The aim of the present study is to synthesize a set of 3,5-disubstituted-2,4-thiazolidinediones as antimicrobial. These compounds were evaluated for their antimicrobial activity and hepatotoxicity. To the date, much research has been directed toward the synthesis and novel uses of 2,4-thiazolidinedione compounds.

**Objective**

- 2,4-Thiazolidinedione derivatives were launched as antidiabetics in 90's. Later the derivatives of 2,4-thiazolidinedione were banned due to hepatotoxicity. To the date, much research has been directed toward the synthesis and novel uses of 2,4-thiazolidinedione compounds.

- The aim of the present study is to synthesize a set of 3,5-disubstituted-2,4-thiazolidinediones as antimicrobial. These compounds were evaluated for their antimicrobial activity.

**Method:** First, the 2,4-thiazolidinedione was substituted at the position of 3 using sodium hydroxide and ethanol and then substituted at the position of 5 in the presence of piperidine by the Knoevenagel condensation method. The structures of the compounds were established on the basis of infrared and nuclear magnetic resonance spectral studies.

**Result:** 3,5-disubstituted-5-benzylidine-2,4-thiazolidinediones derivative was synthesized using benzyl halides and aromatic aldehydes. The results obtained showed that TZ-1 exhibited good activity against *Bacillus subtilis* while no activity against *Escherichia coli*.

**Conclusion:** Attachment of more heterocyclic rings containing Nitrogen on the 3rd position of 2,4-thiazolidinedione can enhance the antimicrobial activity. Addition of more lipophilic agents may increase the bioavailability and efficacy of the drug. Long alkyl chains on the benzylidene ring can also increase the lipophilic character, and further attachment of these kind of agents on benzylidene chain may produce safe and effective compounds in future.

**Keywords:** 2,4-TZD, Antimicrobial, E.coli, Knoevenegel condensation, 3 and 5 disubstituted 2,4-TZD

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**INTRODUCTION**

The increasing identification of antibiotic-resistant pathogens that cause serious infections cannot be ignored. Although the future cannot be predicted with certainty, it is surely possible that an extensive epidemic of resistant bacterial infections could potentially harm millions of people. Given that it takes more than 10 years to establish the efficacy and safety of new compounds, there is an urgent need to restock the antibiotic pipeline. As various infections are the medical challenge, such as gastrointestinal tract GIT infections, HIV, Hepatitis C and urinary tract infections, and the 2,4-thiazolidinediones can be the key to the problem. Due to the resistance and many side effects of antibiotics being used, there is a need for new antimicrobials. Like penicillin has severe side effects, i.e., anaphylaxis and GIT disturbance, and due to acid liability of penicillin, it cannot be given orally. However, 2,4-thiazolidinedione derivatives resemble penicillin's heterocyclic ring but has no acid liability and can be given orally in various infections. Hence, the valuable properties of 2,4-thiazolidinedione compel to synthesize its various derivatives by substituting at position 3 and 5 [1-4].

Why 2,4-thiazolidinedione derivative is chosen for antimicrobial activity.

There are some reasons because of which this nucleus is chosen for antimicrobial activity:

- 2,4-Thiazolidinedione has nitrogen in the heterocyclic ring. It has been proved that whenever nitrogen is present as the member of the heterocyclic ring, it must have antimicrobial activity, for example, beta-lactam ring of penicillin and quinoline and isoquinoline.
- 2,4-Thiazolidinedione is the new antimicrobial which will decrease the chances of resistance [5,6].
MATERIALS AND METHODS

Materials
1. All the chemicals used were procured from C.D.H and HiMedia and purified before use.
2. Melting points were determined on the open capillary tube on Campbell Melting Point Apparatus and are uncorrected.
3. The silica gel used for thin layer chromatography (TLC) was “silica gel G” procured from Merck and was coated on laboratory glass slides.
4. TLC plates were visualized using an iodine chamber.
5. Infrared (IR) spectra were taken using Fourier transform IR Thermo scientific; NICOLET Is10 and KBr disk spectrophotometer. Only principal peaks are reported and expressed in cm$^{-1}$.
6. Proton nuclear magnetic resonance spectra (1H NMR) were recorded on Bruker AC 300 F, 300 MHz spectrophotometers. Chemical shifts are expressed as $\delta$ value (ppm), downfield from tetramethylsilane used as internal standard.
7. While citing 1H NMR data, the following abbreviations have been used S (singlet), d (doublet), t (triplet), and m (multiplet).

Methods

Drying of ethanol
Ethanol of high degree of purity is conveniently obtained by dehydrating commercial ethanol with calcium oxide. In 500 ml RB flask, poured the content of the commercially obtained ethanol, to it add 1 g of calcium oxide and allowed for refluxing. It was then distilled and collected for further use.

Drying of toluene
Commercial toluene may contain methyl thiophenone, which cannot be removed by distillation. It can be purified by treating with concentrated sulfuric acid, but care was taken that temperature was not allowed to rise unduly (<30°C) as toluene is sulfonated more easily than benzene and then store the dry toluene over sodium wire, phosphorus pentaoxide, or calcium hydride.

Drying of acetone
To the 700 ml acetone contained in a liter bottle, a solution of 3 gm of silver nitrate in 20 ml of water as added followed by 10 ml of 1M NaOH solution, and the mixture was shaken for about 10 min. The mix was then filtered dried with calcium sulfate and distilled [7].

Experimental work [7-10]

Step 1: Synthesis of 2,4-thiazolidinedione

Procedure
In a 250 ml three-necked flask a solution containing 56.4 g 0.6 mole of chloroacetic acid in 60 ml of water and 45.6 g (0.6 moles) of thiourea dissolved in 60 ml of water. The mixture was stirred for 15 min. To the contents of the flask were now added slowly 60 ml concentrated hydrochloric acid from dropping funnel, after which the reaction mixture was stirred and refluxed for 10–12 h at 100–110°C. On cooling, the contents of the flask solidified to a mass of clusters of white needles. The product was filtered and washed with water to remove traces of hydrochloric acid and dried. It was re-crystallized from ethanol.

Yield: 85%.

Synthesis of 3-(4’chlorobenzyl)-2, 4-thiazolidinedione

Procedure
In a 250 ml RBF, NaOH (0.8 g, 0.02 moles) was dissolved in 20 ml ethanol. To this solution, 2, 4-thiazolidinedione (0.02 moles) and benzyl bromide were (0.02 moles) added. The resulting reaction mixture was then refluxed for 40 h. Precipitation of salt was observed during the reaction. At the end of the reflux period, ethanol was distilled out, and the residue was extracted from ether and water. The ethereal layer was removed, dried over sodium sulfate and concentrated. The product was dried under reduced pressure and was re-crystallized from ethanol.

Yield: 84.45%; m.p: 97–99°C

TLC: Benzene: Ethyl acetate (4.5:0.5); Rf: 0.64.

Synthesis of 5-benzylidene-N-substituted-2,4-thiazolidinedione derivatives

Synthesis of 5-(4’hydroxy benzylidene)-3-(4’ chlorobenzyl)-2,4-thiazolidinedione

Procedure
In a 250ml 3-necked round bottom flask provided with a Dean-Stark apparatus, 4-hydroxy benzaldehyde (0.188 moles) and 3-chlorobenzyl-2, 4-thiazolidinedione (0.188 moles) were together suspended in dry toluene. To this catalytic amount of piperidine (1ml) was added. The mixture was refluxed with stirring. After complete removal of water and when the temperature crossed 110°C the reaction mixture was stirred for further 1 hr. On cooling, the product precipitated out of toluene. The compound was filtered and washed with cold, dry toluene, and dry ethanol.

Yield: 72%

Melting point - 205°C.

TLC of 5-(4’hydroxy benzylidene)-3-(4’ chlorobenzyl)-2,4-thiazolidinedione (Table 1)

| Compound | Yield (%) | Melting point | Rf value |
|----------|-----------|---------------|----------|
| TZ-1     | 66        | 205°C         | 0.16     |

**Table 1: Characterization data of the synthesized compound**
Spectral analysis

NMR study of 5-(4''hydroxy benzylidene)-3-(4'chlorobenzyl)-2,4-thiazolidinedione (TZ-1)

NMR: δ 4.89 (s,2H,CH2), δ 7.25-7.44 (m,9H,ArH), δ 7.83 (s,1H,OH)

IR study of 5-(4''-hydroxybenzylidene)-3-(4’chlorobenzyl)-2,4-TZD (TZ-1) (Table 2)

Pharmacological activity

We determined 2,4 thiazolidinedione as antimicrobial using the cup plate method. We found very interesting results of inhibition of the growth of Gram-positive as well as Gram-negative bacteria.

Pharmacological activity

Antimicrobial activity [11-13]

Various therapeutic agents which show inhibition of growth of microorganisms such as antibiotics or essential for their growth such as vitamins and amino acids are standardized by microbiological assays.

Any change in the antibiotic molecule which may not be detected by chemical methods will be revealed by a change in the antimicrobial activity, and hence microbiological assays are very useful for resolving doubts regarding a possible change in potency of antibiotics.

The minimal assay is based on a comparison of the inhibition of growth of microorganisms by measured concentrations of the antibiotic to be examined with that produced by known concentrations of a standard preparation of the antibiotic having the known activity.

Two general methods are used for microbiological assays of antibiotics:

Method A: Cylinder plate method or cup plate method
Method B: Turbidimetric method or tube assay method.

The cylinder plate method depends on diffusion of the antibiotic from the vertical cylinder through a solidified agar layer in Petri plate to an extent such that the growth of the added microorganisms is prevented entirely in a zone around the cylinder containing a solution of the antibiotics.

The turbidimetric method depends on the inhibition of growth of microbial culture in a uniform solution of antibiotic in a fluid that is favorable to its rapid growth in the absence of the antibiotic.

Minimum inhibitory concentration is defined as the lowest concentration of an antibiotic that will inhibit the visible growth of microorganisms, and minimum bactericidal concentration is the lowest concentration of antibiotic that will prevent the growth of microorganisms after subculture antibiotic-free media. Any compound showing inhibition zone none exceeding 9 mm diameter and quality of zone better or comparable to ciprofloxacin is considered to be endowed with antibacterial potential.

Procedure

Antimicrobial activity of the synthesized 3,5-disubstituted thiazolidinediones derivative was carried out using cup plate method. The compounds were tested against strain of B. subtilis and E. coli. Stock solution of the synthesized compounds was prepared in dimethyl sulfoxide (DMSO), and the ciprofloxacin was prepared in distilled water. Dilution of the stock solution of synthesized compound was done using DMSO and 3000 µg/ml concentration was prepared in micro test tubes. The stock solution of standard, i.e., ciprofloxacin was diluted in sterile water to give a concentration of 100 µg/ml.

100 µl organism suspensions were seeded on culture plates. Then, in each, the agar plate cavities/well (9 mm) was prepared using a cork borer. 100 µl of the compound was transferred to the wells and ciprofloxacin (100 µg/ml) was added to the remaining well. The plates were incubated at 30°C. The plates were observed for growth at the end of 24–48 h and the zone of inhibition was measured.

Petri dishes showing inhibition zone against B. subtilis (a) and E. coli (b)

Table 2: IR and 1HNMR data

| S. No | Molecule code | IR (cm⁻¹) | 1H NMR (ppm) |
|-------|---------------|-----------|--------------|
| 1     | TZ-1          | 1732.12, 1336.77, 1082.43, 3036.31, 1603.83, 1493.82, 2955.00 | 3.89 (s, 1H), 4.89 (s, 2H), 7.24-7.50 (s, 10H) |

IR: 1723.02 cm⁻¹ (Ketone, C=O), 1508.62 cm⁻¹ and 1493.84 cm⁻¹ (Aromatic C=C), 3346.16 cm⁻¹ (Phenolic OH), 2925.35 cm⁻¹ (Alkane, C-H), 1342.67 cm⁻¹ – 1086.80 cm⁻¹ (Amine C-N). IR: Infrared, 1H NMR: Proton nuclear magnetic resonance spectra
The number 5 (Fig. a and b) is the zone of inhibition shown by 5-(4”-hydroxybenzylidene)-3-(4’chlorobenzyl)-2,4-TZD on both Gram-positive and Gram-negative bacteria. The no. 6 (Fig. a and b) is the zone of inhibition by standard drug ciprofloxacin.

A set of negative and positive controls of growth were also kept for incubation along with test plates. In the plate, for negative growth, the highest volume of DMSO and sterile distilled water was used with no culture being seeded, while in the plate representing maximum growth (positive control), the highest volume of DMSO and sterile distilled water was used along with the organism.

**Summary and discussion**

3,5-disubstituted-5-benzylidine-2,4-thiazolidinediones derivative was synthesized using benzyl halides and aromatic aldehydes. Synthesized compound was characterized by chromatographic methods, IR spectroscopy, and NMR spectroscopy for their structural confirmation. This compound was tested for antimicrobial activity.

Antimicrobial evaluation of the compound was done against *B. subtilis* and *E. coli* using Cup Plate method. The results obtained showed that TZ-1 exhibited good activity against *B. subtilis* while no activity against *E. coli*.

**Conclusion**

Due to some disadvantages of long reaction times, use of large quantities of volatile organic and harsh conditions, therefore, development of an efficient and versatile method is still required.

The work on this project was started with the objective of synthesizing some known 2,4-thiazolidinedione by different methods as reported in literature. Further modifications on benzylidene ring and nitrogen of the 2,4-thiazolidinedione ring may increase antimicrobial activity. Attachment of more lipophilic agents may increase the bioavailability and efficacy of the drug.

Long alkyl chains on the benzylidene ring can also increase the lipophilic character, and further attachment of these kind of agents on benzylidene chain may produce safe and effective compounds in future.

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**Author’s Contributions**

Navjot Singh Sethi: Performed the experiments. D. N. Prasad: Conceived the idea, study design, and finalized the manuscript. Deepak Bhagwat: Performed the clinical studies and helped in the preparation of manuscript. Anuradha, Madhu, and Snageeta: Assisted in experimental work and helped in Statistical Evaluation and Preparation of the manuscript.

**Conflicts of Interests**

There are no conflicts of interest.

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