coli* and *Pseudomonas aeruginosa* by using the disc diffusion method. Ampicillin was used as reference standard for comparing the results. The antibacterial activity of the hetrocyclic derivatives are shown in Table 1.



Scheme 1.

Table 1. Antibacterial Activity of Chalcone and Pyrazoline Derivatives.

Micro organism	Zone of inhibition (mm)						
	Ampicillin	3	4	5	6	7	8
Bacillus subtilis	16	7	8	10	10	11	11
Escherichia coli	18	-	7	12	10	13	7
Pseudomonas aeruginosa	14	8	10	10	12	13	7
Staphylococcus aureus	19	-	8	8	13	11	9
Streptococcus pyogenes	17	7	8	11	15	13	8

The table Showed that chalcone and pyrazoline derivatives of (3) to (8) possess significant activity almost equipotent with the standard ampicillin against both Gram +ve and Gram –ve pathogenic organism. Thus the substituents place a vital role in imparting enhanced antibacterial activity to the compounds.

The screening results indicate that compounds (6) were found to be more active against *S. aureus.* Compounds (7) and (8) were found to be highly active against *B. subtilis*. All other Compounds were found to moderately active against *B. subtilis.* Compound (5), (6), (7) were found to more active against *E. coli.* All other compounds were found to be moderate to less active against *E. coli.*. Compounds (7) was found to be highly active against *P. aeruginosa.* Where as all other Compounds were found to be less active be active against *P. aeruginosa*.

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 2. The following observations were made within the series, Compounds (6) and (7) 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.

Table 2. Antioxidant activity of chalcone and pyrazoline derivatives

S. No.	Compounds	Antioxidant activity (%) DPPH
1	Ascorpic acid	96.32±0.58
2	3	49.65±1.00
3	4	47.10±2.52
4	5	56.58±2.65
5	6	66.39±2.65
6	7	890.25±2.08
7	8	90.56±1.26

6. CONCLUSIONS

Chalcones were prepared from substituted acetophenones and substituted benzaldehydes and condensed with Phenyl hydrazine hydrate in ethanol to get the corresponding pyrazolines (6-8). The compounds were synthesized and characterizes by TLC, melting points, IR spectra. All the synthesized compounds were screened for their antibacterial activities. The *in vitro* antibacterial activity was checked against three Gram positive microorganisms (*S. aureus* and *B. subtilis, Streptococcus pyogenes)* and two Gram negative microorganisms (*E. coli* and *P. aureginosa*). Some of tested compounds exhibited promising antibacterial activities. It is

concluded that the compounds against *Pseudomonas aeruginosa* and *Streptococcus pyogenes* shows very good activity. Compounds against *Bacillus subtilis*, *Escherichia coli* and *Staphylococcus aureus*, shows moderate activity compared with other compounds. The rest of the compounds against rest of the organisms have less activity. All the synthesized compounds (3) to (8) were evaluated for their in-vitro Antioxidant activity by DPPH method. Compounds (6) and (7) showed maximum oxygen scavenging activity which is comparable to ascorbic acid.

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