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Development of Heterocyclic Hybrids: Efficient Two-Step Synthesis and Antimicrobial Efficacy of Pyridine-4-Thiazolidinone Derivatives

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KEYWORDS

Heterocyclic Hybrids, Antimicrobial Efficacy

ABSTRACT:
We have designed a new series of heterocyclic hybrids incorporating motifs of pyridine and 4-thiazolidinedione for their antimicrobial potential. Synthesizing fourteen derivatives confirmed by spectral data using NMR, IR and MS, of which six (Ch2, Ch4, Ch5, Ch7, Ch10, H2) displayed encouraging antimicrobial potential, especially against Gram-positive/ Gram-negative bacteria and certain fungi, with some showing equal or even better efficacy than standard agents. The obtained MIC values are an indication of potent activity. Mechanistic studies also revealed that the pyridine and 4-thiazolidinedione motifs worked synergistically to disorganize microbial cell membranes and inhibit essential enzymes. More, importantly, the compounds showed their in-vitro therapeutic potential. The above findings suggest that these heterocyclic hybrids may represent a novel series of leads against the growing antimicrobial resistance. Further optimization and studies in-vivo are required to comprehensively evaluate their therapeutic profiles. This research shows the rationale and importance of designing multifunctional molecule with enhanced biological activities.

1 | INTRODUCTION

The escalation in the prevalence of antimicrobial resistance is one massive challenge in terms of global health, hence the need for the development of new and potent antimicrobial agents(1). Heterocyclic compounds, especially nitrogen and sulfur atoms containing heterocycles, have been accepted worldwide for their broad spectrum of biological activities, one of which is their antimicrobial properties(2). Recently, much interest has been focused on molecules bearing the pyridine and 4-thiazolidinone motifs because they can interact with microbial targets, thereby interfering with some vital processes in the cell(3). Pyridine derivatives are known to be versatile, and they have been used widely in the development of new drugs for modulation targeting various biological activities especially the antimicrobial activity(4). 4-Thiazolidinones are pharmacologically potent, with a wide range of activities against various microbes, inflammations, and cancer(5). The presence of these two moieties offers crosstalk for use as an antimicrobial scaffold with enhanced activity through synergistic interactions(6).

In the present study, we aimed to synthesize novel heterocyclic hybrids containing two pyridine rings and one 4-thiazolidinone moiety and to check their potential antimicrobial efficacy. The synthesis of these hybrids had been designed through a three-step synthetic route. We, however, optimized the successful completion of a two-step process to make the synthesis more efficient with less reaction time. This modification simply made the synthesis smoother on one side and increased the total yields on the other, making it friendlier toward big-scale production(7). The synthetic approach comprised the cyclization of appropriate precursors to derive the desired hybrids(8). A combination of spectroscopic techniques, amongst others, nuclear magnetic resonance (NMR), infrared (IR) spectroscopy, and mass spectrometry (MS), was used to confirm the structural integrity of the synthesized compounds. All the above methods provided a good insight into the molecular structures so that properly intended hybrids were successfully synthesized(9).

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The synthesized compounds were screened for their antimicrobial activity against different panels of pathogenic bacterial and fungal strains(10). All tested strains were members of both Gram-positive and Gram-negative bacteria, including various fungi. Minimum inhibitory concentration values were computed and compared for each derivative's antimicrobial strength(11). Remarkably, many of the synthesized hybrids showed significant antimicrobial activity, and some had activities similar to or even better than the standard antimicrobial agents. The mechanistic studies showed further reasons for the increased antimicrobial activity(12). These suggested that the synergism of the pyridine and 4-thiazolidinone motifs led to the disorganization of the microbial cell membranes as well as interference in significant enzymatic processes, which ultimately resulted in cell death(13).

It is in the last few years that introduction of green chemistry concepts in medicinal chemistry has become the need to ensure safer, greener drug research and development. This strategy focuses on minimization of the hazardous reagents, energy, waste, and on encouraging atom economy and involving environmentally innocuous solvents. Our approach to the synthesis, which consists of the two-step procedure modified by us, is agreeable to the ideas presented. Solely by decreasing the steps of the reaction to two reaction steps instead of three, we were able to improve both the overall yield and efficiency, and we used as few solvents as we could and we did not have the harsh conditions, which makes it much pre-eminent to scale up and more environmentally friendly. Environmental compatibility of this method is further increased by using relatively green solvent (ethanol) and mild and reusable catalyst (silica gel). In this way, synthetic strategy used here does not only contribute to pharmacological innovation but is a part of sustainable chemical practice.

The novel heterocyclic hybrids prepared in this work, containing pyridine and 4-thiazolidinone motifs, are promising candidates for developing new antimicrobial agents(14). The efficient and scalable production from a three- to two-step synthesis has been optimized to a high degree(15). These data strongly support the prior call for rational design in the development of multitasking molecules optimized for enhanced biological activity, to meet the much-needed new era of therapeutic antimicrobial agents active against resistant pathogens(16).

2| MATERIALS AND METHODS

1. | Chemistry

2.1.1 | Materials and reagents

All chemicals used were manufactured by Sigma Aldrich and S.d. Fine Chem. Limited and were of analytical grade. Each melting point has been determined by the melting point apparatus. With the use of TLC, or thin layer chromatography, the development of every reaction involving the produced molecule was tracked. The following materials were used for TLC: Aluminium sheets, Hexane and ethyl acetate (8:2). TLC plate put in the mixture of hexane and ethyl acetate for solvent running and then removed from the mixture then dry and put in the UV transmitter. The Shimadzu ATR-FTIR Spectrophotometer (ώ, cm^{-1}) was used for the spectroscopic investigation of the infrared (IR). The synthesized compounds' carbon (^{13}C NMR) and proton (^{1}H NMR) spectra were acquired using a BRUKER apparatus running at 500MHz, respectively. Tetramethyl silane was used as the internal standard, and the chemical shift was indicated by the symbol δ=ppm. DMSOd6 was the solvent that was used. Scheme 1 and Scheme 2 shows the route of synthesis, while the description, molecular formula, Mwt, m.p. of the synthesized compounds are summarized in Table 1.

2.1.2 | General procedure for synthesis of 3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Step 1)

A solution of pyridine-2-carbaldehyde (90µl) and pyridine-3-carbaldehyde (90µl) in 10 mL of Ethanol was stirred at 0°C, and 4-aminopyridine (0.09 gm) was introduced, followed by 5 minutes of stirring. Subsequently, Thioglycolic acid (556µl) was added to the reaction, and stirring continued for an additional 5 minutes. The mixture was then subjected to the addition of 0.5 g of silica gel. After reflux for 6.5 hours, the solvent was removed under reduced pressure, and the resulting slurry was purified through flash column chromatography. The final product was obtained in a yield of 89%(17).

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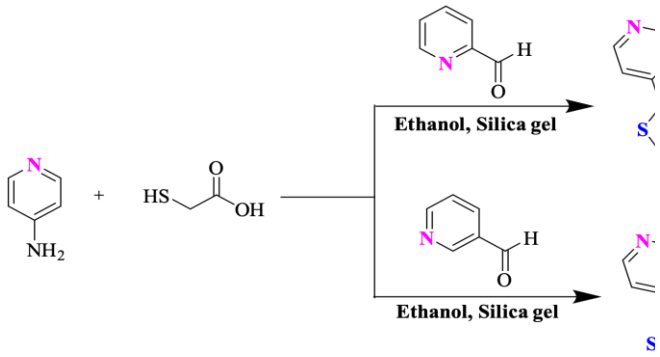
Step 1

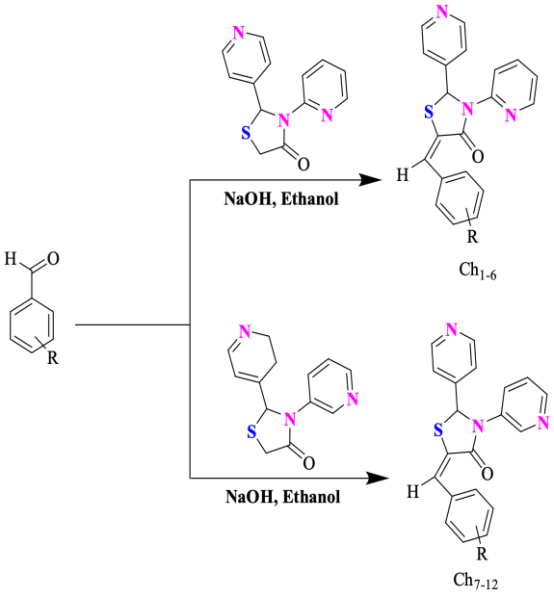


SCHEME 1 Synthetic route of Compounds **H_1** and **H_2**

2.1.3 | General procedure for synthesis of (E)-5-benzylidene-3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Step 2)

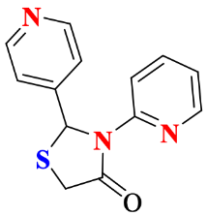
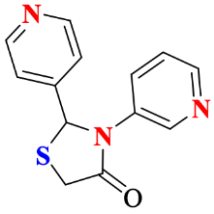
A mixture comprising 0.8 g of 3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one and 3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one 0.6 g of substituted benzaldehyde was stirred in 10 mL of 95% ethanol. A 10% solution of 5 mL NaOH was added to the mixture, which was then stirred at room temperature. Within five minutes, the solution precipitated, forming a solid. The solid was washed thoroughly with cold ethanol(18).

Step 2



SCHEME 2 Synthetic route of Compounds **Ch_{1-6}** and **Ch_{7-12}**

TABLE 1 Description, molecular formula, Mwt, and m.p., of the synthesized compounds. **(Ch_{1-10})**

Compound No.	R	Yield%	M.P. (°C)	M.F	Mwt
H_1		94.2%	357.1°C	$C_{13}H_{11}ON_3S$	257.31
H_2		96.1%	357.1°C	$C_{13}H_{11}ON_3S$	257.31

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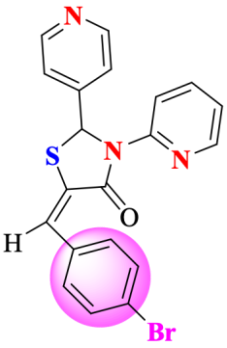
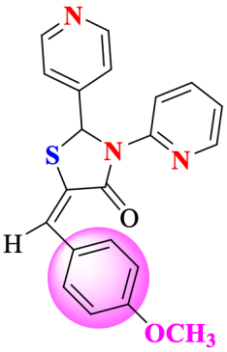
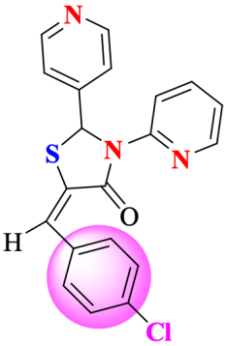
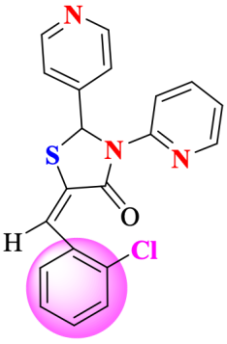
Ch_1		89.1%	545.09°C	C_7H_5OBr	185.02
Ch_2		74.5%	518.79°C	$C_8H_8O_2$	136.15
Ch_3		80.8%	515.21°C	C_7H_5OCl	140.57
Ch_4		78.4%	515.21°C	C_7H_5OCl	140.57

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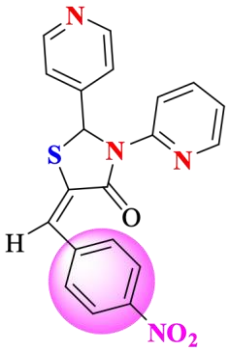
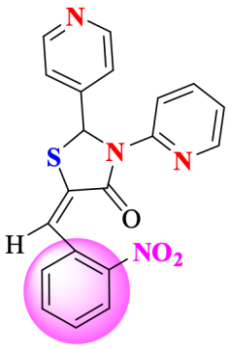
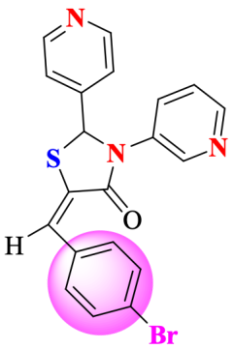
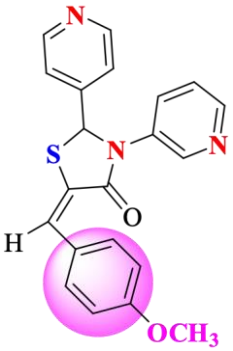
Ch_5		81.3%	524.10°C	$C_7H_5O_3N$	151.12
Ch_6		83.7%	524.10°C	$C_7H_5O_3N$	151.12
Ch_7		92.1%	545.09°C	C_7H_5OBr	185.02
Ch_8		89.2%	518.79°C	$C_8H_8O_2$	136.15

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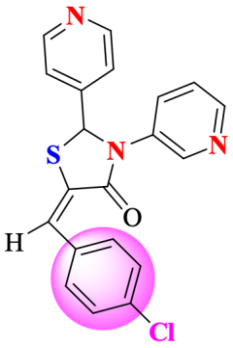
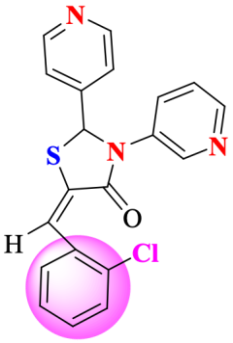
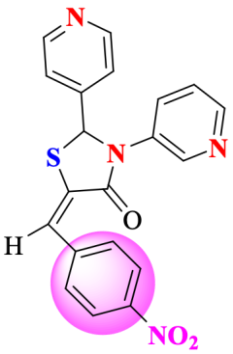
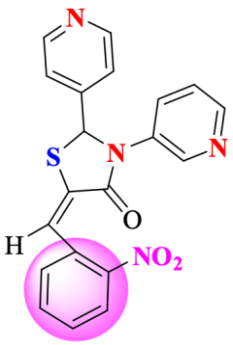
Ch_9		85.5%	515.21°C	C_7H_5OCl	140.57
Ch_{10}		83.6%	515.21°C	C_7H_5OCl	140.57
Ch_{11}		75.9%	524.10°C	$C_7H_5O_3N$	151.12
Ch_{12}		79.8%	524.10°C	$C_7H_5O_3N$	151.12

Synthesis of 3-(pyridine-2-yl)-2-(pyridine-4-yl)thiazolidine-4-one (Compound H_1)
Pale yellow powder, m.p. (357.1) °C, yield 94.2%,

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ATR-FTIR (ύ, cm−1): ATR-FTIR (ύ, cm−1): 1725.23 (C=O) str, 1081.14 (C-N) str, 659.20 (C-H) str, (Supporting Information Figure **S1A**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 9.9(s,1H,NH), 8.8-7.4(m,10H,ArH), 3.6-3.3(m,2H,CH_2) (Supporting Information Figure **S2A**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 171.2 (C=O), 149.8, 146.5, 146.7, 144.1, 138.7, 127.4, 124.4 and 124.2 (Ar-C),72.6 (CH), 33.5 (CH_2) (Supporting Information Figure **S3A**).

Synthesis of 3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound H_2)
Pale yellow powder, m.p. (357.1) °C, yield 96.1%,

ATR-FTIR (ύ, cm−1): 1725.23 (C=O) str, 1074.18 (C-N) str, 680.72 (C-H) str, (Supporting Information Figure **S1B**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.8-7.6(m,10H, ArH), 3.6-3.3(m,2H, CH_2) (Supporting Information Figure **S2B**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 171.2 (C=O), 171.2, 152.3, 149.8, 146.5, 143.6, 141.3, 124.1, 124.2 and 123.8 (Ar-C),72.6 (CH), 33.5 (CH_2) (Supporting Information Figure **S3B**).

Synthesis of 5-(4-bromobenzylidene)-3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_1)
Pale yellow powder, m.p. (545.09) °C, yield 89.1%,

ATR-FTIR (ύ, cm−1): 3022.75 Ar (C-H) str, 1646.69 (C=O) str, 1580.45 Ar (C=C) str, 1318.49 (C-N) str, 821.93 Ar (C-H) str, 726.16 (C-S) str, 691.85 (C-Br) (Supporting Information Figure **S1C**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.3-6.7(m,14H, ArH), (Supporting Information Figure **S2C**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 152.7, 149.8, 148.1, 146.5, 138.3, 134.2, 131.5, 128.6, 125.2, 124.2, 122.3 and 117.9 (Ar-C), 71.1 (CH) (Supporting Information Figure **S3C**).

Synthesis of 5-(4-methoxybenzylidene)-3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_2)
Pale yellow powder, m.p. (518.79) °C, yield 74.5%,

ATR-FTIR (ύ, cm−1): 3000.68 Ar (C-H) str, 1621.23 (C=O) str, 1428.92 (O-CH_3) str, 1336.72 (C-N) str, 659.07 (C-S) str, 691.85 (C-Br) (Supporting Information Figure **S1D**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.8-6.8(m,14H, ArH), 3.4 (s,3H, O-CH_3) (Supporting Information Figure **S2D**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 159.8, 152.7, 149.8, 148.1, 146.5, 138.3, 130.2, 127.5, 125.2, 124.2, 121.5,117.9 and 114.2 (Ar-C), 71.1 (CH), 55.8 (CH_3) (Supporting Information Figure **S3D**).

Synthesis of 5-(4-chlorobenzylidene)-3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_3)
Pale yellow powder, m.p. (515.21) °C, yield 80.8%,

ATR-FTIR (ύ, cm−1): 3047.83 Ar (C-H) str, 1667.25 (C=O) str,1624.65 (C=C) str, 1563.38 Ar (C=C) str, 1336.61 (C-N) str, 748.89 (C-Cl), 709.03 (C-S) str (Supporting Information Figure **S1E**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.3-6.8(m,14H, ArH), (Supporting Information Figure **S2E**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 152.7, 149.8, 148.1, 146.5, 138.3, 133.5, 133.3, 129.0, 128.7, 125.2, 124.2 and 121.5 (Ar-C), 71.1 (CH) (Supporting Information Figure **S3E**).

Synthesis of 5-(2-chlorobenzylidene)-3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_4)
Pale yellow powder, m.p. (515.21) °C, yield 78.4%,

ATR-FTIR (ύ, cm−1): 3000.80 Ar (C-H) str, 1620.90 (C=O) str, 1416.97 Ar (C=C) str, 1336.77 (C-N) str, 779.80 (C-Cl), 658.60 (C-S) str (Supporting Information Figure **S1F**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.3-6.8(m,14H, ArH), (Supporting Information Figure **S2F**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 152.7, 149.8, 148.1, 146.5, 138.3, 134.0, 133.0, 129.9, 129.3, 128.7, 127.8, 126.7, 125.2, 124.2, 121.5 and 117.9 (Ar-C), 71.1 (CH) (Supporting Information Figure **S3F**).

Synthesis of 5-(4-nitrobenzylidene)-3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_5)
Pale yellow powder, m.p. (524.10°C) °C, yield 81.3%,

ATR-FTIR (ύ, cm−1): 3342.32 Ar (C-H) str, 1633.28 (C=O) str, 1515.83 (N=O) str, 1394.89 Ar (C=C) str, 1347.01 (C-N) str, 873.90 (C-S) str (Supporting Information Figure **S1G**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.3-6.8(m,14H, ArH), (Supporting Information Figure **S2G**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 152.7, 149.8, 148.1, 147.1, 146.5, 141.3, 138.3, 129.0, 125.2, 124.2, 123.8, 121.5 and 117.9 (Ar-C), 71.1 (CH) (Supporting Information Figure **S3G**).

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Synthesis of 5-(2-nitrobenzylidene)-3-(pyridin-2-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_6)
Pale yellow powder, m.p. (524.10°C) °C, yield 83.7%,

ATR-FTIR (ύ, cm−1): 3231.48 Ar (C-H) str, 1622.82 (C=O) str, 1454.08 (N=O) str, 1335.67 (C-N) str, 658.81 (C-S) str (Supporting Information Figure **S1H**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.3-6.4(m,14H, ArH), (Supporting Information Figure **S2H**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 152.7, 149.8, 148.1, 147.7, 146.5, 138.3, 134.7, 128.8, 127.3, 125.2, 124.2, 123.8, 121.5 and 117.9 (Ar-C), 71.1 (CH) (Supporting Information S1 Figure **S3H**).

Synthesis of 5-(4-bromobenzylidene)-3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_7)
Pale yellow powder, m.p. (545.09) °C, yield 92.1%,

ATR-FTIR (ύ, cm−1): 3021.92 Ar (C-H) str, 1647.08 (C=O) str, 1595.04 (C=C) str, 1580.67 (C-N) str, 69162 (C-Br) (Supporting Information Figure **S1I**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.3-6.8(m,14H, ArH), (Supporting Information Figure **S2I**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 149.8, 148.2, 146.5, 140.6, 138.3, 134.2, 131.5, 128.6, 125.2, 124.7, 124.2 and 122.3 (Ar-C), 71.1 (CH) (Supporting Information Figure **S3I**).

Synthesis of 5-(4-methoxybenzylidene)-3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_8)
Pale yellow powder, m.p. (518.79) °C, yield 89.2%,

ATR-FTIR (ύ, cm−1): 2981.40 Ar (C-H) str, 1597.79 (C=O) str, 1511.26 (C=C) str, 1420.67 (O-CH_3) str, 1336.80 (C-N) str (Supporting Information Figure **S1J**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.2-6.7(m,14H, ArH), 3.9-3.4(s,3H, O-CH_3) (Supporting Information
Figure **S2J**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 159.8, 149.8, 148.2, 146.5, 140.6, 138.3, 138.3, 135.1, 130.2, 127.5, 125.2, 124.7, 124.2 and 114.2 (Ar-C), 71.1 (CH), 55.8 (CH_3) (Supporting Information S1 Figure **S3J**).

Synthesis of 5-(4-chlorobenzylidene)-3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_9)
Pale yellow powder, m.p. (515.21) °C, yield 85.5%,

ATR-FTIR (ύ, cm−1): 2980.90 Ar (C-H) str, 1666.76 (C=O) str,1624.03 (C=N) str, 1586.09 Ar (C=C) str, 1336.47 (C-N) str, 748.69 (C-Cl), 707.60 (C-S) str (Supporting Information Figure **S1K**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 7.8-6.8(m,14H, ArH), (Supporting Information Figure **S2K**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 149.8, 148.2, 140.6, 138.8, 138.3, 135.1, 133.5, 133.3, 129.0, 128.7, 125.2, 124.7 and 124.2 (Ar-C), 71.1 (CH) (Supporting Information Figure **S3K**).

Synthesis of 5-(2-chlorobenzylidene)-3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_{10})
Pale yellow powder, m.p. (515.21) °C, yield 83.6%,

ATR-FTIR (ύ, cm−1): 2981.82 Ar (C-H) str, 1620.81 (C=O) str, 1453.34 Ar (C=C) str, 1337.19 (C-N) str, 779.70 (C-Cl), 667.12 (C-S) str (Supporting Information Figure **S1L**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.0-6.8(m,14H, ArH), (Supporting Information Figure **S2L**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 152.7, 149.8, 148.2, 146.5, 140.6, 138.8, 138.3, 135.1, 134.0, 133.0, 129.9, 129.3, 127.8, 126.7, 125.2, 124.7 and 124.2 (Ar-C), 71.1 (CH) (Supporting Information S1 Figure **S3L**).

Synthesis of 5-(4-nitrobenzylidene)-3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_{11})
Pale yellow powder, m.p. (524.10°C) °C, yield 75.9%,

ATR-FTIR (ύ, cm−1): 2981.72 Ar (C-H) str, 1649.90 (C=O) str, 1604.25 (C=N) str, 1517.06 (N=O) str, 1413.12 (C-N) str, 1346.44 Ar (C=C) str, 852.22 (C-S) str (Supporting Information Figure **S1M**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.3-6.8(m,14H, ArH), (Supporting Information Figure **S2M**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 149.8, 148.2, 147.1, 146.5, 141.3, 140.6, 138.8, 138.3, 135.1, 129.0, 125.2, 124.7, 124.2 and 123.8 (Ar-C), 71.1 (CH) (Supporting Information Figure **S3M**).

Synthesis of 5-(2-nitrobenzylidene)-3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one (Compound Ch_{12})
Pale yellow powder, m.p. (524.10°C) °C, yield 79.8%,

ATR-FTIR (ύ, cm−1): 2980.59 Ar (C-H) str, 1622.46 (C=O) str, 1453.15 Ar (C=C) str, 1336.05 (N=O) str, 658.93 (C-S) str (Supporting Information Figure **S1N**).

^{1}HNMR (500MHz, $DMSO_{d6}$, δ, ppm): 8.5-

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6.4(m,14H, ArH), (Supporting Information Figure **S2N**).

^{13}CNMR (500MHz, $DMSO_{d6}$, δ, ppm): 162.3, 149.8, 148.2, 147.7, 146.5, 140.6, 138.8, 138.3, 135.1, 134.7, 128.8, 127.3, 125.2, 124.7, 124.2 and 123.8 (Ar-C), 71.1 (CH) (Supporting Information Figure **S3N**).

2.2 | Biology

1. | In vitro antimicrobial activity

Evaluation techniques

➢ To evaluate antimicrobial activity, certain requirements must be met:

1. The drug and test organisms must have close contact.
2. Conditions for microbes to flourish must be created.
3. The study should be conducted under identical conditions.
4. Maintaining a sterile and aseptic atmosphere is crucial.

➢ Several techniques can be used to evaluate antibacterial activity:

1. Turbidometric technique
2. Agar dilution technique
3. Serial dilution process
4. Agar Diffusion Method

➢ The agar diffusion method can be applied using the following techniques:

1. Agar Cup technique
2. Paper Disc technique
3. Agar Ditch method

We utilized the Borth Dilution Method to assess the antibacterial activity. This method is a non-automated in vitro bacterial susceptibility test that provides a quantitative result for the amount of antimicrobial agents required to inhibit the growth of specific microorganisms. The test is conducted in tubes and can also be performed using plastic trays in a microdilution format.

Determination of minimal inhibition concentrations by micro broth dilution method

Please take note of the following information:

➢ Materials and methods:

1. All synthesized drugs were used for antibacterial test procedures.
2. All necessary controls were included:
   - Drug Control
   - Vehicle Control
   - Agar Control
   - Organism Control
   - Known Antibacterial Drugs Control
   - All MTCC cultures were tested against the above-mentioned known and unknown drugs.
   - Mueller Hinton Broth was used as a nutrient to grow and dilute the drug suspension for the test bacteria.
   - The inoculum size for the test strain was adjusted to 10^8 CFU (Colony Forming Unit) per milliliter by comparing the turbidity.
   - Common standard strains procured from the Institute of Microbial Technology, Chandigarh were used for screening antibacterial and antifungal activities.

E.coli	*P.aeruginosa*	*S.aureus*	*S. pyogenus*
MTCC443	MTCC1688	MTCC96	MTCC442

C. albicans	*A.niger*
MTCC227	MTCC282

- DMSO was used as a diluent or vehicle to achieve the desired concentration of drugs to test upon standard bacterial strains.
- The median inhibitory concentration (IC50) value was determined.
- The percentage (%) of bacterial growth inhibition is calculated as [(Ac-At)/Ac] × 100, where Ac is an average of six replicates of light absorption values at wavelength - nm of the negative controls, and At is an average of six

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replicated light absorption values at wavelength - nm of the samples.

- The IC50 value is calculated using the linear relation between the inhibitory probability and concentration logarithm according to the method of Sakuma. The IC50 value is expressed as the mean ± standard deviation of three independent experiments.

Minimal inhibition concentration [MIC]

The 'Broth Dilution Method' for determining Minimum Inhibitory Concentration (MIC) offers several advantages, one of which is its ability to readily determine the MIC. Here are the steps involved:

1. Prepare serial dilutions for primary and secondary screening.
2. Inoculate the control tube containing no antibiotic by evenly spreading a loopful over a quarter of a medium suitable for the test organism's growth. Incubate the tubes at 37°C overnight.
3. Read the MIC of the control organism to verify the accuracy of the drug concentrations.
4. Record the lowest concentration that inhibits the growth of the organism as the MIC.
5. Compare the amount of growth from the control tube before incubation (representing the original inoculum).

Methods used for primary and secondary screening:

For each synthesized drug, a stock solution was created by diluting it to a concentration of 2000 micrograms/ml.

- In the primary screening, the synthesized drugs were tested at concentrations of 1000 micro/ml, 500 micro/ml, and 250 micro/ml. The active drugs identified in this screening were then further tested at a second set of dilutions against all microorganisms.
- The active drugs from the primary screening were diluted to concentrations of 200 micro/ml, 100 micro/ml, 50 micro/ml, 12.5 micro/ml, and 6.250 micro/ml for the secondary screening.
- The MIC (minimum inhibitory concentration) was determined by identifying the highest dilution that resulted in at least a 99% inhibition zone. It's important to note that the test results can be affected by the size of the inoculum, and the test mixture should contain 10^8 organisms/ml.
- A summary table of the study results is provided below for easy reference and a better understanding of the findings.

TABLE 2 Antimicrobial activity (MICs, µg/mL) of (ch_{1-12})

MINIMAL BACTERICIDAL CONCENTRATION (µg/mL)					MINIMAL FUNGICIDAL CONCENTRATION (µg/mL)	
Compound No.	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. pyogenus*	*C. albicans*	*A.niger*
H_1	200	100	125	125	1000	500
H_2	100	**62.5**	100	125	>1000	1000
Ch_1	125	250	125	125	500	>1000
Ch_2	**62.5**	100	125	125	500	1000
Ch_3	100	125	250	100	1000	1000
Ch_4	100	**62.5**	125	200	500	>1000
Ch_5	125	250	250	100	**250**	500

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Ch_6	125	250	100	125	500	1000
Ch_7	250	100	**62.5**	100	**250**	1000
Ch_8	100	100	100	200	500	500
Ch_9	250	125	100	100	500	1000
Ch_{10}	125	250	**62.5**	100	**250**	>1000
Ch_{11}	100	100	250	250	1000	500
Ch_{12}	125	250	125	250	1000	500
Drug	Micromolar (µg/mL)					
Gentamycin	0.05	1	0.25	0.5	-	-
Ampicillin	30	-	40	25	-	-
Chloramphenicol	50	50	50	50	-	-
Ciprofloxacin	25	25	50	50	-	-
Norfloxacin	10	10	10	10	-	-
Nystatin	-	-	-	-	100	100
Griseofulvin	-	-	-	-	500	100

3 | RESULTS AND DISCUSSION

3.1 | Chemistry

The ATR-FTIR spectra (Supporting Information Figure S1) revealed that Compound **H_1** had a characteristic absorption band at ύ=1726.29 cm^{-1} for (C=O), at ύ=1575.42 cm^{-1} for (C=N) stretching and at ύ =1350.09 cm^{-1} for (C-N) stretching. Compound **Ch_1** had the following absorption peaks: ύ=1646.69cm^{-1} for (C=O) stretching, ύ=1580.45cm^{-1} for Aromatic (C=C) stretching and ύ=691.85cm^{-1} for (C-Br) stretching. Compound **Ch_3** had the following absorption peaks: ύ=3047.83cm^{-1} for Aromatic (C-H) stretching, ύ=1667.25cm^{-1} for (C=O) stretching and ύ=1563.65cm^{-1} for Aromatic (C=C) stretching. Compound **Ch_8** had the following absorption peaks: ύ=2981.40cm^{-1} for Aromatic (C-H) stretching, ύ=1597.79cm^{-1} for (C=O) stretching, ύ=1511.36cm^{-1} for Aromatic (C=C) stretching and ύ=1420.67cm^{-1} for (OCH_3) stretching. Compound **Ch_{12}** had the following absorption peaks: ύ=2980.59cm^{-1} for Aromatic (C-H) stretching, ύ=1622.46cm^{-1} for (C=O) stretching, ύ=1453.15cm^{-1} for Aromatic (C=C) stretching and ύ=1336.05cm^{-1} for (N=O) stretching.

The ^{1}HNMR spectra (Supporting information Figure S2) of the synthesized compound **H_1** showed a distinct multiplet at δ=8.8-7.4 and 3.6-3.3 ppm for Aromatic proton and (CH and CH_2 group). Compound **H_2** showed multiplet at 8.8-6.7 and 3.6-3.3 ppm for Aromatic proton and (CH and CH_2 group). compound **Ch_2** showed distinct singlet band at δ=3.42 ppm for (OCH_3). Compound **Ch_4** showed distinct multiplet band at δ=8.3-6.8 ppm for aromatic proton. Compound **Ch_8** showed multiplet at δ=8.2-6.7 and 3.9-3.4 for Aromatic proton and (CH and CH_2 group).

The ^{13}CNMR spectra (Supporting Information Figure S3) exhibited that compound **H_1** showed signals at δ=171.2 ppm for (C=O), 72.6 ppm for (CH) and 33.5 ppm for (CH_2) groups. Compound **Ch_5** showed a signal at δ=162.3-123.8 ppm due to Aromatic carbon and 71.1 ppm for alkyl carbon. Compound **H_2** showed signals at δ=171.2 ppm for (C=O), 72.6 ppm for (CH) and 33.5 ppm for (CH_2) groups. Compound **Ch_8** showed signals at δ=162.3-124.2 ppm for Aromatic carbon, 71.1 ppm for

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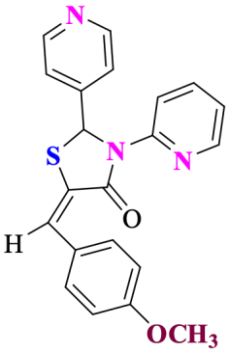
(CH) and 55.8 for (CH_3).

3.2 | In vitro Anti-microbial study results

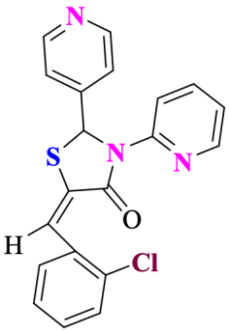
The antibacterial activity of many produced compounds was evaluated against a range of bacterial and fungal species. The activity of the produced compounds against bacteria was assessed by measuring minimum inhibitory concentrations and used the broth dilution technique. The examination made use of standard medications such as gentamycin, chloramphenicol, ampicillin, ciprofloxacin, norfloxacin, griseofulvin, and nystatine. The results displayed in Table 2 revealed that most of the tested compounds had varied inhibitory effects on the growth of both Gram-positive and Gram-negative bacterial strains. Ch_2 containing pyridine showed good activity compared to Gentamycin against *E.coli*. Ch_4 and H_2 containing pyridine showed good activity compared to Gentamycin against *P. Aeruginosa*. Ch_7 and Ch_{10} containing pyridine showed good activity compared to Gentamycin against *S. aureus.* However, the rest of the compounds were either poor or moderately active against all of the organisms with higher MIC values. Additionally, new derivatives were tested as potential antifungal agents. Compound Ch_5, Ch_7 and Ch_{10} containing Pyridine were found to be as effective as the reference drug Griseofulvin against *C. albicans.* However, other compounds did not show satisfactory results.

4 | CONCLUSION

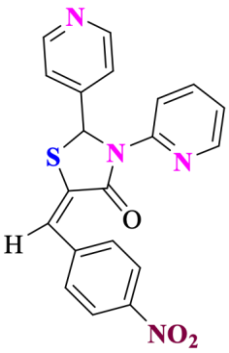
In the first step Pyridine-2-carbaldehdye and Pyridine-3-carbaldehyde raect with 4-aminopyridine and thioglycolic acid in the presence of ethanol and silica gel to give 3-(pyridin-2-yl)-2-(pyridine-4-yl)thiazolidine-4-one**(H_1)** and gives the 3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one**(H_2)** product. In the second step 3-(pyridin-2-yl)-2-(pyridine-4-yl)thiazolidine-4-one**(H_1)** and gives the 3-(pyridin-3-yl)-2-(pyridin-4-yl)thiazolidin-4-one**(H_2)** react with substituted aldehyde in the presence of ethanol and NaOH and give Thiazolidinone derivatives. The structures of the new compounds were confirmed bt different spsctroscopic techniques. Compound **Ch_4** and **H_2** demonstrated excellent activity against *P. Aeruginosa* at a concentration of 62.5µg/mL. Compound **Ch_7** and **Ch_{10}** demonstrated excellent activity against *S. Aureus* at a concentration of 62.5µg/mL. Compound **Ch_2** exhibited potency against *E.coli* at a concentration of 62.5µg/mL. While compound **Ch_5**, **Ch_7** and **Ch_{10}** showed good antifungal activity. These findings suggest that these compounds hold promise for further reserach in the development of novel antimicrobial agents. The structure of all active compounds is provided below.



Ch_2 (MIC=62.5µg/mL) *E.coli*



Ch_4 (MIC=62.5µg/mL) *P. aeruginosa*



Ch_5 (MIC=250µg/mL) *C. albicans*

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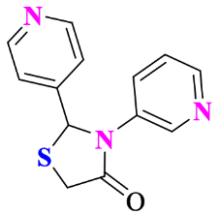
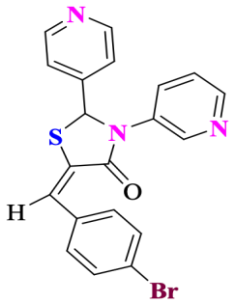
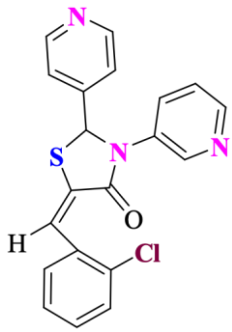


H₂ (MIC=62.5µg/mL) *P. aeruginosa*



Ch₇ (MIC=62.5µg/mL) *S. Aureus*
(MIC=2505µg/mL) C. albicans



Ch₁₀ (MIC=62.5µg/mL) *S. Aureus*
(MIC=2505µg/mL) C. albicans

The synthesized thiazolidinone derivatives not only show considerable prospective in biological terms, but also exemplify benefits of using green chemistry tenets in current drug design. The simplified two-step reaction did not only enhance the efficiency and the yield of the reaction but also decreased the use of unnecessary amounts of solvents, reagents and purifications. The use of ethanol rather than toxic solvents, and silica gel catalyst that is weak, can be recycled, represent an advance towards environmentally friendly synthetic methods. This finding between biological activity and environmental efficiency highlights the higher worth of incorporating green chemistry into early-stage antimicrobial drug discovery to make such chemicals better candidates not only in the further development of the therapeutic but to enable inexpensive manufacturing of the drug at scale, as well as eth-environmentally sensitive manufacturing.

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