Synthesis and Antimicrobial Activity of Novel 5-Arylidene-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto azo hydrazide Derivatives

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ABSTRACT
Ethyl N-Succinimido acetate was synthesized by equimolar condensation of succinimide and ethylchloroacetate by refluxing in presence of anhydrous potassium carbonate. Then Ethyl N-succinimido acetate was refluxed with hydrazine hydrate in presence of 1. 4-dioxane to obtain N-Succinimido acetylhydrazide, which was further treated with benzaldehyde to get phenyl succinimido acetylhydrazide, this compound was cyclized with mercaptoacetic acid to get the 3-(2-phenyl 4-oxo-1,3-thiazolidine) acetalizimo succinimide which was reacted with various aldehyde to get the 5-Arylidene-3-(2-phenyl 4-oxo-1,3-thiazolidine) succinimido aceto hydrazide. The formation of title compounds confirmed by physical and spectral data. The synthesized compounds were subjected to microbiological screening.

Keywords: Thiazolidinone derivatives, 5-Arylidene-3-(2-phenyl 4-oxo-1,3-thiazolidine) succinimido aceto hydrazide, antimicrobial activity.

INTRODUCTION
Heterocyclic synthesis has emerged as a powerful technique for generating new molecules useful for drug discovery. Heterocyclic compounds provided scaffolds on which pharmacophore can arrange to yield potent and selective drugs. Heterocyclic compounds containing sulphur and nitrogen atoms represent a very important group of organic compounds, which exhibit significant biological activity and show various pharmacological effects.

These classes of compounds are known as ‘Thiazolidines’, which contain both ‘thio’ (sulphur) and ‘azo’ (nitrogen) atoms in a cyclic 5 membered ring. Along with these two heteroatoms, a ‘ketone’ group was introduced to form a novel ring system called ‘Thiazolidinone’, which has given a big blow to the bacterial and fungal resistance by many of the drugs and antibiotics. In the recent past years, attention has been focused on synthesis of substituted heterocycles and their analogs, due to their increasing medicinal importance. The presence of linkage N-C-S in thiazolidinone is believed to account for antifungal activity and better chemotherapeutic agent against awful threats.

The historical importance of thiazolidinones was emphasized during the period 1941-45, when work on the structure of penicillins showed the presence of thiazolidine ring in it. Compounds carrying the thiazolidinone ring have been reported to demonstrate wide range of pharmacological activities, like antibacterial, antifungal, anti-inflammatory, analgesic, antitubercular, anticonvulsant, antihistaminic, anaesthetic, antithyroid, antiparkinsonism, anticancer, antimalarial, etc.

We synthesized some newer 5-Arylidene-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide Derivatives(5a-5j) from succinimide by using different aldehydes. The synthesized compounds have shown satisfactory spectral data which are in conformity of the proposed structures. All the synthesized compounds were screened for antimicrobial activity.

MATERIALS AND METHODS
The chemicals and reagents used in the present project were of AR and LR grade, procured from Aldrich, Hi-Media, Merck, Sigma and Ranbaxy. Melting points of the synthesized compounds were determined by open capillary method and were uncorrected. IR spectral analysis was carried out using FTIR–8400S, SHIMADZU. 1HNMR spectral analysis were carried out using instrument amx-400 and the solvent used was deuterated chloroform and dimethyl sulfoxide. The mass spectral data were recorded from LCMS 2010A, SHIMADZU.

METHODOLOGY
Step 1: Synthesis of Ethyl N-succinimido acetate (I): Equimolar mixture of succinimide (0.05 mol, 4.95 g) & ethylchloroacetate (0.05 mol, 6.1 ml) was taken in 500 ml round bottom flask. To this mixture dry acetone (70 ml) and ethanol (40 ml) were added. The reaction mixture was refluxed in presence of
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anhydrous K$_2$CO$_3$ for about 9 hours in water bath. Then cooled & poured the mixture into ice-cold water where the ester of N-succinimidoethylacetate gets precipitated. Product was filtered and dried in oven at 125 °C.

**Step 2: Synthesis of N-Succinimido acetylhydrazide (2):** Equimolar mixture of ethyl N-succinimidoacetate (0.05 mol, 9.25 g) & hydrazine hydrate (0.05 mol, 1.6 ml) was taken in round bottom flask. To this reaction mixture, 1, 4-dioxan (50 ml) was added and refluxed about 5 hrs maintaining the temperature 60-70°C. Soft solid mass had appeared which was filtered, dried and recrystallized with ethanol.
Step-3 Synthesis of phenyl succinimido acetylhydrazide (3): N-Succinimido acetylhydrazide (0.05 mol, 8.55 g) taken in round bottom flask along with 30 ml ethanol. Benzaldehyde (0.05 mol, 5.3 ml) was dissolved in 30 ml ethanol and added slowly for about 20 min with vigorous stirring, maintaining the temperature at 40-50°C. Added four drops of glacial acetic acid and allowed to reflux for further 3 hrs. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystallized from ethanol.

Step-4 Synthesis of 3-(2-phenyl 4-oxo-1, 3-thiazolidine) acetamido succinimide (4): Phenyl succinimido acetylhydrazide (0.02 mol, 5.18 g) was taken in round bottom flask and mercaptoacetic acid (0.02 mol, 1.84 ml) was added. To this mixture a pinch of anhydrous zinc chloride (0.02 mol, 1.84 ml) was added. This mixture was refluxed for about 10 hrs in a heating mantle and allowed to cool, filtered and dried. The product was recrystallised from chloroform.

Synthesis of 5-benzylidene-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5a): Equimolar 3-(2-Phenyl 4-oxo-1,3-thiazolidine) acetamido succinimide (0.05 mol 15.05 gm) & Benzaldehyde (0.05 mol 5.3 ml) were taken in round bottom flask along with 50 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 8 hours. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystalized from ethanol.

Synthesis of 5-(2-hydroxybenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidine) succinimido aceto hydrazide (5c): Equimolar 3-(2-Phenyl 4-oxo-1, 3-thiazolidine) acetamido succinimide (0.05 mol 15.05 gm) & 2-hydroxybenzaldehyde (0.05 mol 6.1 gm) were taken in round bottom flask along with 60 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 10 hours. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystalized from ethanol.

Synthesis of 5-(3-methoxybenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5d): Equimolar 3-(2-Phenyl 4-oxo-1, 3-thiazolidine) acetamido succinimide (0.05 mol 15.05 gm) & 3-methoxybenzaldehyde (0.05 mol 6.8 gm) were taken in round bottom flask along with 60 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 9 hours. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystalized from ethanol.

Synthesis of 5-(4-chlorobenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5e): Equimolar 3-(2-Phenyl 4-oxo-1, 3-thiazolidine) acetamido succinimide (0.05 mol 15.05 gm) & 4-chlorobenzaldehyde (0.05 mol 7.025 gm) were taken in round bottom flask along with 55 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 8 hours. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystalized from ethanol.

Synthesis of 5-(3,4,5-trimethoxybenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5f): Equimolar 3-(2-Phenyl 4-oxo-1,3-thiazolidine) acetamido succinimide (0.05 mol 15.05 gm) & 3,4,5-trimethoxybenzaldehyde (0.05 mol 9.8 gm) were taken in round bottom flask along with 60 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 9 hours. Poured the mixture into 250 ml ice-cold water.
and stirred. Precipitate obtained was filtered, dried and recrystallized from ethanol.

**Synthesis of 5-(2,4-dichlorobenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidine) succinimido aceto hydrazide (5g):** Equimolar 3-(2-Phenyl 4-oxo-1,3-thiazolidine) acetamido succinimide (0.05 mol 15.05gm) & 2,4-dichlorobenzaldehyde (0.05 mol 8.75gm) were taken in round bottom flask along with 55 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 8 hours. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystallized from ethanol.

**Synthesis of 5-(4-N, N-dimethylaminobenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5h):** Equimolar 3-(2-Phenyl 4-oxo-1,3-thiazolidine) acetamido succinimide (0.05 mol 15.05gm) & 4-N,N-dimethylaminobenzaldehyde (0.05 mol 7.45gm) were taken in round bottom flask along with 50 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 8 hours. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystallized from ethanol.

**Synthesis of 5-(3-methoxy-4-hydroxybenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidine) succinimido aceto hydrazide (5i):** Equimolar 3-(2-Phenyl 4-oxo-1,3-thiazolidine) acetamido succinimide (0.05 mol 15.05gm) & 3-methoxy-4-hydroxybenzaldehyde (0.05 mol 7.6gm) were taken in round bottom flask along with 50 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 8 hours. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystallized from ethanol.

**Synthesis of 5-(3,4-dimethoxybenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidine) succinimido aceto hydrazide (5j):** Equimolar 3-(2-Phenyl 4-oxo-1,3-thiazolidine) acetamido succinimide (0.05 mol 15.05gm) & 3,4-dimethoxybenzaldehyde (0.05 mol 8.3gm) were taken in round bottom flask along with 60 ml dioxane in presence of four drops of sodium ethoxide and allowed to reflux for approximate 9 hours. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystallized from ethanol.

**Antibacterial activity:** Antibacterial activity of the synthesized compounds was determined by the cup-plate method against the gram-positive organisms *Staphylococcus aureus*, *Bacillus subtilis* and gram-negative organisms *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella* at 100µg/ml concentration. The bacteria were subcultured on Nutrient Agar medium. The petridishes were incubated at 37°C for 24hr. Ampicillin (10 mcg/disc) (Std.1) and Ciprofloxacin (30mcg/disc) (Std.2) were used as standards. The results are presented in Table 2.

**Antifungal activity:** The antifungal activity of the synthesized compounds was carried out against the fungi *Candida albicans* and *Aspergillus niger* at 100µg/ml concentration. The fungi were subcultured in Sabouraud Dextrose Agar medium. The fungal susceptibility testing was done by cup-plate method using Fluconazole (10 mcg/disc) (Std.1), Amphotericin B (100 units/disc) (Std.2) and Clotrimazole (100 mcg/disc) as std.3. The petridishes were incubated for 48hr at 25°C. The results are presented in Table 2.

**RESULTS AND DISCUSSION**

**Ethyl N-succinimido acetate (1):** (m.p. 135°C), IR (KBr), CM<sup>1</sup>: 2862 (-CH₂-C str), 1622 (-CO-N-CO), 1276 (O=C-COOC₂H₅), 1210 (-N-CH₂-)

**N-Succinimido acetylhydrazide (2):** (m.p. 158°C), IR (KBr), CM<sup>1</sup>: 3163 (NH-NH₂ str), 2858 (-CH₂-C str), 1658 (CONH amide), 1625 (-CO-N-CO), 1276 (C=O), 1230 (-N-CH₂- str)

**Phenyl succinimido acetylhydrazide (3):** (m.p. 146°C), IR (KBr), CM<sup>1</sup>: 3163 (-NH- str), 2887 (-CH₂-C), 1664 (CONH amide), 1623 (CH=NH str), 1600 (-CO-N-CO), 1210 (-N-CH₂- Ar str); 1H NMR (CDCl₃): δ 11.5 (1H, N=CH), δ 8.7 (1H, CONH amide), δ 7.51-7.8 (6H, Ar-H), δ 2.5 (2H, -CO-CH₂-); MS: (m/z): 260 (M+1), 259 M<sup>+</sup> and other peaks are 245, 189, 171 & 91

**3-(2-Phenyl 4-oxo-1,3-thiazolidine) acetamido succinimide (4):** (m.p. 77°C), IR (KBr), CM<sup>1</sup>: 3360 (-NH- str), 2879 (-N-CH₂-S), 1720 (C=O), 1685 (CONH), 1600 (-CO-N-CO ), 1552 (C=C- Ar str), 690 (-CH₂-S-CH); 1H NMR (CDCl₃): δ 8.6 (1H, CONH), δ 7.1-7.8 (6H, Ar-H), δ 6.1 (1H, N-CH₂-Ar), δ 3.6-3.7 (2H, S-CH₂), δ 3.8-3.9 (2H, N-CH₂), δ 1.2 (2H, CO-CH₂-); MS: (m/z): 333 (M<sup>+</sup>) & other peaks are 208,155,111,93.

**5-benzylidene-3-(2-phenyl 4-oxo-1,3-thiazolidine) succinimido aceto hydrazide (5a):** (m.p. 154°C), IR (KBr), CM<sup>1</sup>: 2896 (-N-CH₂-S str), 1718 (C=O cyclic), 1660 (CONH), 1635 (C=C=CH, benzylidene), 1556 (-C=C- Ar str), 684 (-CH₂-S-CH); 1H NMR (CDCl₃): δ 8.68 (1H, CONH), δ 6.9-7.3 (12H, Ar-H), δ 3.4 (2H, S-CH₂), δ 1.2 (2H, CO-CH₂-); MS: (m/z): 421 M<sup>+</sup> & other peaks are 364, 341, 325, 94.
5-(2-Nitrobenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidin-2-yl) Succinimido aceto hydrazide (5b): (m.p. 169°C), IR (KBr), CM^1: 2887 (-N=CH-S str), 1682 (C=O cyclic), 1600 (-CO-N-CO str.), 1558 (-C=C- Ar str), 1328 (NO2-C Ar str), 684 (-CH2-S-CH) 5-(2-Hydroxybenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidin-2-yl) Succinimido aceto hydrazide (5c): (m.p. 142°C), IR (KBr), CM^1: 3200 (C-OH Ar str), 2868 (-N=CH2-S), 1732 (C=O cyclic), 1604 (-CO-N-CO), 1642 (CONH), 1542 (-C=C- Ar str), 678 (-CH2-S-CH); 1H NMR (CDCl3): δ 8.68 (1H, CONH amide), δ 8.4 (1H, OH-Ar), δ 7.7 (3H, Ar-H), δ 3.4 (2H, S-CH2), δ 1.2 (2H, CO-CH2-); MS: (m/z): 438 (M+1), 437 (M^+), 381, 197. 5-(3-Methoxybenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidi-2-yl) Succinimido aceto hydrazide (5d): (m.p. 161°C), IR (KBr), CM^1: 2935 (-N=CH2-S str), 1770 (C=O cyclic), 1622 (-CONH), 1604 (-CO-N-CO), 1573 (-C=C- Ar str), 1245 (-N=CH2-), 692 (-CH2-S-CH) 5-(4-Chlorobenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidin-2-yl) Succinimido aceto hydrazide (5e): (m.p. 174°C), IR (KBr), CM^1: 2877 (-N=CH2-S str), 1718 (C=O cyclic), 1604 (-CO-N-CO), 1544 (-C=C- Ar str), 1093 (Cl-C Ar str), 688 (-CH2-S-CH); 1H NMR (CDCl3): δ 8.6 (1H, CONH amide), 7.5 (2H, Cl near Ar-H), 7.2-7.7 (8H, Ar-H), 3.53 (2H, S-CH2), 3.4 (2H, N=CH2), 1.58-1.6 (2H, CO-CH2-); MS: (m/z): 456 (M+1), 419, 399, 94. 5-(3,4,5-Trimethoxybenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidin-2-yl) Succinimido aceto hydrazide (5f): (m.p. 171°C), IR (KBr), CM^1: 2839 (-N=CH2-S str), 1710 (C=O cyclic), 1619 (-CO-N-CO), 1579 (-C=C- Ar str), 1233 (-N=CH2-), 690 (-CH2-S-CH) 5-(2,4-Dichlorobenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidin-2-yl) Succinimido aceto hydrazide (5g): (m.p. 152°C), IR (KBr), CM^1: 2923 (-N=CH2-S), 1680 (C=O), 1583 (-CO-N-CO), 1220 (-N=CH2-), 1103 (-C=C-Cl Ar str), 684 (-CH2-S-CH) 5-(4-N,N-dimethylaminobenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidin-2-yl) Succinimido aceto hydrazide (5h): (m.p. 207°C), IR (KBr), CM^1: 3164 (-N=CH2- Ar str), 2896 (-N=CH2-S), 1660 (CONH), 1600 (-CO-N-CO), 1554 (-C=C- Ar str), 688 (-CH2-S-CH) 5-(3-Methoxy-4-hydroxybenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidin-2-yl) Succinimido aceto hydrazide (5i): (m.p. 186°C), IR (KBr), CM^1: 3342 (OH-C Ar str), 2879 (-N=CH2-S str), 1720 (C=O cyclic), 1685 (CONH), 1600 (-CO-N-CO), 1546 (-C=C- Ar str), 688 (-CH2-S-CH) 5-(3, 4-Dimethoxybenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidin-2-yl) Succinimido aceto hydrazide (5j): (m.p. 182°C), IR (KBr), CM^1: 2880 (-N=CH2-S str), 1720 (C=O cyclic), 1610 (-CO-N-CO), 1480 (-C=C- Ar str), 692 (-CH2-S-CH) 

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<th>Compound Code</th>
<th>Ar</th>
<th>Mol. Formula</th>
<th>% Yield</th>
<th>Rf*</th>
<th>M.P.</th>
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<td>5a</td>
<td>Benzaldehyde</td>
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<td>2-Nitrobenzaldehyde</td>
<td>C6H4OxN=S</td>
<td>74%</td>
<td>0.53</td>
<td>169°C</td>
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<td>5c</td>
<td>2-Hydroxybenzaldehyde</td>
<td>C6H4OxN=S</td>
<td>61.80%</td>
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<td>142°C</td>
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<tr>
<td>5d</td>
<td>3-Methoxybenzaldehyde</td>
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<td>51.5%</td>
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<td>4-Chlorobenzaldehyde</td>
<td>C6H4OxN=Cl</td>
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<td>0.61</td>
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<td>5f</td>
<td>3,4,5-Trimethoxybenzaldehyde</td>
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<td>58.90%</td>
<td>0.66</td>
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<tr>
<td>5g</td>
<td>2,4-Dichlorobenzaldehyde</td>
<td>C6H4OxN=Cl2</td>
<td>64.10%</td>
<td>0.68</td>
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<td>5h</td>
<td>4-N,N-Dimethylaminobenzaldehyde</td>
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<td>71.50%</td>
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<td>3-Methoxy-4-Hydroxybenzaldehyde</td>
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<td>60.20%</td>
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<td>182°C</td>
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*Stationary Phase: Silica Gel G  
Mobile Phase: Chloroform: Acetone : : 9:1

**Antibacterial activity:**  
Most of the compounds exhibited mild to moderate antibacterial activity against all the microbes (*S.aureus, B.subtilis, E.coli, P.aeruginosa, Shigella*) tested. All the compounds have shown antibacterial activity as indicated by the diameter of zone of inhibition (Table-2). Among them compound 5g was found to possess highest activity against Gram positive and 5e against Gram negative organism compared to other derivatives.

Antifungal activity:
The antifungal activity of the compounds was determined against two fungal species. Most of the compounds showed reasonable antifungal activity against both the strains (C. albicans, A. niger) tested.

Table 2: Biological Activity Data of the Synthesized Compounds

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<th>Compound Code</th>
<th>Zone of Inhibition (in mm)</th>
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<td>B.subtilis</td>
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<td>5a</td>
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<td>5b</td>
<td>11</td>
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<tr>
<td>Std 3</td>
<td>--</td>
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<tr>
<td>Control</td>
<td>NI</td>
</tr>
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Note: Average zone diameter in mm of triplicates
NI: No inhibition
Control: DMSO

CONCLUSION
The derivatives of 5-(4-Chlorobenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5e) and 5-(2,4-Dichlorobenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5g) show potent antimicrobial activity. With these encouraging results, all the synthesized compounds can be further explored for detailed microbiological and pharmacological investigation to arrive at possible newer potent drugs.

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