

Synthesis and Antimicrobial Activity of Novel 5-Arylidene-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto 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) acetamido 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 threats¹.

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, analgesic², antitubercular³, anticonvulsant⁴, antihistaminic, anaesthetic, antithyroid, antiparkinsonism, anticancer, antimalarial, etc⁵.

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 activity⁶⁻⁹.

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.

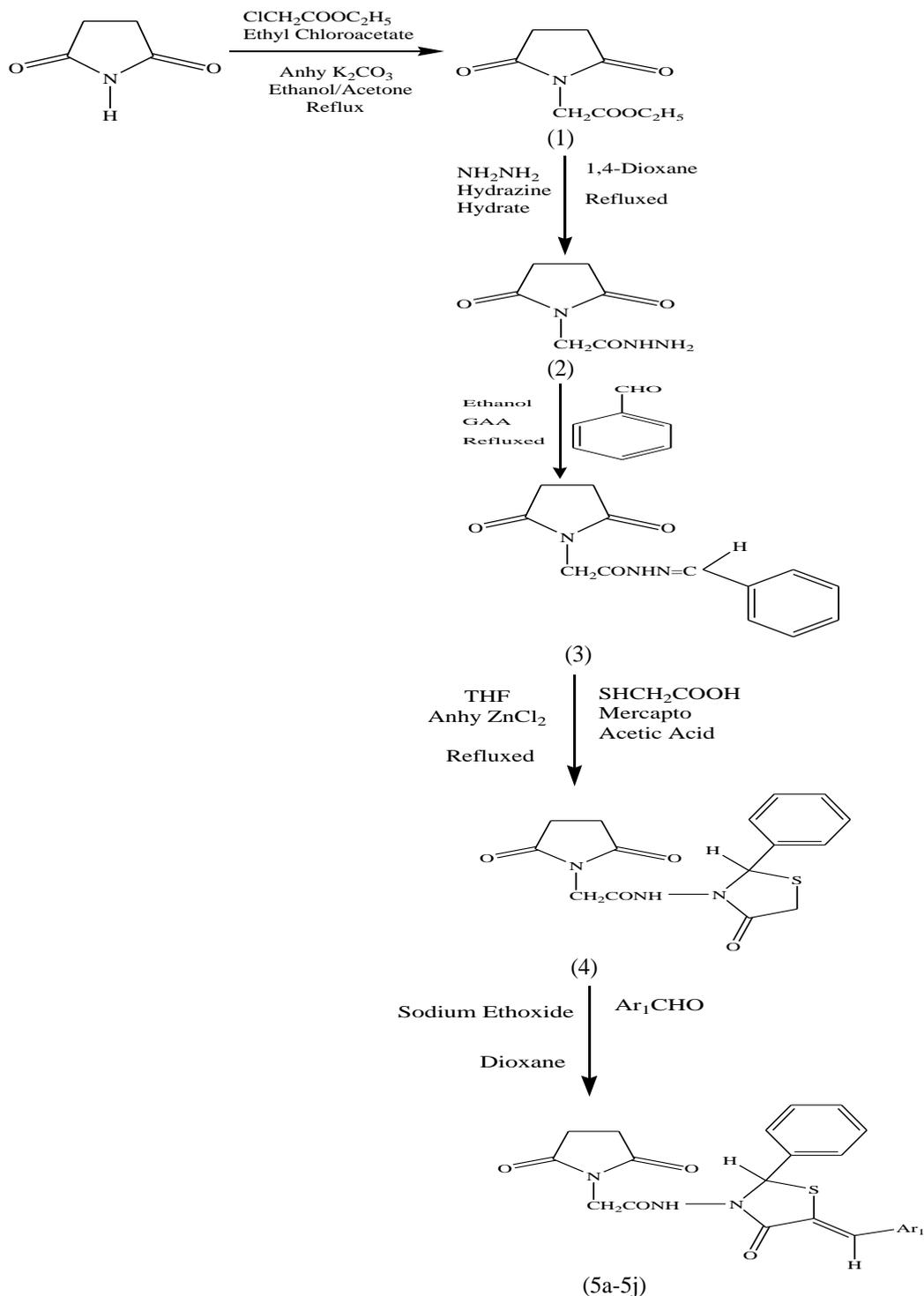
METHODOLOGY

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

anhydrous K_2CO_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 $125^\circ C$.

Step 2: Synthesis of N-Succinimido acetylhydrazide (2): Equimolar mixture of ethyl N-succinimido

acetate (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-dioxane (50 ml) was added and reflux about 5 hrs maintaining the temperature $60-70^\circ C$. Soft solid mass had appeared which was filtered, dried and recrystallized with ethanol.



Compound	Ar₁
5a	benzaldehyde
5b	2-nitrobenzaldehyde
5c	2-hydroxybenzaldehyde
5d	3-methoxybenzaldehyde
5e	4-chlorobenzaldehyde
5f	3,4,5-Trimethoxybenzaldehyde
5g	2,4-dichlorobenzaldehyde
5h	4-N,N-dimethylaminobenzaldehyde
5i	3-methoxy-4-hydroxybenzaldehyde
5j	3,4-dimethoxybenzaldehyde

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, 5.3ml) 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 and THF (60 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 15.05gm) & Benzaldehyde (0.05 mol 5.3ml) 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-(2-nitrobenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5b): Equimolar 3-(2-Phenyl 4-oxo-1, 3-thiazolidine) acetamido succinimide (0.05 mol 15.05gm) & 2-nitrobenzaldehyde (0.05 mol 7.55gm) 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 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-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 15.05gm) & 2-hydroxybenzaldehyde (0.05 mol 6.1gm) 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 recrystallized 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 15.05gm) & 3-methoxybenzaldehyde (0.05 mol 6.8gm) 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-(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 15.05gm) & 4-chlorobenzaldehyde (0.05 mol 7.025gm) 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-(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 15.05gm) & 3,4,5-trimethoxybenzaldehyde (0.05 mol 9.8gm) 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^{-1} :** 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^{-1} :** 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^{-1} :** 3163 (-NH- str), 2887 (-CH₂-C), 1664 (CONH amide), 1623 (CH=N 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⁺ 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^{-1} :** 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-CH-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⁺) & 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^{-1} :** 2896 (-N-CH₂-S str), 1718 (C=O cyclic), 1660 (CONH), 1635 (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⁺ & other peaks are 364, 341, 325, 94.

5-(2-Nitrobenzylidene)-3-(2-phenyl 4-oxo-1,3-thiazolidine) succinimido aceto hydrazide (5b): (m.p. 169°C), **IR (KBr), CM^{-1} :** 2887 (-N-CH₂-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-thiazolidine) 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 7-7.3 (8H, 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-thiazolidine) 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 (-NCH₂-), 692 (-CH₂-S-CH)

5-(4-Chlorobenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) 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-thiazolidine) 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 (-NCH₂- str), 690 (-CH₂-S-CH)

5-(2,4-Dichlorobenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5g): (m.p. 152°C), **IR (KBr), CM^{-1} :** 2923 (-N-CH₂-S), 1680 (C=O), 1583 (-CO-N-CO), 1220 (-NCH₂-), 1103 (-C-C-Cl Ar str), 684 (-CH₂-S-CH)

5-(4-N,N-dimethylaminobenzylidene)-3-(2-phenyl 4-oxo-1, 3-thiazolidine) succinimido aceto hydrazide (5h): (m.p. 207°C), **IR (KBr), CM^{-1} :** 3164 (-N-(CH₃)₂ 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-thiazolidine) 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-thiazolidine) 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)

Table 1: Physical Data of the Synthesized Compounds

Compound Code	Ar ₁	Mol. Formula	% Yield	R _f *	M.P.
5a	Benzaldehyde	C ₂₂ H ₁₉ O ₄ N ₃ S	62.50 %	0.47	154°C
5b	2-Nitrobenzaldehyde	C ₂₂ H ₁₈ O ₆ N ₄ S	74 %	0.53	169°C
5c	2-Hydroxybenzaldehyde	C ₂₂ H ₁₉ O ₅ N ₃ S	61.80 %	0.49	142°C
5d	3-Methoxybenzaldehyde	C ₂₃ H ₂₁ O ₅ N ₃ S	51.5%	0.39	161°C
5e	4-Chlorobenzaldehyde	C ₂₂ H ₁₈ O ₄ N ₃ Cl	63.10%	0.61	174°C
5f	3,4,5-Trimethoxybenzaldehyde	C ₂₅ H ₂₅ O ₇ N ₃ S	58.90%	0.66	171°C
5g	2,4-Dichlorobenzaldehyde	C ₂₂ H ₁₇ O ₄ N ₃ Cl ₂	64.10%	0.68	152°C
5h	4-N,N-dimethylaminobenzaldehyde	C ₂₄ H ₂₄ O ₄ N ₄ S	71.50%	0.74	207°C
5i	3-methoxy-4-Hydroxybenzaldehyde	C ₂₃ H ₂₁ O ₆ N ₃ S	60.20%	0.70	186°C
5j	3,4-Dimethoxybenzaldehyde	C ₂₄ H ₂₃ O ₆ N ₃ S	46.45%	0.51	182°C

*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

Compound Code	Zone of Inhibition (in mm)						
	B.subtilis	S.aureus	E.coli	P.aeruginosa	Shigella	C.albicans	A.niger
5a	12	13	16	14	11	18	10
5b	11	15	21	13	11	13	11
5c	14	13	18	12	13	9	11
5d	14	14	15	10	8	12	13
5e	13	14	20	19	12	13	8
5f	10	15	16	15	12	10	12
5g	17	18	15	19	13	15	9
5h	10	13	15	10	10	10	11
5i	15	16	19	18	12	8	10
5j	12	14	15	12	10	9	12
Std 1	3	4	NI	9	6	23	17
Std 2	33	37	30	27	40	31	8
Std 3	--	--	--	--	--	18	23
Control	NI	NI	NI	NI	NI	NI	NI

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 result, 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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