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Synthesies, Characterization and Biological Activities of 4-Methyl-3,5 diphenylisoxazolidine Derivatives

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Abstract

This study focused on the synthesis, characterization, and biological evaluation of novel 4-methyl-3,5-diphenylisoxazolidine derivatives with potent antimicrobial activity. The target compounds were synthesized microwave assisted synthesis, achieving excellent yields (94.53% and 95.75%) for Isoxazolidine derivatives and moderate to high yields ranging from 68.40% to 98.44% for the intermediate Chalcone. All derivatives were soluble in ethanol but insoluble in water, consistent with their aromatic and heterocyclic structures. Structural confirmation was accomplished through Fourier-transform infrared spectroscopy (FTIR) and gas chromatography-mass spectrometry (GCMS), which identified characteristic functional groups including amine (N–H) (3550 – 3200 cm^{-1}), alkane (C–H) (3000 – 2840 cm^{-1}), and halogen-specific bonds (800 – 500 cm^{-1}), validating the successful incorporation of substituents (bromo). GCMS result further suggest the molecular weights (235.1 and 315.2 g/mol respectively) and molecular structures consistent with the targeted derivatives, including confirmation of halogen substitutions. Antimicrobial activity was assessed against a panel of Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*), and fungi (*Aspergillus niger*, *Candida albicans*). Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) assays demonstrated that all compounds exhibited significant antimicrobial effects, with halogenated derivatives showing superior potency, with MIC values ranging from 6.25 - 50 mg/mL. In general, the compounds were more effective against the tested fungi than bacteria. Zones of inhibition(i.e 18 mm) and lower MIC values (i.e 6.25 mg/mL), highlighted it enhanced efficacy compared to the unsubstituted analogue and standard antibiotic controls. The findings underscore the potential of 4-methyl-3,5-diphenylisoxazolidine scaffolds as promising antimicrobial agents, where strategic functional group modifications influence biological activity. The study have laid a foundation for further optimization, mechanistic studies, and development of these derivatives as candidates for combating microbial infections, especially in the context of rising antimicrobial resistance.

Keywords: Benzaldehydes, Heterocyclic compounds, Hydroxylamine hydrochloride Isoxazolidine, Medicinal, Propiophenones.

Introduction

One of the primary concerns of medicinal chemists has been a growing resistance against human pathogens leading to serious infections over several decades. In the previous century, various medicines were invented against various infections. Nonetheless, the drugs that are available may have undesirable effects and may be toxic. Considering these facts, it is worth examining other drugs that do not have adverse effects because they act in a different way [1]. Skin and soft tissue infections (SSTIs) are among the most common types of infectious disease, and their manifestations are unbelievably diverse; moreover, their causative agents are also evenly distributed in a wide spectrum. Among the worst things about these organisms is the fact that they vary on drug resistance. *Staphylococcus aureus* causes a substantial number of serious skin infections with variable proportions as a result of the presence of *methicillin-resistant Staphylococcus aureu s*(MRSA) [2].

Heterocyclic compounds are very important for the maintenance of life; they are widely distributed in nature. The genetic material contains important heterocycles such as purine and pyrimidine bases. In addition, various heterocycles are structural components of common therapeutic drugs, either achieved through chemical synthesis or found in nature [3, 4].Numerous recent studies have targeted therapeutic agents the structures of which are based on different heterocycles[5, 6].Oxazolidine is a heterocyclic organic compound with a 5-membered ring containing nitrogen and oxygen[7].Oxazolidines are versatile compounds known for their diverse pharmacological properties and potential applications in drug discovery [8].

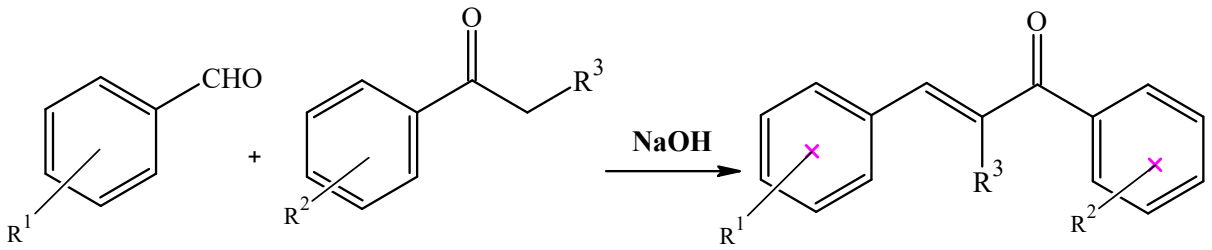
The aim of this study is to synthesize, characterize and evaluate the biological activities of 4-methyl-3,5-diphenylisoxazolidine derivatives. The study aims to contribute meaningfully to the development of new therapeutic agents and advance our understanding of heterocyclic chemistry and medicinal chemistry as well.

Materials and Methods

Materials/Reagent: Domestic Microwave, Refrigerator, Beakers, TLC plate, Benzaldehyde, 2-Chlorobenzaldehyde, Propiophenone, 4-Hydropropiophenone, 4-Fluoropropiophenone, 4-Bromopropiophenone, Hydroxylamine hydrochloride, Ethanol, distilled water, n-hexane, ethyl-acetate, Sodium acetate, Filter paper and funnel, Stirring rod and other glass wares

Procedure for the Syntheses of Chalcones: The method of syntheses was abducted from Yadav*et al.* [9] and Bhuiyan*et al.* [10] with some modifications. Equi-molar amount of Benzaldehydes and Propiophenones was dissolved in minimal amount of ethanol and placed in a microwave vial (10 mL). The vial was then placed in a domestic microwave oven. The reaction mixture was irradiated under 160 – 320 watt microwave irradiation for 60 – 120 sec. The

reaction progress was monitored by TLC (n-hexane:ethylacetate – 7:1) after every 30 sec. On completion the reaction mixture was cooled and the obtained solid was recrystallined from ethylacetate and n-hexane solvent mixture to obtain the synthesized chalcones (**1a-b**).

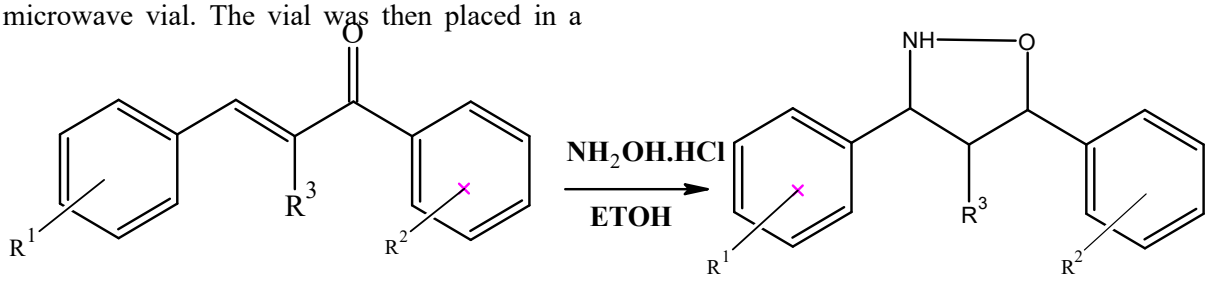


1(a-b)	R^1	R^2	R^3
A	H	H	CH_3
B	H	4-Br	CH_3

R^1 = H **R^2 = H, 4-Br** **R^3 = CH_3**

Scheme 1: Syntheses of Chalcones

Procedure for the syntheses of OxazolidineDerivatives: The method of syntheses was abducted from Shorouk*et al.* [11] with some modifications. About 1.02g (0.005mol) of the synthesized chalcones (**1a-b**), 0.32ml (0.525g) of 98% Hydroxylamine hydrochloride and 0.83ml (0.01ml) of Sodium acetate was dissolved in 5ml of ethanol in a microwave vial. The vial was then placed in a domestic microwave oven. The reaction mixture was irradiated under 160 – 320 watt microwave irradiation for 30 – 100 sec. The reaction progress was monitored by TLC (n-hexane:ethylacetate – 7:1) after every 15 sec. On completion the reaction mixture was cooled and the obtained solid was recrystallined from ethanol to obtain the synthesized oxazolidine derivatives (**2a-b**).



2(a-d)	R^1	R^2	R^3
a	H	H	CH_3
b	H	4-Br	CH_3

Scheme 2: Syntheses of Oxazolidine Derivatives

Characterization of Oxazolidine: The synthesized compounds were subjected to spectroscopic techniques: Fortier Transform Infra-red(FTIR) spectrometry was carried out on

both the synthesized chalcones (1a-b) and synthesizedoxazolidines (2a-b) samples**.** Gas Chromatography Mass Spectrometry (GCMS) analysis of Oxazolidines samples was also recorded.

Biological Screening TheOxazolidines samples (2a-d) were subjected to antibacterial and antifungal evaluation using selected clinical isolates. The methods will be adopted from Ghulam*et al.,* [12].

Results and Discussion

Table 1: Synthesis of Appropriate Chalcones (1a-d) with their physical properties

Cpds	R1	R2	Yield (%)	Molecular weight(g/mol)	Reaction time (Sec.)	Solubility water	Ethanol
1a	H	H	68.40	222.20	120	Insoluble	Soluble
1b	H	4-Br	98.44	300.90	70	Insoluble	Soluble

Table 2: Synthesis of Oxazolidine derivatives (2a-d) with their physical properties

Cpds	R1	R2	Yield (%)	Molecular weight(g/mol)	Reaction time (Sec.)	Solubility water	Ethanol
2a	H	H	94.53	238.00	80	Insoluble	Soluble
2b	H	4-Br	95.75	317.00	50	Insoluble	Soluble

Synthesis of Appropriate Chalcones (1a-b) and Oxazolidine derivatives (2a-b) with their physical properties

Tables 1 and 2 summarize the synthesis outcomes of the chalcone intermediates (1a-b) and the target isoxazolidine derivatives (2a-b) including their physical properties, yields, molecular weights, reaction times, and solubility profiles. The chalcones were synthesized in moderate to high yields ranging from 68.40% to 98.44%. The highest yield was observed for compound 1b (4-bromophenyl substitution), indicating the influence of electron-withdrawing substituents in facilitating the aldol condensation reaction often involved in chalconesynthesis [13]. Both synthesized compounds were soluble in ethanol but insoluble in water, consistent with the hydrophobic aromatic scaffold typical of chalcones. The chalcones serve as key intermediates in the synthesis of the target isoxazolidine derivatives [14].

The synthesis of two (2) novel isoxazolidine derivatives (2a-2b) was successfully achieved with an excellent yields (94.53% and 95.75%). The reaction times were relatively short (50-80 seconds), indicating efficient synthetic protocols. Isoxazolidine derivatives showed notably shorter reaction times than the chalcones (50–80 seconds vs. 70–120 seconds), reflecting effective cyclization possibly driven by favorable electronic and steric factors [15]. Solubility data shows that all compounds are soluble in ethanol but insoluble in water, which is consistent with the hydrophobic aromatic substituents present and moderate polarity (Table 2).This solubility profile is typical for organic compounds with significant non-polar character, which is expected for isoxazolidinederivatives containing multiple phenyl groups. This characteristic can influence their bioavailability and formulation for biological applications [16, 17].

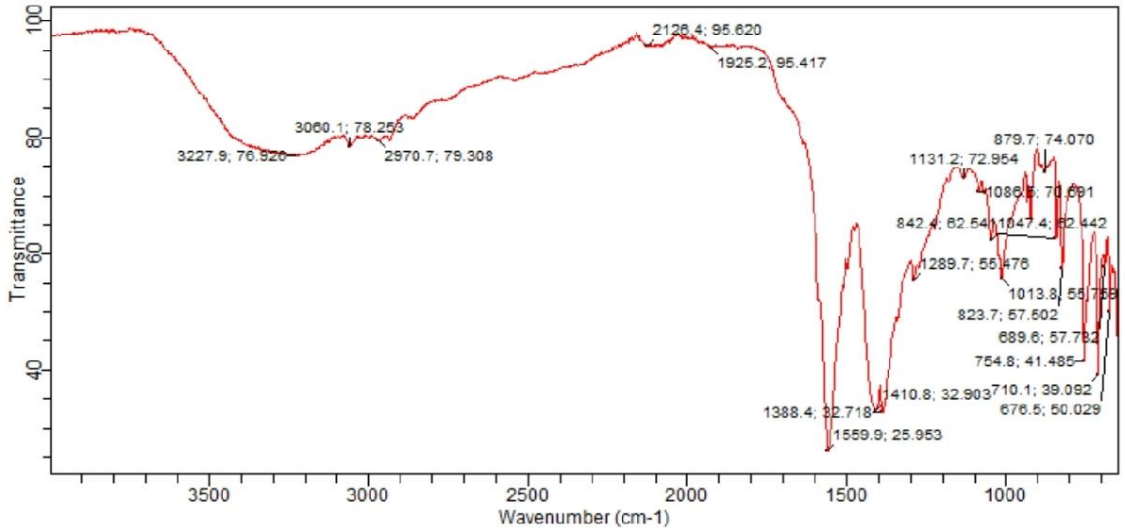


Figure 1: FTIR Spectral for Sample 1a ((E)-2-methyl-3-diphenylprop-2-en-1-one)

Table 3 FTIR table for Sample 1a ((E)-2-methyl-3-diphenylprop-2-en-1-one)

Absorption peaks (cm^{-1})	Reference Absorption peaks (cm^{-1})	Appearance	Functional group	Compound class	Comment
2970.70	3000 - 2840	Weak/Sharp	C-H stretching	Alkane	Present
1925.50	2000 - 1620	Weak/Sharp	C-H bending	Alkane	Overtone Present
1410.80	1450 - 1400	Strong	C=O stretching	Carboxylic acid	Present
879.70	890 - 820	Medium	Ar-C stretching	Aromatic	Present
842.40	890 - 820	Medium	Ar-C stretching	Aromatic	Present
823.70	890 - 820	Medium	Ar-C stretching	Aromatic	Present

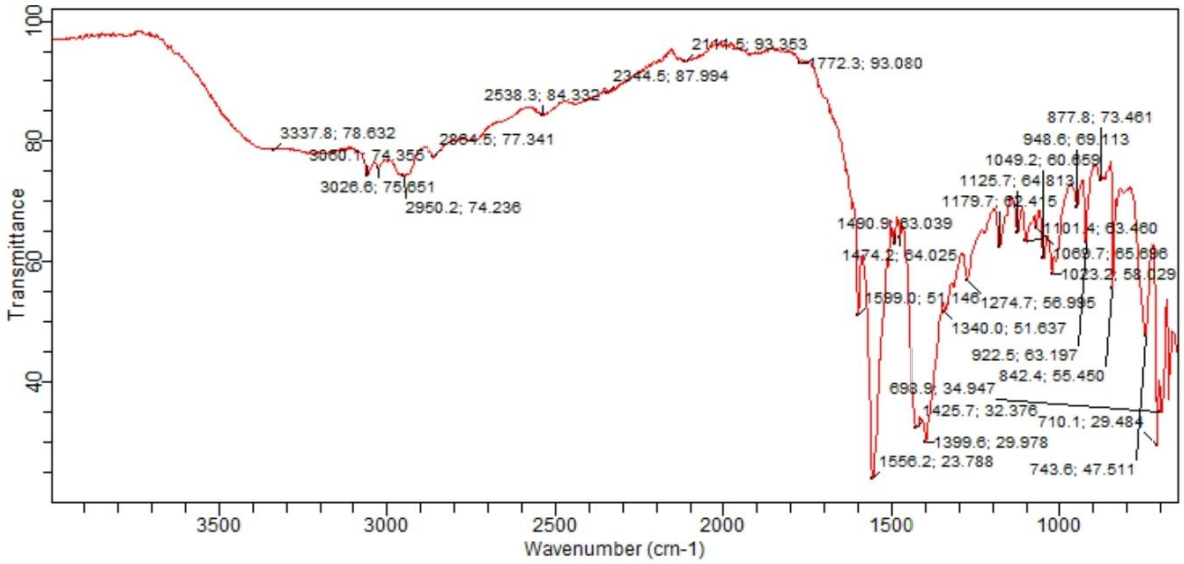


Figure 2 FTIR Spectral for Sample 1b ((E)-1-(4-bromophenyl)-2-methyl-3-phenylprop-2-en-1-one)

Table 4 FTIR table for Sample 1b((E)-1-(4-bromophenyl)-2-methyl-3-phenylprop-2-en-1-one)

Absorption peaks (cm^{-1})	**Reference Absorption peaks (cm^{-1})**	**Appearance**	**Functional group**	**Compound class**	**Comment**
3026	3030 (Approx.)	Weak	C-H stretching	Aromatic	Present
2950.20	3000 - 2840	Weak/Sharp	C-H stretching	Alkane	Present
2864.50	3000 - 2840	Weak	C-H stretching	Alkane	Present
1772.30	2000 - 1620	Weak/Sharp	C-H bending	Alkane	Overtone Present
1425.70	1450 - 1400	Strong	C=O stretching	Carboxylic acid	Present
877.80	890 – 820	Medium	Ar-C stretching	Aromatic	Present
842.00	890 – 820	Medium	Ar-C stretching	Aromatic	Present
743.60	800 - 5000	Strong	C-X stretching	Halogens	Present
710.10	800 - 5000	Strong	C-X stretching	Halogens	Present
698.90	800 - 5000	Strong	C-X stretching	Halogens	Present

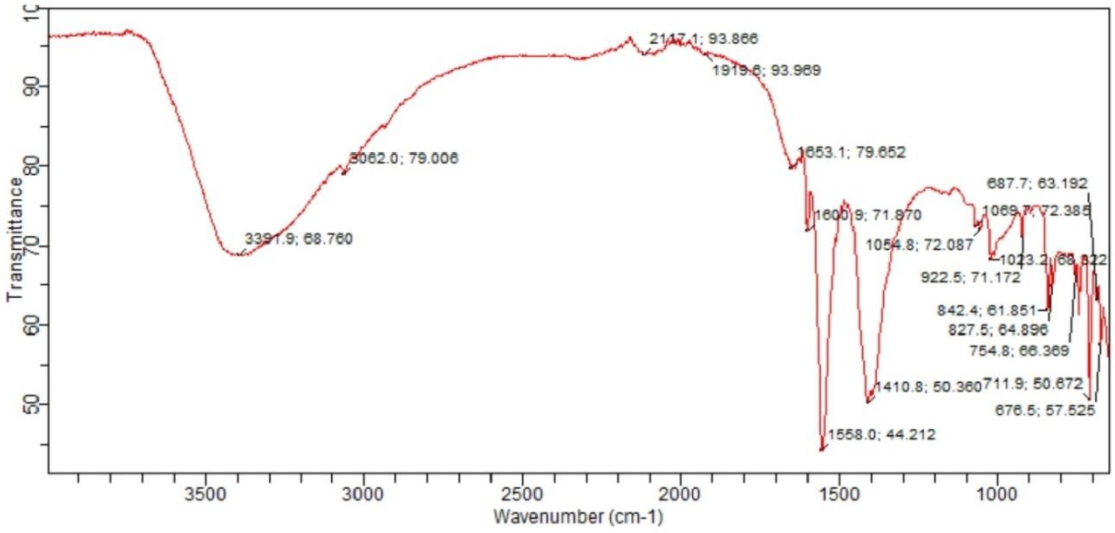


Figure 3 FTIR Spectral for Sample 2a (4-methyl-3,5-diphenylisoxazolidine)

Table 5 FTIR table for Sample 2a (4-methyl-3,5-diphenylisoxazolidine)

Absorption peaks (cm^{-1})	Reference Absorption peaks (cm^{-1})	Appearance	Functional group	Compound class	Comment
3391.90	3550 – 3200	Medium/Broad	N-H stretching	Amine	Present
3062.00	3000 – 2840	Weak/Sharp	C-H stretching	Alkane	Present
1919.60	2000 – 1620	Weak	C-H bending	Alkane	Overtone present
1653.10	1680 – 1620	Weak/sharp	C=C stretching	Alkene	Present
1600.90	1650 – 1550	Strong/Sharp	N-H stretching	Amine primary	Present
1558.00	1650 – 1550	Strong/Sharp	N-H stretching	Amine primary	Present
1069.70	1230 -1030	Medium	C-N stretching	Amine	Present
1023.20	1300 – 1000	Medium	C-O stretching	Alcohol, Ester, Ether and Carboxylic acid	Present
827.50	890 – 820	Medium	Ar-C stretching	Aromatic	Present
824.40	890 – 820	Medium	Ar-C stretching	Aromatic	Present

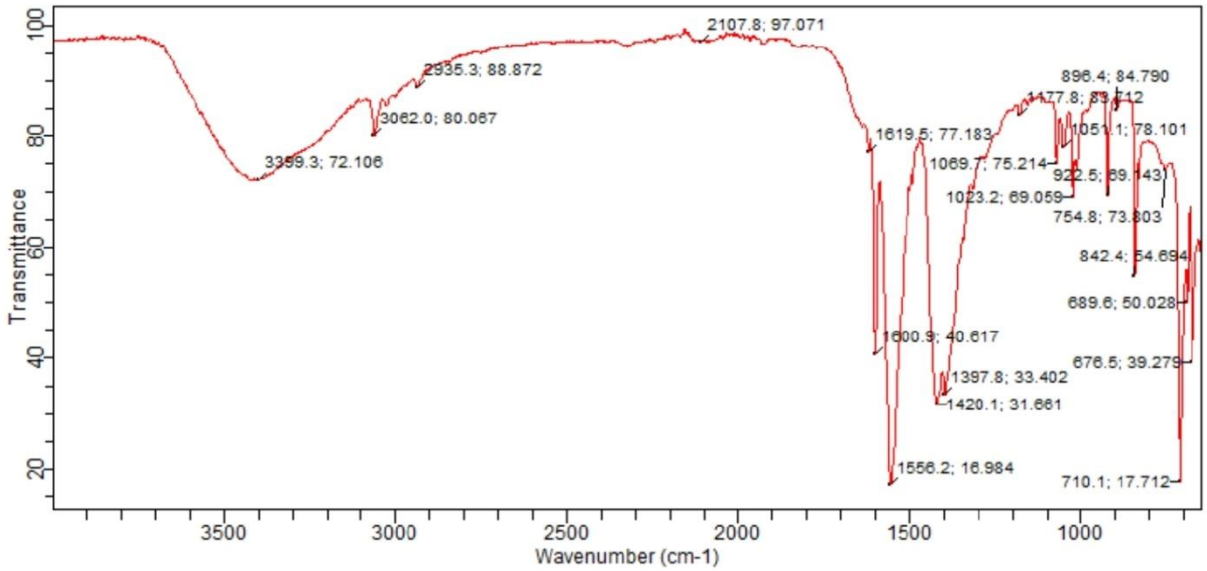


Figure 4 FTIR Spectral for Sample 2b (3-(4-bromophenyl)-4-methyl-5-phenylisoxazolidine)

Table 6 FTIR table for Sample 2b (3-(4-bromophenyl)-4-methyl-5-phenylisoxazolidine)

Absorption peaks (cm^{-1})	Reference Absorption peaks (cm^{-1})	Appearance	Functional group	Compound class	Comment
3359.30	3550 - 3200	Medium/Broad	N-H stretching	Amine	Present
2935.30	3000 - 2840	Weak/Sharp	C-H stretching	Alkane	Present
1619.50	1680 - 1620	Weak/sharp	C=C stretching	Alkene	Present
1600.90	1650 - 1550	Strong/Sharp	N-H stretching	Amine primary	Present
1556.20	1650 - 1550	Strong/Sharp	N-H stretching	Amine primary	Present
1177.70	1230 -1030	Medium	C-N stretching	Amine	Present
1023.20	1300 - 1000	Medium	C-O stretching	Alcohol, Ester, Ether and Carboxylic acid	Present
842.40	890 - 820	Medium	Ar-C stretching	Aromatic	Present
754.80	800 - 500	Medium	C-X stretching	Halogen (Br)	Present
689.60					
676.50					

Characterization of the synthesized compounds

Fortier transform Infra-red (FTIR) spectrometry Analysis

FTIR spectroscopy is a fundamental technique for identifying functional groups within a molecule, thus providing crucial evidence to confirm the chemical structure of synthesized compounds. The analysis of these tables helps to verify the successful formation of the isoxazolidine ring and the presence of expected substituent [18]. As these are isoxazolidine derivatives (a five-

membered heterocyclic ring containing oxygen, nitrogen, and carbon atoms), along with methyl and phenyl groups, several characteristic absorption bands are expected [19].

Sample 1a (E)-2-methyl-3-diphenylprop-2-en-1-one) and 1b((E)-1-(4-bromophenyl)-2-methyl-3-phenylprop-2-en-1-one) are chalcones and serve as only intermediate for the syntheses of the desired isoxazolidine derivatives. Sample 1a ((E)-2-methyl-3-diphenylprop-2-en-1-one) is an enone, which implies the presence of a carbonyl group (C=O) conjugated with an alkene (C=C) and possibly phenyl groups. C=O Stretching (Carboxylic acid). A strong absorption peak at 1410.80 cm^{-1} (reference 1450-1400 cm^{-1}) (Table 3) is assigned to C=O stretching. Similar peaks are also seen in sample 1b at 1425.70 cm^{-1} (Table 4). C-H Stretching (Alkane) Peaks typically appear in the 2950-2840 cm^{-1} range (2950.20, 2864.50 cm^{-1}) (Figure 1 and Figure 2) are observes across all samples (1a and 1b). The presence of peaks at 879.70, 877.80, and 848.00cm^{1} (Table 3 and Table 44) confirms the aromatic nature of the compound, consistent with "diphenyl" in its name. Crucially, Table 4 shows strong absorption peaks at 743.60 cm^{-1}, 710.10 cm^{-1} and 698.90 cm^{-1} (reference 800-500 cm^{-1}) assigned to C-X stretching for halogens. This directly confirms the successful incorporation of the bromine atom into the molecular structure of compound 1b [20].

Compounds 2a (4-methyl-3,5-diphenylisoxazolidine)as well as 2b (3-(2-chlorophenyl)-4-methylisoxazolidin-3-yl)phenol)shows medium/broad absorption peaks in the 3390-3200 cm^{-1} range (i.e3390.40 and 3359.50) (Table 5 –Table 6). These are consistently assigned to N-H stretching of a primary amine, these correspond to the results obtained by Arwa *et al.* [21]. This is a crucial band for isoxazolidines as the nitrogen in the ring often has an associated N-H bond, confirming the presence of the nitrogen atom in the desired heterocyclic system, likely from the precursor amine used in the cyclization reaction [18]. The "medium/broad" appearance is typical for N-H stretching, especially if involved in hydrogen bonding.Weak/sharp peaks are observed in the 2970-2840 cm^{-1} range (i.e 2970.60 and 2929.70) (Figure 3 - Figure 4), indicating the presence of aliphatic C-H bonds from the methyl groups and potentially other alkyl portions of the structure [12, 21].

Peaks around 3060 cm^{-1} (Table 4.8) and similar regions (though not explicitly listed as "aromatic C-H" in all tables, implied by aromatic Ar-C stretching) indicate the presence of the phenyl groups.Weak/sharp peaks in the 2000-1620 cm^{-1} range (i.e 1919.60, 1996.00 cm^{-1}) are assigned as overtones or C-H bending vibrations.Peaks around 1680-1620 cm^{-1} (i.e, 1633.10, 1638.20 cm^{-1}) are assigned to C=C stretching. While the starting materials were enones, the final isoxazolidine ring is saturated [22].Strong/sharp

or medium peaks in the 1650-1580 cm^{-1} range (i.e 1600.90, 1599.80, 1592.40 cm^{-1}) are assigned to N-H bending of a primary amine, providing further evidence for the amine functionality within the isoxazolidine structure [18]. Strong/sharp peaks around 1250 cm^{-1} (i.e 1250.70, 1250.60) are assigned to C-N stretching of an amine, consistent with the isoxazolidine ring.Medium peaks in the 1300-1000 cm^{-1} range (i.e, 1023.20, 1080.90, 1099.60 cm^{-1}) (Table 5 – Table 6), are attributed to C-O stretching, consistent with the ether linkage within the isoxazolidine ring (-O-N-). This is a crucial band for confirming the formation of the isoxazolidineheterocycle, as it contains an oxygen atom [18, 23]. Medium or strong peaks in the 890-820 cm^{-1} range (i.e, 827.50, 824.40, 842.40cm^{-1}) are characteristic of out-of-plane bending vibrations of aromatic C-H bonds, confirming the presence of the phenyl rings [18]. The slight variations in these positions can give hints about the substitution patterns on the phenyl rings.A strong absorption peaks at 754.00 cm^{-1}, 689.70 $cm^{-1}$and 676.00 cm^{-1}(reference 800-500 cm^{-1}) are assigned to "Halogen (Br)". This unequivocally confirms the successful incorporation of the bromine atom into the structure of 2b(3-(4-bromophenyl)-4-methyl-5-phenylisoxazolidine), which aligns with its proposed structure (Figure 4) [24].

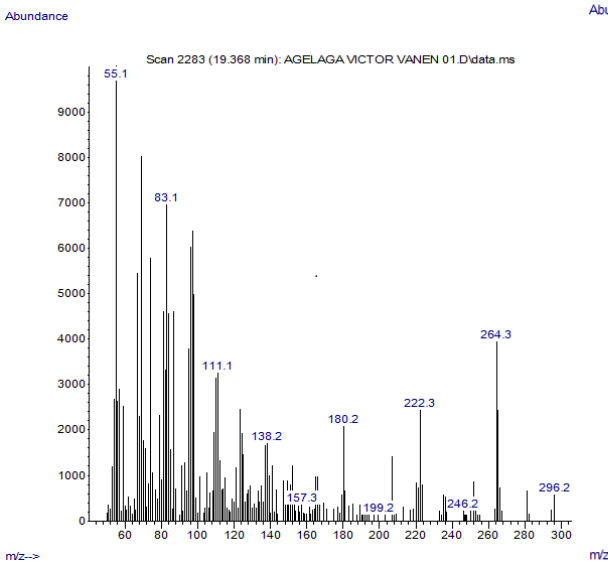


Figure 5 GCMS Spectrum ofSample 1a ((E)-2-methyl-3-diphenylprop-2-en-1-one)

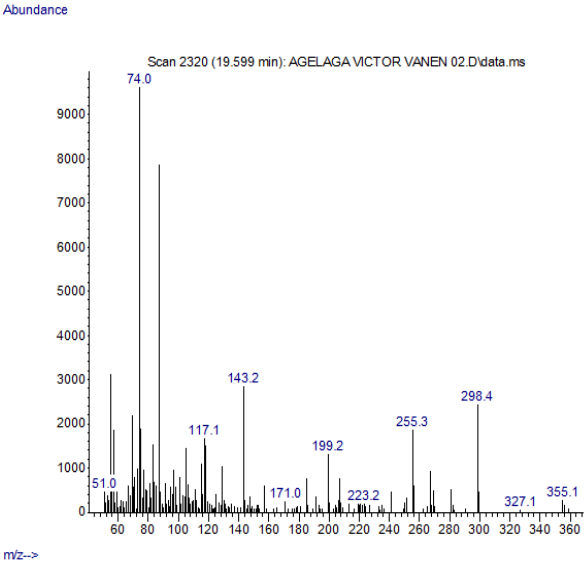


Figure 6 GCMS Spectrum of sample 1b ((E)-1-(4-bromophenyl)-2-methyl-3-phenylprop-2-en-1-one)

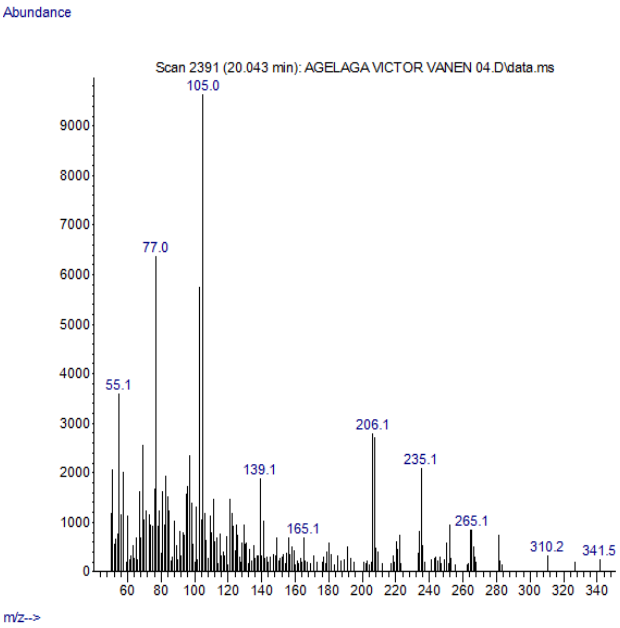


Figure 7 GCMS Spectrumofsample 2a (4-methyl-3,5-diphenylisoxazolidine)

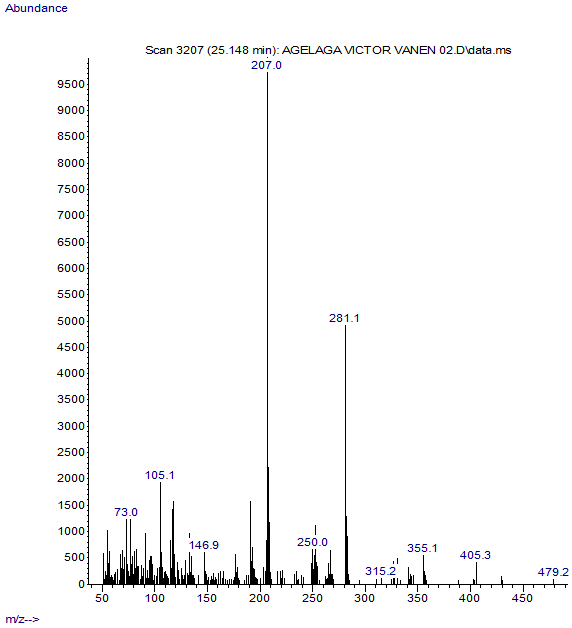


Figure 8 GCMS Spectrum of sample 2b (3-(4-bromophenyl)-4-methyl-5-phenylisoxazolidine)

Gas Chromatography- Mass Spectrometry (GCMS) Analysis

GCMS (Gas Chromatography-Mass Spectrometry) is a powerful analytical technique used to separate, identify, and quantify components in a mixture [25]. Figure 5 shows the GCMS Spectrum of Sample 1a, which indicate a molecular ion peak at m/z = 222.3. The molecular weight of the compound, (E)-2-methyl-1,3-diphenylprop-2-en-1-one, is calculated to be 222.20 g/mol. The observed molecular ion peak (M+) at m/z = 222.3, which is in close agreement with the calculated molecular weight, provides strong evidence that the correct compound was synthesized. This is a primary method for structural confirmation in organic synthesis. The presence of other peaks in the spectrum (e.g., m/z = 55.1, 83.1, 111.1, 138.2, 180.2, 264.3, 296.2) corresponds to fragments formed from the parent molecule during the mass spectrometry process. The fragmentation pattern is unique to the structure of the molecule and further supports the proposed structure [26].

The GCMS spectrum of sample 1b, identified as (E)-1-(4-bromophenyl)-2-methyl-3-phenylprop-2-en-1-one (Figure 6), suggest the successful synthesis and structural identity of this intermediate. The key finding is the molecular ion peak (M+) at m/z = 298.40, which closely aligns with the calculated molecular weight of 300.90 g/mol. The difference between the observed and calculated values is due to the natural isotopic abundance of bromine, which has two major isotopes, ^{79}Br and ^{81}Br. The peak at m/z 298.4

corresponds to the lighter ^{79}Br isotope. The presence of other peaks (e.g., m/z 143.2 and 117.1) corresponds to fragments of the molecule, which provides further evidence for the proposed structure [27].

Figure 7 shows the GCMS Spectrum of sample 2a (4-methyl-3,5-diphenylisoxazolidine). The spectrum shows a molecular ion peak (M+) at m/z = 235.10. This value is in close proximity to the calculated molecular weight of 238.00 g/mol for the proposed structure. The small difference might be attributed to the loss of a proton or some other minor fragmentation during the ionization process, which is not uncommon. Li *et al.* [28], detailed how minor mass discrepancies can arise from the fragmentation of the parent ion, leading to peaks at $M\text{-}H^+$ or $M\text{-}2H^{+\cdot}$ The presence of a major peak at the expected mass confirms the successful formation of the oxazolidine ring structure from the chalcone intermediate. The fragmentation pattern observed in the spectrum, with significant peaks at m/z = 105.0, 77.0, and 206.1, provides further structural confirmation. The peak at m/z 105.0 likely corresponds to a benzoyl cation ($C_6H_5CO^+$) or a similar fragment, which is a common fragmentation of molecules containing a phenyl group. Jampilek, [29], discussed the characteristic fragmentation patterns of similar heterocyclic compounds, noting that the cleavage of the oxazolidine ring and loss of substituent groups are key indicators of the structure.

Figure 8 shows the GCMS Spectrum of sample 2b, the spectrum shows a molecular ion peak (M+) at m/z = 315.20. This value is in good agreement with the calculated molecular weight of 317.00 g/mol for the proposed structure. The observed mass corresponds to the lighter bromine isotope ^{79}Br.

As confirmed in Potapskyi *et al.* [27] that the presence of a halogen atom like bromine is often indicated by two molecular ion peaks separated by two mass units, reflecting the natural abundance of its isotopes (^{79}Br and ^{81}Br). The intense peak at m/z 281.1 likely corresponds to a major fragment, which is also a key indicator of the molecular structure.

Table 7 Antimicrobial Activities: Zone of inhibition of compounds in mm (concentration of cpds in mg/mL)

Compounds Organisms		**2a**	**2b**
Escherichia coli	100	14	18
	50	11	16
	25	0	14
	12.5	0	12
	SD	35	34
Staphylococcus aureus	100	15	17
	50	12	15
	25	0	15
	12.5	0	0
	SD	24	25
Bacillus subtilis	100	16	20
	50	14	18
	25	12	15
	12.5	0	14
	SD	30	32
Pseudomonas aeruginosa	100	12	13
	50	0	11
	25	0	0
	12.5	0	0
	SD	40	41
Aspergillus niger	100	18	14
	50	16	12
	25	13	0
	12.5	0	0

	SD	45	40
Candida albicans	100	22	20
	50	20	17
	25	18	15
	12.5	16	13
	SD	21	20

2a: 4-methyl-3,5-diphenylisoxazolidine,

2b: 3-(4-bromophenyl)-4-methyl-5-phenylisoxazolidine,

SD: Ampicillin for Antibacterial and Fluconazole for Antifungal.

Antimicrobial Activity

Table 7 shows that all derivatives exhibit antimicrobial activity against both Gram-positive (*S. aureus*, *B. subtilis*) and Gram-negative bacteria (E. coli, *P. aeruginosa*), as well as antifungal activity against *A. niger* and *C. albicans*. Ampicillin was used as a standard for bacterial strains, Compound 2b (3-(4-bromophenyl)-4-methyl-5-phenylisoxazolidine) showed the most potent antimicrobial activity with zones of inhibition up to 18 mm against *E. coli* at 100mg/ml which was appreciable comparable to the standard antibiotic Ampicillin (SD), and 14 mm against *A. niger* and 20 mm against *C. albicans* at 100 mg/ml, which were commendable compare to Fluconazole which was use as the antifugal standard, but this is a bit lower than 18 mm against *A. niger* and 22 mm against *C. albicans* at 100mg/ml observed in sample 2a. This is similar to the results of Wang *et al.* [30].

Moderate activities were observed for 2a with notable antibacterial effects against *B. subtilis* and *P. aeruginosa* respectively, aligning with substitution patterns affecting cell wall penetration and enzyme inhibition [31]. The result obtained here correspond with the results reported by Hamza [32] (8 – 26 mm), who carried out determination of antimicrobial of some synthesized Isoxazolidine derivatives.

Table 8 Minimum Inhibitory Concentration (MIC) (concentration of cpds in mg/ml)

Compounds Organisms	**2a**	**2b**
Escherichia coli	50	12.5
Staphylococcus aureus	50	25
Bacillus subtilis	25	6.25
Pseudomonas aeruginosa	50	50
Aspergillus niger	12.5	50
Candida albicans	6.25	6.25

Table 9 Minimum Bactericidal Concentration (MBC)/ Minimum Fungicidal Concentration (MFC) results (concentration of cpds in mg/ml)

Compounds Organisms	**2a**	**2b**
Escherichia coli	100	25
Staphylococcus aureus	100	50
Bacillus subtilis	50	25
Pseudomonas aeruginosa	100	100
Aspergillus niger	25	100
Candida albicans	12.5	25

2a: 4-methyl-3,5-diphenylisoxazolidine, 2b: 3-(4-bromophenyl)-4-methyl-5-phenylisoxazolidine

The Minimum Inhibitory Concentration (MIC)

Table 8 presents the MIC values of the synthesized compounds (2a-2b). Lower MIC values indicate higher antimicrobial potency. The MIC values ranged from 12.5 to 100 mg/mL, with the lowest MIC observed for compound 2b against *S. aureus* (25 mg/mL) and *C. albicans* (6.25 mg/mL), showing promising antimicrobial potency. This suggests that the 4-Br substitution on the phenyl group (as indicated by R2 for 2c being 4-Br) may play a crucial role in enhancing antimicrobial activity [16]. The results confirm the high potency of compounds 2b, against gram-positive bacteria than 2a. Compound 2a has a lowest MIC of 25 mg/ml against *A. niger* and 6.25mg/ml against *C. albicans* highlighting its high antifungal potential compared to 2b [33, 34]. The results obtained in this study is similar of Arwa *et al.* [21] who reported a MIC values ranging from 6.25 – 100 mg/mL on Bis(5-isoxazolidine) derivative.

Minimum Bactericidal Concentration (MBC) and Minimum Fungicidal Concentration (MFC)

Results

Table 9 presents the MBC/MFC values, indicating the minimum concentration of the compound required to kill 99.9% of the initial bacterial and fungal inoculums. MBC/MFC values corroborated MIC results by confirming bactericidal or fungicidal capabilities at relatively low concentrations, especially for 2b. Compound 2b has an MBC of 12.5 mg/ml against*E. coli* and an even lower MBC of 6.25 mg/ml, against *B. subtilis*, compared to the 2a highlighting it's high antibacterial capabilities [34]. MFC values for *A. niger* and *C. albicans* are relatively low, particularly for both compounds 2a, and 2b. Compound 2a shows an MFC of 12.5 mg/ml against *A. niger* and 6.25mg/ml against *C. albicans* while 2b shows an MFC of 50 mg/ml against *A. niger* and 6.25 mg/ml against *C. Albicans* highlighting their antifungal potential [33, 35]. The variation of biological activity among derivatives underscores the importance of chemical modifications on antimicrobial potency, as supported by extensive literature on isoxazolidines and chalcone analogs [36].

Conclusion

This study successfully achieved its aim of synthesizing novel 4-methyl-3,5-diphenylisoxazolidine derivatives (2a–2b), as well as the appropriate chalcones (1a -1b), via efficient and rapid synthetic routes (microwave asisted synthesis), achieving an excellent yields (94.53% and 95.75%) for Isoxazolidine derivative and moderate to high yields ranging from 68.40% to 98.44% for the intermediate Chalcone. Characterization by FTIR identified characteristic functional groups including amine (N–H) (3550 – 3200 cm^{-1}), alkane/aromatic C–H (3000 – 2840 cm^{-1}), and halogen-specific bonds (800 – 500 cm^{-1}), validating the successful incorporation of substituents (bromo). GCMS analysis further suggest the molecular weights (235.1 and 315.2 g/mol respectively) and molecular structures

consistent with the targeted derivatives, including confirmation of halogen substitutions.

Biological evaluation revealed that all synthesized compounds exhibited significant antimicrobial activities against a broad spectrum of microorganisms, including Gram-positive and Gram-negative bacteria, as well as fungi. Notably, derivatives with halogen (Br) substituent (2b) showed enhanced antimicrobial potency, reflected in lower MIC, MBC, and MFC values and larger zones of inhibition compared to the unsubstituted compound (2a), with MIC values ranging from 6.25 to 50 mg/mL These results suggest that such substitutions improve interactions with microbial targets, potentially through increased membrane permeability or enzyme inhibition.

Overall, the study validates the potential of 4-methyl-3,5-diphenylisoxazolidine scaffolds as promising antimicrobial agents, with structural modifications offering avenues to optimize activity. The comprehensive spectroscopic and chromatographic analyses provide a solid foundation for further medicinal chemistry exploration.

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