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SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF NOVEL N-{3-CHLORO-2-(SUBSTITUTEDPHENYL)-4-OXAZETIDIN-1-YL} ACETAMIDO SUCCINIMIDE DERIVATIVES

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ABSTRACT

N-{3-Chloro-2-(substitutedphenyl)-4-oxazetidin-1-yl} acetamido succinimide**(4a-4j)** were synthesized by cyclization of substituted phenyl succinimido acetylhydrazide**(3a-3j)** and triethylamine by dissolving in dioxane, when chloroacetyl chloride was added dropwise. Substituted phenyl succinimido acetylhydrazide**(3a-3j)** were synthesized by condensation of various substituted aromatic aldehydes with N-succinimido acetylhydrazide**(2)**, which was synthesized by equimolar mixture of ethyl N-succinimido acetate**(1)** and hydrazine hydrate by refluxing in presence of 1,4-dioxane. Ethyl N-succinimido acetate**(1)** was synthesized by equimolar condensation of succinimide and ethyl chloroacetate by refluxing in presence of anhydrous potassium carbonate. The formation of title compounds were established by physical and spectral studies. Further the synthesized compounds were subjected to microbiological screening.

KEYWORDS: Azetidinone derivatives, oxazetidine derivatives, antimicrobial activity.

INTRODUCTION

The discipline of medicinal chemistry is devoted to the discovery and development of new agents for treating diseases. Most of this activity is directed to the new natural or synthetic organic compounds. To provide an understanding of the principle of medicinal chemistry, it is necessary to consider the physicochemical properties used to develop new pharmacologically active compounds and their mechanism of action, the drug metabolism

including possible biological activities of the metabolites, the importance of stereochemistry in drug design and the methods used to determine what space a drug occupies.[1]

A large number of medicinal compounds which have been discovered belong to a major class of heterocycles containing nitrogen and/or sulphur. The versatile synthetic applicability and biological activity of these heterocycles has helped the medicinal chemist to plan, organize and implement new approaches towards discovery of novel drugs.

Azetidinone and Thiazolidinone derivatives were reported to possess antibacterial, antifungal, antituberculor activity, anti-HIV, analgesic, anti inflammatory and ulcerogenic activity. Benzimidazole derivatives were reported to possess antibacterial, antifungal, anti-inflammatory, antiviral, antitumor, antioxidant and antihelmintic activities.[2]

A large number of azetidinone containing β-lactam rings and 4-thiazolidinone are known to exhibit various biological activities like antifungal and antibacterial activities. More particularly and recently these types of compounds have been found in the treatment of Tuberculosis and other chemotherapeutic diseases.[3] We synthesized some newer N-{3-chloro-2-(substitutedphenyl)-4-oxazetidin-1-yl} acetamido succinimide derivatives(4a-4j)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.[4,5,6,7,8]

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, ^{1}HNMR 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.

METHODOLGY

Step 1: Synthesis of Ethyl N-succinimido acetate (1)

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˚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-dioxan (50 ml) was added and reflux about 5 hrs maintaining the temperature 60-70˚C. Soft solid mass had appeared which was filtered, dried and recrystalized with ethanol.

Step-3: General method of synthesis of substituted phenyl succinimido acetylhydrazide (3a-3j)

N-Succinimido acetylhydrazide (0.05 mol, 8.55 g) taken in round bottom flask along with 30 ml ethanol. Various aromatic aldehydes (0.05 mol) were 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 3-4 hrs. Poured the mixture into 250 ml ice-cold water and stirred. Precipitate obtained was filtered, dried and recrystalized from ethanol.

Step-4: General method of synthesis of N-{3-chloro-2-(substitutedphenyl)-4-oxazetidin-1-yl} acetamido succinimide (4a-4j)

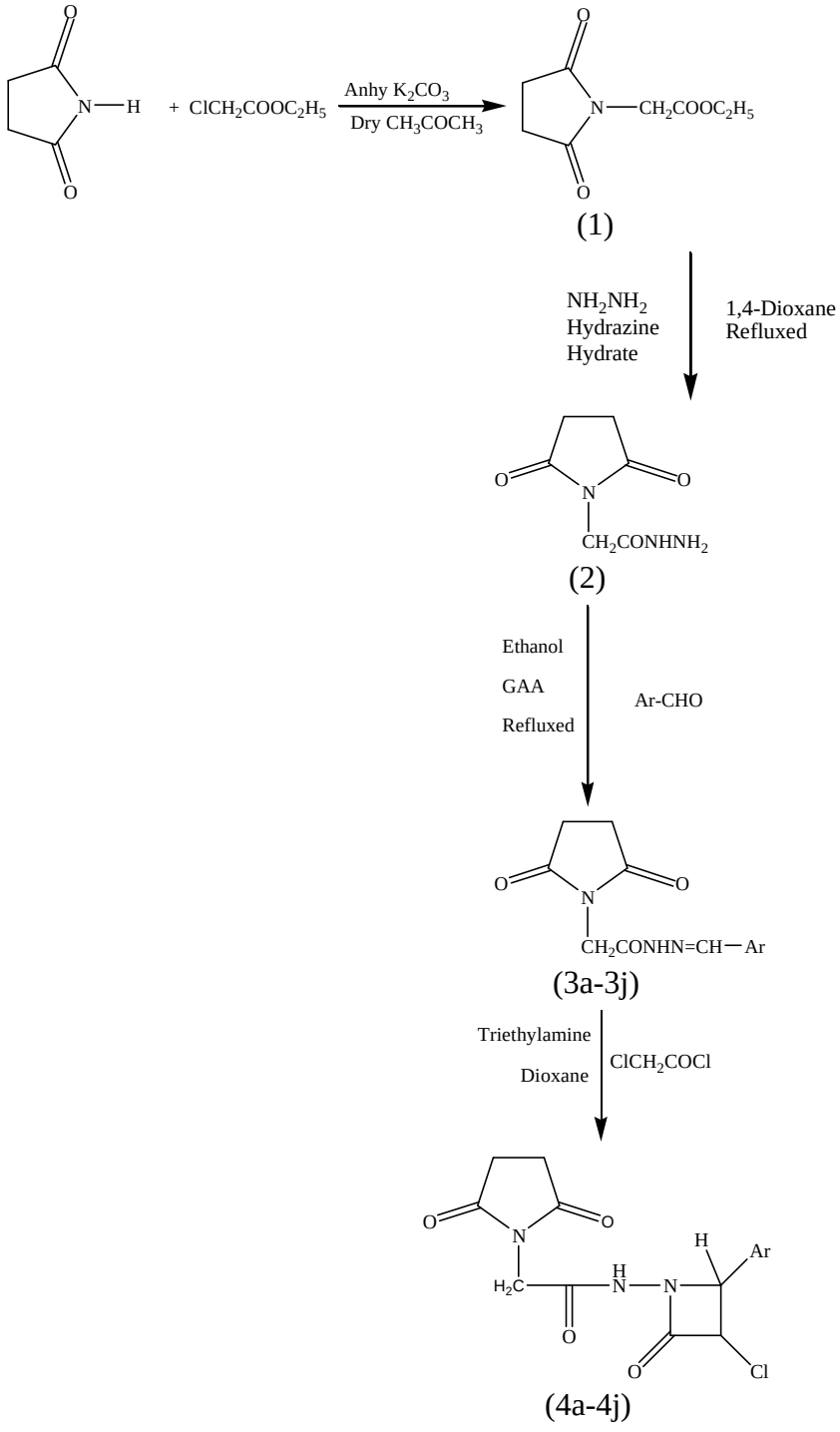
A mixture of substituted phenyl succinimido acetylhydrazide (0.02mol) and triethylamine (0.02mol, 2.02ml) was dissolved in dioxane (50ml), cooled and stirred. To this well stirred cooled solution chloroacetyl chlroride (0.025mol, 2.83ml) was added dropwise within a period of 20minutes. The reaction mixture was then stirred for an additional 3hrs and then reflux for 4-6hrs. The reaction mixture was cooled and excess of solvent distilled off and the residue was poured into ice-cold water. A solid obtained was filtered and which was recrystalized from ethanol.

Ethyl N-succinimido acetate (1): (m.p. 135°C), **IR (KBr), CM^{-1}:** 2862 (-CH_2-C str), 1622 (-CO-N-CO), 1276 (O=C-$COOC_2H_5$), 1210 (-N-CH_2-).

N-Succinimido acetylhydrazide (2): (m.p. 158°C), **IR (KBr), CM^{-1}:** 3163 (NH-NH_2 str), 2858 (-CH_2-C str), 1658 (CONH amide), 1625 (-CO-N-CO), 1276 (C=O), 1230 (-N-CH_2-str).

Phenyl succinimido acetylhydrazide (3a): (m.p. 146°C), **IR (KBr), CM^{-1}:** 3163 (-NH- str), 2887 (-CH_2-C), 1664 (CONH amide), 1623 (CH=N str), 1600 (-CO-N-CO), 1210 (-N-CH_2- Ar str); **1H NMR ($CDCl_3$)**: 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-CH_2-); **MS: (m/z):** 260 (M+1), 259 M^+ and other peaks are 245, 189, 171 & 91.

Annexure-1



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

2-Nitrophenyl succinimido acetylhydrazide (3b): (m.p. 215˚C), **IR (KBr), CM^{-1}:** 3095, 3043 (Ar CH str), 1650, 1635 (C=O str), 1600 (-CO-N-CO), 1340 (NO_2-C Ar str), 846 (C-N str).

2-Hydroxyphenyl succinimido acetylhydrazide (3c): (m.p. 113˚C), **IR (KBr), CM^{-1}:** 3199 (O-H str), 3004 (Ar CH str), 1681 (C=O str), 1612 (-CO-N-CO), 1554 (-C=C- Ar str).

3-Methoxyphenyl succinimido acetylhydrazide (3d): (m.p. 184˚C), **IR (KBr), CM^{-1}:** 3062 (Ar CH str), 2898 (Alkyl C-H str.), 1663 (C=O str), 1592 (-CO-N-CO), 1552 (-C=C- Ar str), 1260 (-NCH_2 -).

4-Chlorophenyl succinimido acetylhydrazide (3e): (m.p. 135˚C), **IR (KBr), CM^{-1}:** 3097 (Ar CH str), 2993 (Alkyl C-H str.), 1676 (C=O str), 1629 (-CO-N-CO), 1535 (-C=C- Ar str), 1041 (C-Cl).

3,4,5-Trimethoxyphenyl succinimido acetylhydrazide (3f): (m.p. 142˚C), **IR (KBr),CM^{-1}:** 3062, 3010 (Ar CH str), 2943 (Alkyl C-H str.), 1683 (C=O str), 1587 (-CO-N-CO), 1542 (-C=C- Ar str), 1234 (-NCH_2 -).

2,4-Dichlorophenyl succinimido acetylhydrazide (3g): (m.p. 132˚C), **IR (KBr), CM^{-1}:** 3053 (Ar CH str), 2995, 2960 (Alkyl C-H str.), 1703, 1662 (C=O str), 1591 (-CO-N-CO), 1542 (-C=C- Ar str), 1099 (C-Cl).

4,N,N-Dimethylaminophenyl succinimido acetylhydrazide (3h): (m.p. 200˚C), **IR (KBr), CM^{-1}:** 3172 {-N-$(CH_3)_2$ Ar str.}, 3026 (Ar CH str), 1654 (C=O str), 1579 (-CO-N-CO), 1556 (-C=C- Ar str).

3-Methoxy-4-hydroxyphenyl succinimido acetylhydrazide (3i): (m.p. 145˚C), **IR (KBr), CM^{-1}:** 3232 (OH-C Ar str.), 3060 (Ar CH str), 2983, 2948 (Alkyl C-H str.), 1663 (C=O str), 1592 (-CO-N-CO), 1552 (-C=C- Ar str), 1282 (-NCH_2 -).

3,4-Dimethoxyphenyl succinimido acetylhydrazide (3j): (m.p. 155˚C), **IR (KBr), CM^{-1}:** 3062 (Ar CH str), 2898 (Alkyl C-H str.), 1663 (C=O str), 1592 (-CO-N-CO), 1552 (-C=C- Ar str), 1260 (-NCH_2 -).

N-{3-Chloro-2-phenyl-4-oxazetidin-1-yl} acetamido succinimide (4a): (m.p. 207˚C), **IR (KBr), CM^{-1}:** 3060 (Ar CH str), 1670 (C=O str), 1585 (-CO-N-CO), 1550 (-C=C- Ar str), 1298 (-NCH_2 -), 754 (-C-Cl str.); **1H NMR ($CDCl_3$)**: δ 8.6 (1H, CONH), δ 7.1-7.8 (5H, Ar-H), δ 6.1 (1H, N-CH-Ar), δ 3.6 (1H, CH-Cl), δ 3.8-3.9 (2H, N-CH_2) , δ 1.2 (4H, CO-CH_2-); **MS: (m/z):** 336, 337 (M+1), 181, 91.

N-{3-Chloro-2-(2-nitrophenyl)-4-oxazetidin-1-yl} acetamido succinimide (4b): (m.p. 185˚C), **IR (KBr), CM^{-1}:** 3083, 3053 (Ar CH str), 1701, 1685 (C=O str), 1575 (-CO-N-CO), 1560 (-C=C- Ar str), 1417 (Ar C-NO_2 str.), 1296 (-NCH_2 -), 837 (-C-Cl str.).

N-{3-Chloro-2-(2-hydroxyphenyl)-4-oxazetidin-1-yl} acetamido succinimide (4c): (m.p. 156˚C), **IR (KBr), CM^{-1}:** 3045 (Ar CH str), 1622 (C=O str), 1571 (-CO-N-CO), 1271 (CO str. of OH), 748 (-C-Cl str.); **1H NMR ($CDCl_3$)**: δ 8.78 (1H, CONH), δ 8.71 (1H, Ar-OH), δ 7.4-7.9 (4H, Ar-H), δ 7.4 (1H, N-CH-Ar), δ 3.2 (2H, N-CH_2), δ 2.8 (1H, CH-Cl) , δ 1.3 (4H, CO-CH_2-); **MS: (m/z):** 351, 354(M+2), 198, 157, 91, 79.

N-{3-Chloro-2-(3-methoxyphenyl)-4-oxazetidin-1-yl} acetamido succinimide (4d): (m.p. 213˚C), **IR (KBr), CM^{-1}:** 2945 (Ar CH str), 1681 (C=O str), 1600 (-CO-N-CO), 1556 (-C=C- Ar str),1286 (-NCH_2 -), 1261 (C-O of arylalkylether), 784 (-C-Cl str.).

N-{3-Chloro-2-(4-chlorophenyl)-4-oxazetidin-1-yl} acetamido succinimide (4e): (m.p. 201˚C), **IR (KBr), CM^{-1}:** 1701 (C=O str), 1602 (-CO-N-CO), 1527 (-C=C- Ar str), 1348 (-NCH_2 -), 784, 740 (-C-Cl str.); **1H NMR ($CDCl_3$)**: δ 8.1-8.6 (1H, CONH), δ 7.8-7.9 (2H, Ar-

H), δ 7.6-7.7 (2H, Cl near to Ar-H), δ 7.2-7.6 (1H, N-CH-Ar), δ 3.3 (2H, N-CH_2), δ 2.5 (1H, CH-Cl) , δ 1.2 (4H, CO-CH_2-); **MS: (m/z):** 370, 372 (M+2), 214, 91, 57.

N-{3-Chloro-2-(3,4,5-trimethoxyphenyl)-4-oxazetidin-1-yl} acetamido succinimide (4f): (m.p. 175˚C), **IR (KBr), CM^{-1}:**3010 (Ar CH str), 2943, 2840 (Alkyl C-H str.), 1683 (C=O str), 1587 (-CO-N-CO), 1506 (-C=C- Ar str), 1330 (-NCH_2 -), 1234 (C-O of arylalkylether), 730 (-C-Cl str.).

N-{3-Chloro-2-(2,4-dichlorophenyl)-4-oxazetidin-1-yl} acetamido succinimide (4g): (m.p. 163˚C), **IR (KBr), CM^{-1}:** 3091 (Ar CH str), 1726, 1691 (C=O str), 1579 (-CO-N-CO), 1562 (-C=C- Ar str), 1276 (-NCH_2 -), 781, 705 (-C-Cl str.).

N-{3-Chloro-2-(4-N,N-dimethylaminophenyl)-4-oxazetidin-1-yl} acetamido succinimide (4h): (m.p. 242˚C), **IR (KBr), CM^{-1}:** 3091 (Ar CH str), 2916, 2891 (Alkyl C-H str.), 1697 (C=O str), 1577 (-CO-N-CO), 1562 (-C=C- Ar str), 1276 (-NCH_2 -), 783, 705 (-C-Cl str.).

N-{3-Chloro-2-(3-methoxy-4-hydroxyphenyl)-4-oxazetidin-1-yl} acetamido succinimide (4i): (m.p. 180˚C), **IR (KBr), CM^{-1}:** 3209 (Phenolic OH str.), 3082, 3094 (Ar CH str), 2975, 2943 (Alkyl C-H str.), 1666 (C=O str), 1591 (-CO-N-CO), 1517 (-C=C- Ar str), 1288 (-NCH_2 -), 752 (-C-Cl str.).

N-{3-Chloro-2-(3,4-dimethoxyphenyl)-4-oxazetidin-1-yl} acetamido succinimide (4j): (m.p. 211˚C), **IR (KBr), CM^{-1}:** 3010 (Ar CH str), 2943, 2840 (Alkyl C-H str.), 1683 (C=O str), 1587 (-CO-N-CO), 1506 (-C=C- Ar str), 1330 (-NCH_2 -), 1234 (C-O of arylalkylether), 730 (-C-Cl str.).

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 aeuroginosa, Shigella* at 100µg/ml concentration. The bacteria were subcultured on Nutrient Agar medium. The petridishes were incubated at 37ºC for 24hr. Ampicillin(10 mcg/disc) (Std.1) and Ciprofloxacin (30mcg/disc) (Std.2) were used as standards. The results are presented in Table2.

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 25ºC. The results are presented in Table 2.

RESULTS AND DISCUSSION

A number of N-{3-chloro-2-(substitutedphenyl)-4-oxazetidin-1-yl} acetamido succinimide derivatives were synthesized by using different substituted aromatic aldehyde to show mild to moderate activity. The characterizations of final products were checked by melting point, TLC, IR, ^{1}H NMR and Mass spectra.

Table 1: Physical data of N-{3-chloro-2-(substitutedphenyl)-4-oxazetidin-1-yl} acetamido succinimide (4a-4j)

Comp. Code	Ar-CHO	Mol. Formula	M.W. (g)	% Yield	R_f*	M.P.
4a	Benzaldehyde	$C_{15}H_{14}O_4N_3Cl$	335.5	64.70%	0.67	207˚C
4b	2-Nitrobenzaldehyde	$C_{15}H_{13}O_6N_4Cl$	380.5	71.35%	0.71	185˚C
4c	2-Hydroxybenzaldehyde	$C_{15}H_{14}O_5N_3Cl$	351.5	66.85%	0.64	156˚C
4d	3-Methoxybenzaldehyde	$C_{16}H_{16}O_5N_3Cl$	365.5	68.40%	0.55	213˚C
4e	4-Chlorobenzaldehyde	$C_{15}H_{13}O_4N_3Cl_2$	370	62.41%	0.81	201˚C
4f	3,4,5-Trimethoxybenzaldehyde	$C_{18}H_{20}O_7N_3Cl$	425.5	68.15%	0.63	175˚C
4g	2,4-Dichlorobenzaldehyde	$C_{15}H_{12}O_4N_3Cl_3$	404.5	69.20%	0.81	163˚C
4h	4-N,N-dimethylaminobenzaldehyde	$C_{17}H_{19}O_4N_4Cl$	378.5	71.33%	0.84	242˚C
4i	3-methoxy-4-Hydroxybenzaldehyde	$C_{16}H_{16}O_6N_3Cl$	381.5	58.98%	0.56	180˚C
4j	3,4-Dimethoxybenzaldehyde	$C_{17}H_{18}O_6N_3Cl$	395.5	72.70%	0.74	211˚C

*Stationary Phase: Silica Gel G

Mobile Phase: Chloroform: Acetone:: 9:1.

Table 2: Biological activity of N-{3-chloro-2-(substitutedphenyl)-4-oxazetidin-1-yl} acetamido succinimide (4a-4j)

Comp. Code	Zone of Inhibition (in mm)						
	B.subtilis	S.aureus	E.coli	P.aeuroginosa	Shigella	C.albicans	A.niger
	50 µg	50 µg	50 µg	50 µg	50 µg	50 µg	50 µg
4a	12	14	17	15	12	19	11
4b	17	21	20	14	13	14	10
4c	13	16	18	11	12	10	9
4d	12	14	14	13	9	11	14
4e	15	17	21	20	14	15	10

4f	11	13	15	16	12	12	11
4g	17	19	18	20	15	18	13
4h	13	14	16	11	11	15	12
4i	15	16	20	19	11	10	9
4j	10	12	14	10	10	9	8
Std 1	4	5	NI	8	6	22	20
Std 2	32	36	29	30	37	27	18
Std 3	--	--	--	--	--	17	20
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 N-{3-chloro-2-(2-nitrophenyl)-4-oxazetidin-1-yl} acetamido succinimide (4b), N-{3-chloro-2-(4-chlorophenyl)-4-oxazetidin-1-yl} acetamido succinimide (4e) and N-{3-chloro-2-(2,4-dichlorophenyl)-4-oxazetidin-1-yl} acetamido succinimide (4g) show potent antimicrobial activity. With this 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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