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Synthesis And Evaluation Of Antibacterial Activity Of Thiazolidine 2, 4-Dione Derivatives

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Abstract: Microbial infection has increased dramatically in recent years due to change life style and resistance. Bacteria have been the cause of many diseases. New targets are necessary to develop anti-microbial drugs thizolidine-2, 4-dione ring is recently becoming versatile scaffold because of ability of this ring to deliver variety of therapeutic actions.

Thiazolidine- 2, 4-dione is heterocyclic ring system having many biological activities.In this study we have made an attempt to synthesis derivatives which have thiazolidine-2, 4-dione ring as core ring. Thiazolidine-2, 4-dione at 3rd position of Nitrogen is substituted with variety of aryl, alkyl groups it causes good antibacterial activity. Derivatives were synthesized and characterized by IR, NMR and MASS Spectrometric technique and Antibacterial activity was determined.

Index Terms **- Thiazolidine 2, 4-dione, Benzene sulphonyl chloride, antibacterial**

I. INTRODUCTION

Thiazolidine dions are peroxisome proliferator activated receptor that improves insulin sensitivity in skeletal muscle. Thiazolidine dions are hypoglycemic agent. The activity of compounds depends upon their structure and different structural requirement are needed for effective activity against different targets.Electron withdrawing group on benzylidene of thiazolidene dione can improve antibacterial activity. This can be considered as most important target for antibacterial activity and trying to synthesis derivatives and evaluate their biological activity.

EXPERIMENTAL WORK

General remarks:

All the melting points were determined by open capillary method and are uncorrected.Infrared spectrums were recorded by ATR (Attenuated Total Reflectance) spectrophotometer (Bruker)from KTHM College, Nashik. Proton Nuclear Magnetic Resonance (NMR) were recorded (Bruker Avance 300) from Diya Lab, Mumbai. Mass Spectroscopy were recorded (PeSciex API 2000) from Diya Lab, Mumbai. Thin Layer Chromatography was performed to monitoring the reactions. The spot were visualized inUV chamber.

STEP I: **Synthesis of thiazolidine2, 4-Dione**.

Two solutions were prepared: a) In 250 ml beaker, mixture of chloroacetic acid (5.64 gm, 0.06mol) in 6 mlof water. b) In another beaker, mixture of thiourea in 6ml of water.

Above mixtures (a&b) were mixed together in 100ml RBF. The mixture was stirred for 15 min. at RT. To the resulting mixture 6 ml of concentrated HCl added slowly with the aid of separating funnel. The mixture was refluxed for 8-10 hr at

100-110℃, progress of reaction was monitored by TLC. After completion of reaction, cooled the content of flask, cluster of colourless crystalline product was separate out and washed with water to remove traces of HCl and dried.[9]

Yield: 86% **Mobile Phase:** Benzene: methanol (3:2)

Melting point: 120-121°C **Colour:**White crystalline **Nature:** solid

Confirmatory test for thiazolidine2, 4-Dione

Table No. 1 Confirmatory test of thiazolidine-2,4-dione

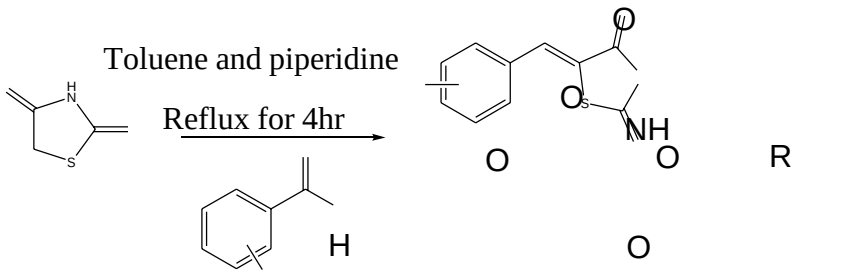
Sr. no	**Carboxylic acid test**	**Observation**	**Inference**
1	5ml of 10% $NaHCO_3$ solution + sample	No effervescence of CO_2	COOH group absent
2	5ml $NaHCO_3$ +sample + HCl	Precipitate was not observed	COOH absent

STEP II:

***Synthesis of 5 arylidene thiazolidine-2, 4-dione*General procedure:**

In a 250 ml 3-necked round bottom flask aromatic aldehydes (1 mol) and Thiazolidine-2,4-dione (1 mol) were together suspended in dry toluene. To this catalytic amount of piperidine (1 ml) 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 precipitate out from toluene.The compound was filtered and washed with cold, dry toluene and then recrystallized from dry ethanol. Reaction monitored by TLC.



TZD 2a-e

R = CL, Br. I, NO2,H,CH3O

R

Synthesis of derivatives:

1. ***Synthesis of 2a: 5 benzylidene thiazolidine-2,4-dione***

   Yield: 74% **Melting point:** 250-251℃

2. ***Synthesis of 2b: 5(4-methoxybezylidene) thiazolidine-2,4-dione***

   Yield: 80% **Melting point:** 217-218℃

3. ***Synthesis of 2c: 5(P-chlorobenzylidene) thiazolidine-2,4-dione***

   Yield: 88% **Melting point:** 223-224℃

4. ***Synthesis of 2d: 5(O-chlorobenzylidene) thiazolidine-2,4-dione***

   Yield: 85% **Melting point:** 179-180℃

5. ***Synthesis of 2e: 5(4-flurobenzylidene) thiazolidine-2,4-dione***

   Yield: 80% **Melting point:** 210-211℃

Step 3: Synthesis of 5-(substituted benzylidene)-3-phenyl sulphonyl thiazolidine-2,4-dione

General procedure:

To the stirred solution of 5-arylidene 2,4-thiazolidinedione(5mmol) in DCM (20ml), triethylamine (7.5mmol) was added at room temperature and stirring was continued for 15 min. Then to this solution phenyl sulphonyl chloride (7.5 mmol) was added drop wise with constant stirring. After the addition was completed the reaction mass was further stirred for 15-20 hr. At room temperature progress of the reaction was monitored by TLC on completion of the reaction dichloromethane was removed by Rota evaporator and obtained crude solid product was poured in ice cold water. Then solid thus obtained was filteredvaccum dried and crystallized from ethanol.[23]

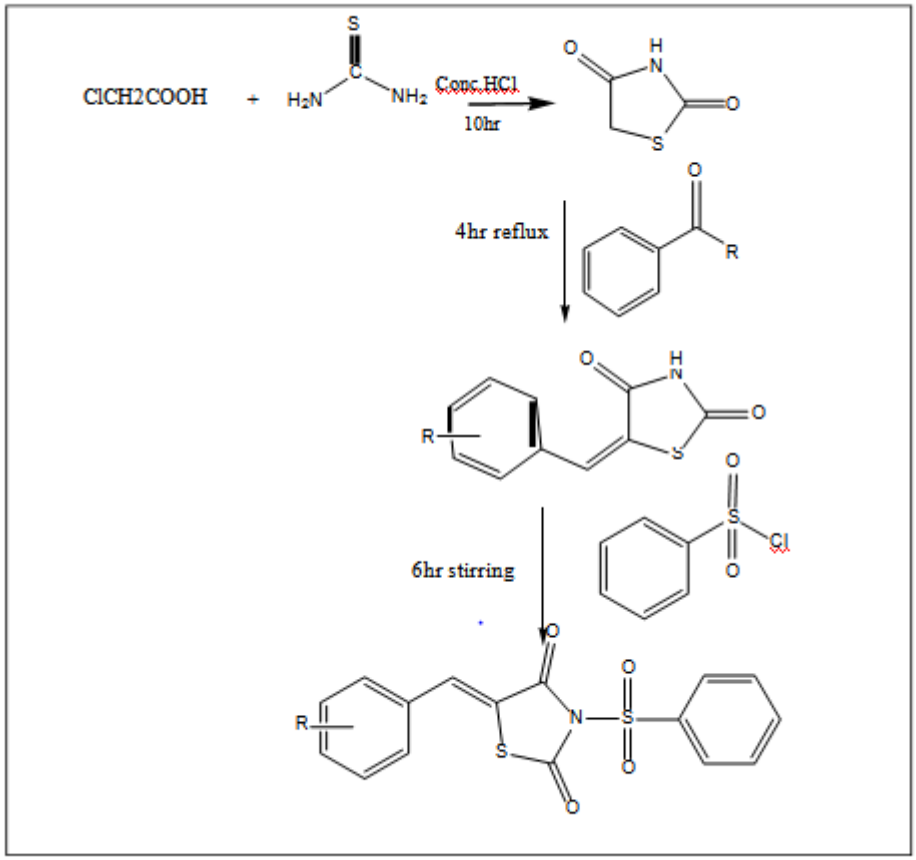


Table no. 2 Synthesized derivatives

Sample ID	code	Derivatives
B-TZD	**3a**	5-benzylidene 3-phenyl suplhonyl thiazolodine 2.4-dione
4-MTZD	**3b**	5-(4-methoxy benzylidene)-3-phenyl sulphonyl thiazolidine 2,4-dione
P-CTZD	**3c**	5-(P-chloro benzylidene)-3-phenyl sulphonyl thiazolidine 2,4-dione
O-CTZD	**3d**	5-(O-chloro benzylidene)-3-phenyl sulphonyl thiazolidine 2,4-dione
4-FTZD	**3e**	(4-fluro benzylidene)-3-phenyl sulphonyl thiazolidine2,4-dione

ANTIBACTERIAL ACTIVITY

Disc diffusion method:

Disc diffusion method was used to determine antibacterial activity of all test compounds. Saturated solution of test sample was prepared in DMSO. Ampicillin was used as standard to compare the activity of test compounds. Following procedure were used to carry out the study.

Principle

The method is based on the principle that disc of filter paper are impregnated with a single concentration of different antibiotics or other chemicals, diffuse disc into agar. The selected antibiotics disc placed on previously inoculated agar plate with bacteria. During incubation period, antibiotics diffuse outward from the disc into the agar. This will create concentration gradient in agar medium which depend on solubility of chemical and its molecular size. The absence of growth of organism around the antibiotics disc shows respected organism susceptible to the antibiotics and presence of growth around the antibiotics disc shows organism is resistant to particular antibiotics. The area of no growth around the disc called as zone of inhibition.

Susceptibility Test Procedure

Media Preparation: Mueller Hinton Agar used in disc diffusion method for rapidly growing aerobic organism. Mueller Hilton supplemented with 5% sterile defibrinated blood. Mueller Hinton agar + 2% glucose + 0.5 mcg/ml methylene blue was used for bacterial culture.

Inoculum Preparation: Fungal cultures inoculum is prepared by picking five distinct colonies (approximately 1 mm from 24 hrs old culture grown on saturated dextrose agar and incubated at 35±2 ºC). Those colonies are suspended in 5 ml of sterile 0.85% saline. Swirl the resulting suspension and adjust the turbidity to yield 1×10^6-5×10^6 cells/ml.

Test Procedure:

- Dip a sterile cotton swab into the standardized bacterial suspension.
- Remove excess inoculum by lightly pressing the swab against the tube wall at a level abovethat of the liquid.
- Inoculate the agar by streaking with the swab containing the inoculums
- Rotate the plate by 60° and repeat the rubbing procedure. Repeat two times. This will ensurean even distribution of the inoculums
- Allow the surface of the medium to dry for 3-5 minutes but not longer than 15 minutes toallow for absorption of excess moisture.
- Aseptic techniques should be used while applying disc.
- While depositing the disc care should be taken that their centers should be at least 24 mmapart.
- In case of fastidious organisms the distance should be at least 30 mm
- Incubate plates in an inverted position at 35±2 °C temperature and examine each plate 20-24hrs of incubation.

Result and Interpretation: Measure the zone of inhibition.

RESULT AND DISCUSSION:

The thiazolidine-2, 4-dione ring is one of the versatile scaffolds which facilitate the synthesis of many derivatives belonging to various therapeutic categories. Thiazolidine-2, 4-dione is important nucleus in the antidiabetic therapy. But recent studies which are previously covered in literature reviews show potential of this ring by which it can be converted into different derivatives. Malarial diseases, antifungal, anti-obesity, anticancer, viral diseases and antimicrobials are those categories which crave for develop more potent and

safe medicines. Drug development is more focused to find newer leads and target for new drug development. Keeping this scenario in mind the derivatives were planned. Thiazolidine-2,4-dione is highly active heterocycle. Due to various type of biological activity found in thiazolidine-2,4- dione derivatives, objectives has been taken to synthesize thiazolidine-2,4- dione in order to evaluate their pharmacological activity. A literature survey revealed that the presence of a substituted aromatic ring at position 5^{th} and N-3 substitution on thiazolidine-2, 4-dione nucleus is necessary for antibacterial and antifungal activity. An attempt was made to synthesize derivatives of 5(substituted benzylidene)thiazolidine-2, 4-dione combined with Benzene sulphonyl chloride and evaluate for their antibacterial activity.

The investigation was planned in the following manner.

- Synthesis of thiazolidine-2, 4-Dione.
- Synthesis of 5(substituted benzylidene) thiazolidine-2,4-dione.
- Synthesis of 5(substituted benzylidene) 3-benzene sulphonyl thiazolidine 2,4-dione.
- Establishment of structures of targeted compounds on the basis of IR, NMR and Mass spectrum.
- Evaluation of targeted compound for their antimicrobial activity by disc diffusion method on MuellerHington agar media.

➢ Thiazolidine-2,4-dione was synthesized by reacting chloroacetic acid and thiourea in presence of concentrated HCl.This is **2^{nd} order substitution reaction** & subsequent removal of HCl. Cleavage occurs by intermolecular nucleophilic substitution. At last, hydrolysis in position of the imino group catalysed by the consequent formation of HCl. & leads to formation of thiazolidine-2,4-dione.

➢ 5(substituted benzylidene) thiazolidine-2, 4-dione was synthesized by reacting benzaldehyde or substituted benzaldehyde with piperidine catalyst which follows the **knoevengel condensation/ aldol condensation reaction.** The reaction initiated by formation of iminium ion. Derivatives from **2a-e** were prepared.

➢ Synthesis of 5(substituted benzylidene)-3phenyl thiazolidine-2, 4-dione was reacted with benzene sulphonyl chloride in dichloromethane at ambient temperature and then formation of targeted compound from **3a-e.**

➢ ***Result of the intermediate compound:***

Table.No. 3: Result of intermediate compound

Sr.no.	Code	Molecular formula	Molecular wt (g/mol)	Melting point ℃	RF Value	% Yield	Colour
1	1	$C_3H_3NO_2S$	117.12	120-121°C	0.44	86	white
2	2a	$C_{10}H_7O_2NS$	205	250-251°C	0.63	74	yellow
3	2b	$C_{11}H_9O_3NS$	235	217-218°C	0.5	80	yellow
4	2c	$C_{10}H_6O_2NSCl$	239	223-224°C	0.5	88	white
5	2d	$C_{10}H_6O_2NSCl$	239	179-180°C	0.56	85	white
6	2e	$C_{10}H_6O_2NSF$	222	210-211°C	0.56	80	yellow

Result of Targeted compound:

Table No. 7.3: Result of targeted compound

Sr. no.	Code	Molecular formula	Molecular Wt (g/mol)	Melting point ℃	Rf Value	% Yield	Colour
1	3a	$C_{16}H_{11}O_4NS_2$	345	180-181	0.54	88	Yellow
2	3b	$C_{17}H_{13}O_5S_2N$	375	180	0.75	83	Yellow
3	3c	$C_{16}H_{10}O_4NS_2Cl$	379	179-180	0.72	82	Yellow
4	3d	$C_{16}H_{10}O_4NS_2Cl$	379	159-160	0.80	79	Yellow
5	3e	$C_{16}H_{10}O_4NS_2F$	362	180	0.79	84	Yellow

Spectral interpretation of targeted synthesized compound:

The final compound and intermediate were purified and their structure was established by IR, NMR & Massspectrum.

TZD: Starting material used for synthesis of TZD contain acid group. TZD was confirmed by doing carboxylic acid test. Sample was added to 5ml of 10% $NaHCO_3$ solution, it was observed that no effervescence of CO_2 means COOH group was absent

(86%) MP 120-121℃; IR 1641.42 C=O(amide), 3122.75 N-H, 607.58 C-S, 1328.95 C-N, 1394.53 CH_2

5-benzylidene thiazolidine-2,4-dione(2a):

6-$C_{10}H_7O_2NS$; 74%; Rf 0.63; Mp 250-251℃; IR 3100 N-H, 1700 C=O, 1600 C=O (amide), 800 C-S.

5-(Benzylidene) 3-phenyl sulphonyl Thiazolidine 2,4-dione(3a):

$C_{16}H_{11}O_4NS_2$; 88%; RF 0.54; Mp 180-181°C;IR 1700 C=(cyclic amide), 1600 C=O (amide), 850 C-S, 1197

S=O, 2750 C-H; MS m/z 345.1, 203$(M-H)^+$, 90.7, 144

5-(4-Methoxybenzylidene)-3-phenyl sulphonyl Thiazolidene-2,4-dione(3b):

$C_{17}H_{13}O_5S_2N$; 83%; RF 0.75; Mp 180-181°C; IR 1595.13 C=O(amide), 1716.65 C=O(cyclic amide),1008.87

S=O,1330.88 C-N,1485.19 C=H, 821 C-S; MS 376$(M+H)^+$, 222, 122, 74.5

5-(P-chlorobenzylidene)-3-phenyl sulphonyl Thiazolidene-2,4-dione(3c):

$C_{16}H_{10}O_4NS_2Cl$, (82%); RF 0.72; Mp 179-180 ℃; IR 1595.13 C=O, 1182.36 C-N, 1330.88 C-S, 1087.85

S=O, 690.52-729.09 C-Cl, 2765.92 C-H; MS 380 $(M+H)^+$, 143, 180, 74.1, 201, 102; NMR of compound 3c

Displayed multiple complexes at δ 7.71-7.76 for benzene. Singlet for CH at δ 1.19, Doublet for a & a' at δ

8.09-8.11 and also doublet for b & b' at δ 7.31-7.32 and singlet for CH_2 at δ 1.19

5-(O-chlorobenzylidene)-3-phenyl sulphonyl Thiazolidene-2,4-dione(3d):

$C_{16}H_{10}O_4NS_2Cl$; (79%); RF 0.80; Mp 159-160 ℃ ; IR 1604.77 C=O (amide), 1091 C-N, 1327.03 C-S,

1049.28 S=O, 759.95 C-Cl; MS m/z 380.4 $(M+H)^+$, 102.1; NMR of compound 3d displayed multiple complex at δ7.52-7.63 for benzene. Triplet of b & b' at δ 7.32-7.44 and doublet of a & a' found at δ 8.09-8.12.

5-(4-flurobenzylidene)-3-phenyl sulphonyl Thiazolidene-2,4-dione(3e):

$C_{16}H_{10}O_4NS_2F$; (84%); RF 0.79; Mp 180 ℃ ; IR 1589.78 C=O, 1193 C-N, 1390.68 C-S, 1089.78 S=O,

1448.54 C-F; MS m/z 361.9(base peak); NMR of compound 3e displayed multiple complex at δ 7.31-7.42 for benzene. Doublet of a & a' at δ 7.73-7.76 and doublet of b & b' found at δ 8.09-8.11. Singlet for CH at δ 3.083.

CONCLUSION:

- In this synthesis, 5(substituted benzylidene)3-benzene sulphonyl thiazolidine 2,4-dione derivatives weresynthesized and characterized by IR, NMR and Mass spectra.
- All the synthesized compounds have screened for their antibacterial activity.
- Antibacterial activity checked ampicillin, by Disc diffusion method on Mueller Hington Agar media.
- Activity of compounds was compared by using as standard drug.
- Compound O-CTZD shows maximum zone of inhibition (**28mm**) on Staphylococcus aureus and nozone of inhibition on E. coli. Than other derivatives.
- Compound 4-FTZD shows zone of inhibition (**24mm**) on Staphylococcus aureus and no zone of inhibition on E.coli.
- Compound P-CTZD shows similar zone of inhibition as compared with ampicillin.

From the antibacterial studies it has been conclude that my synthesized compound shows promising zone ofinhibition against **gram +ve** bacteria and no zone of inhibition against **gram -ve** bacteria.

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