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Enantioselective Construction of Novel Bis(5-Isoxazolidine) Scaffolds and Their In Silico Evaluation for Antioxidant and Antimicrobial Potential

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ABSTRACT

A series of newly synthesized mono-5-isoxazolidine and bis(5-isoxazolidine) derivatives were obtained as bicyclic cycloadducts. These compounds were rationally designed and constructed through a 1,3-dipolar cycloaddition between nitrones and 3,9-divinyl-2,4,8,10-tetraoxaspiro(5.5)undecane, with the goal of discovering new antimicrobial and antioxidant agents. The resulting isoxazolidines were thoroughly characterized using FT-IR, ^{13}C-NMR, and ^{1}H-NMR spectroscopic techniques. The physicochemical properties—including lipophilicity and predicted bioactivity scores—were evaluated using computational methods. Pharmacokinetic parameters, such as absorption, distribution, metabolism, and excretion (ADME), were also predicted in silico. Biological screening demonstrated that many of the synthesized compounds possess notable antimicrobial activity against a range of bacterial strains, including the Gram-negative species Pseudomonas aeruginosa and Escherichia coli, as well as the Gram-positive species Streptococcus pyogenes and Staphylococcus aureus. Several compounds also exhibited antifungal activity against Candida albicans, Aspergillus niger, and Aspergillus clavatus. In many cases, the compounds performed better than standard reference drugs. Antioxidant evaluation at different concentrations revealed that selected cycloadducts displayed strong, moderate, or weak radical-scavenging properties. Molecular docking studies further supported the biological findings, showing that the synthesized molecules bind effectively—often more favorably than the standard drug—to the active site of pantothenate synthetase (PDB ID: 2X3F).

Keywords: Isoxazolidines, Bicycloadducts, Nitrones, 1,3-Dipolar cycloaddition reaction, Drug-likeness, Docking study

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Introduction

The steady rise in infections caused by bacterial and fungal pathogens—driven largely by the widespread and often indiscriminate use of antibiotics—has led to increasing antimicrobial resistance, greatly diminishing therapeutic effectiveness and contributing significantly to global morbidity and mortality [1]. In response to the rapid emergence of resistant strains, the development of new and potent antibacterial and antifungal agents incorporating innovative chemical scaffolds has become essential [2, 3]. Among these, various heterocyclic families, including isoxazolidines, have demonstrated substantial biological potential [4]. Isoxazolidine derivatives are known to exhibit diverse pharmacological properties, such as antibacterial and antifungal activities [5], as well as anticancer and anti-inflammatory effects [6].

Nitrones represent an important class of 1,3-dipoles frequently used as precursors in [3 + 2] cycloaddition reactions with alkenes, enabling the straightforward synthesis of enantiomerically enriched heterocycles such as isoxazolidines [7, 8]. These isoxazolidines can be readily transformed into other biologically relevant structures—including amino sugars [9], β-lactams, alkaloids [10, 11], and amino acids [12]—via reductive cleavage of the N–

O bond [13]. Due to the accessibility of this heterocycle through 1,3-dipolar cycloaddition [14], the isoxazolidine framework is particularly valuable in the design of structurally modified drug candidates.

The nitrone–alkene (3 + 2) cycloaddition is one of the most extensively explored reactions of this type. Because electron-donating or electron-withdrawing substituents on the alkene polarize the C=C double bond, both the C–C and C–O bonds are formed simultaneously. This polarization influences molecular orbital interactions during the transition state—specifically the HOMO(dipole)–LUMO(dipolarophile) or LUMO(dipole)–HOMO(dipolarophile) interactions—ultimately dictating the regio- and stereochemical outcome of the reaction. Typically, electron-rich dipolarophiles favor the formation of 5-substituted isoxazolidines, whereas those bearing electron-withdrawing groups such as –CN, –CHO, or –CO_2R predominantly yield 4-substituted isoxazolidines [8]. Such electronic effects also guide the cycloaddition through either endo or exo transition states. Domingo [15] further demonstrated that regio- and stereoselectivity are strongly influenced by the size of the reacting partners. Simple nitrones tend to favor endo selectivity, whereas bulkier nitrones and dipolarophiles promote an exo approach.

Bis(isoxazolidines), characterized by two isoxazolidine rings arranged symmetrically around a central unit, are obtained by 1,3-dipolar cycloaddition of nitrones to symmetrical dipolarophiles containing two double bonds [16]. These bis-adducts have numerous industrial and pharmaceutical applications, including roles in the synthesis of therapeutic agents, agrochemicals, and polymeric materials [17]. They also share many biological activities reported for mono-isoxazolidines, such as antibacterial and antifungal effects.

In the present work, we report the design, synthesis, and characterization of a new series of mono-5-isoxazolidines and bis(5-isoxazolidines) (compounds 3 and 4) prepared through 1,3-dipolar cycloaddition of C-aryl-N-methyl nitrones (1a–l) with the symmetric alkene 3,9-divinyl-2,4,8,10-tetraoxaspiro(5.5)undecane **(Figure 1)**. Our study aims to contribute to ongoing drug discovery efforts by generating bioactive heterocycles with potential antioxidant and antibacterial properties. The regio- and stereoselective aspects of the cycloaddition reactions are discussed in detail. The synthesized compounds were screened in vitro for their antibacterial and antioxidant activities. Additionally, their pharmacokinetic and pharmacodynamic profiles were predicted using SwissADME, while molecular docking analyses were conducted on the most active derivatives to assess their suitability as future therapeutic candidates.

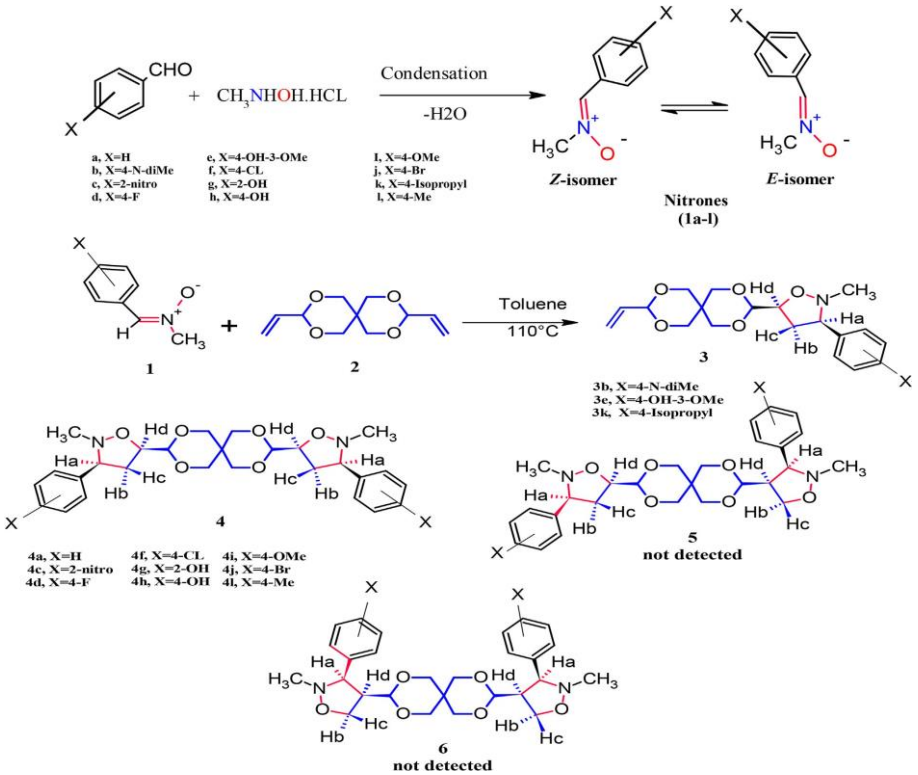

Figure 1. Synthesis of nitrone (1a-j) and of isoxazolidines derivatives. (1) Nitrones (1a-l); (2) bis-dipolarophile; (3) mono-5-isoxazolidines isomer**;** (4) (bis-5 isoxazolidine isomer); (5) (bis-4,5- isoxazolidine isomer); (6) (bis-4 isoxazolidine isomer).

Materials and Methods

Chemicals and physical measurements

All reagents and chemicals were purchased from Sigma-Aldrich and were of high purity (99–99.9%). Reaction progress was monitored by thin-layer chromatography (TLC) using a benzene–methanol solvent system (8:2, v/v) as the mobile phase. Melting points were determined on a CL-726 digital apparatus (IndiaMART Member Since, Noida, India). FTIR spectra were recorded on a Nicolet iS10 spectrophotometer (Thermo Scientific, Waltham, MA, USA) equipped with a diamond ATR accessory. Each spectrum was obtained from 32 scans at a resolution of 4 cm^{-1} over the range of 4000–500 cm^{-1}. ^{1}H and ^{13}C NMR spectra were acquired on a JEOL ECP-400 spectrometer (Tokyo, Japan) operating at 400 MHz. Elemental composition (C, H, N, S) was analyzed using an EA 300 elemental analyzer.

Physicochemical and ADME properties

SwissADME (http://www.swissadme.ch/) [3, 18–20] is an online platform used to calculate physicochemical descriptors and predict pharmacokinetic behavior, including ADME-related properties. It also provides insight into drug-likeness and medicinal chemistry parameters, making it a valuable tool in early-stage drug discovery. The synthesized isoxazolidine derivatives were evaluated for several key physicochemical features, including (1) molecular weight within the range of 150–500 g/mol; (2) hydrogen bond acceptors fewer than 10; (3) rotatable bonds between 0 and 9; (4) hydrogen bond donors fewer than 5; (5) fraction of sp^3 carbons (Fsp^3) between 0.25 and 1; and (6) topological polar surface area (TPSA) between 20 and 160 $Å^2$. Predicted pharmacokinetic and drug-likeness profiles were also assessed, covering parameters such as blood–brain barrier (BBB) permeability, gastrointestinal absorption (GI), skin permeability, plasma P-glycoprotein (P-gp) interactions, and binding or inhibitory effects on major human cytochrome P450 enzymes (CYP1A2, CYP2D6, CYP2C19, CYP3A4, and CYP2C9), which collectively mediate approximately 90% of drug metabolism [21]. These enzyme interactions are essential, as they influence both xenobiotic and endogenous compound metabolism. Drug-likeness and medicinal chemistry characteristics for all synthesized derivatives are summarized in **Table 3**. Additionally, bioavailability radar plots were generated, where the pink region represents the optimal physicochemical range. A compound whose radar profile falls entirely within this region is considered to exhibit favorable drug-like properties [22]. Pharmacokinetic performance was further analyzed using the BOILED-Egg model **(Figure 10)**, which visualizes passive gastrointestinal absorption and blood–brain barrier penetration.

Antimicrobial assay

The microorganisms used to assess antimicrobial activity included both bacterial and fungal strains. The bacterial panel consisted of two Gram-positive species—Streptococcus pyogenes (MTCC 442) and Staphylococcus aureus (MTCC 96)—and two Gram-negative species—Pseudomonas aeruginosa (MTCC 1688) and Escherichia coli (MTCC 443). The fungal strains evaluated were Aspergillus niger (MTCC 282), Aspergillus clavatus (MTCC 1323), and Candida albicans (MTCC 227). All strains were procured from the Institute of Microbial Technology (IMTECH), Chandigarh.

Antimicrobial efficacy was determined using the minimum inhibitory concentration (MIC) assay based on the macro-dilution double-dilution technique [23]. Dimethyl sulfoxide (DMSO, 5%) served as the solvent for preparing the test compound solutions. Serial dilutions of the synthesized compounds were prepared at concentrations of 1000, 500, 250, 200, 100, 50, 25, 12.5, and 6.25 µg/mL in labeled test tubes. Each tube received 1 mL of nutrient broth followed by inoculation with 10 µL of the corresponding bacterial or fungal suspension. The tubes were incubated at 37 °C for 18–24 hours, after which microbial growth was assessed by observing turbidity and compared against reference standards.

Ampicillin and chloramphenicol were employed as standard antibacterial agents due to their broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Nystatin and griseofulvin were used as reference antifungal drugs, as they are well-established agents for the treatment and prevention of fungal and yeast infections.

Antioxidant assay

A Shimadzu double-beam UV–Visible spectrophotometer was employed to measure absorbance. A 2 mM solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) was prepared in 95% ethanol, and the test samples were similarly

dissolved in 95% ethanol. A blank solution consisted of 2 mL of 95% ethanol. For each measurement, 1 mL of the DPPH solution was mixed with 1 mL of the test sample, and the absorbance was recorded at 515 nm. Ascorbic acid served as the reference antioxidant (positive control). All measurements were performed in triplicate. The samples were analyzed at eight concentrations (1, 2, 5, 10, 25, 50, 100, and 200 µg/mL). The IC_{50} values were determined from the corresponding dose–response curves and are summarized in **Table 3**.

Molecular docking

Previous studies have reported that isoxazolidine derivatives possess notable antibacterial properties. Methicillin-resistant Staphylococcus aureus (MRSA) remains a significant global health concern due to its well-documented multidrug resistance [24]. For molecular docking studies, the three-dimensional crystal structure of the S. aureus Sar2676 protein (PDB ID: 2X3F), which contains no amino acid sequence mutations and has a resolution of 1.95 Å, was obtained from the Protein Data Bank (http://www.rscb.org) [25]. Docking simulations were subsequently conducted using the Molecular Operating Environment (MOE 2015.10) software.

Pre-docking preparation

Both the synthesized compounds (ligands) and the target protein were prepared, optimized, and energy-minimized using the MOE software suite. The ligands were constructed using the MOE builder module, followed by 3D protonation, assignment of partial charges, and energy minimization employing the MMFF94x force field with an RMS gradient of 0.05 kcal/mol/$Å^2$. The optimized ligand structures were then saved as a database file in MOE format.

Preparation of the target protein (PDB ID: 2X3F) followed previously reported protocols [26]. During preprocessing, water molecules, the B chain, and the SO_4 group were removed. The structure was subsequently 3D-protonated, energy-minimized, and corrected to generate a refined model consisting of 4464 atoms. The active site of the protein was identified using the Site Finder module within MOE.

Docking methodology

Molecular docking was performed following the methodology described previously [27]. The Triangle Matcher placement algorithm was used in combination with the London dG scoring function, applying the default MOE docking parameters (function 1). During the refinement step, the protein receptor was kept rigid while the ligands were allowed full flexibility. Each ligand was permitted to generate up to five binding poses with the active site. The docking scores of the best-fit poses for interactions with the active site of PDB ID 2X3F were subsequently recorded.

General procedure for the preparation of C-aryl-N- methyl nitrones(1a-l)

Nitrones were obtained following a modified version of the method described in reference [28]. N-Methylhydroxylamine hydrochloride (750 mg, 9 mmol) was first dissolved in 20 mL of absolute ethanol with gentle stirring and heated to 40 °C. To ensure complete solubilization, an additional 10 mL of ethanol was added dropwise. Sodium acetate (737 mg, 9 mmol) was then introduced to neutralize the hydrochloride. Subsequently, the substituted benzaldehyde was added gradually to the reaction mixture, which was stirred at room temperature in the dark for 24 hours. The reaction progress was monitored using TLC. After completion, the mixture was filtered, dried over anhydrous magnesium sulfate, concentrated, and the crude nitrone was purified by recrystallization from hot benzene.

Preparation of C-(phenyl)-N-methylnitrone(1a)

Benzaldehyde (913mg) was used for the synthesis as described in the general procedure. Yield 80%; M.p.80–82 °C; R_f 0.45, IR(KBr): 3389 (aromat. H), 3061 (CH_3), 1695(C=N),1594-1442(aromatic.C=C), 1158 (N^+-O^-),1096(C–N), 838, 781, 687 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.00–7.37 (m,5H, aromat. H),7.15 (CH = N) 3.76 (s, 3H, N–CH_3), ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 55.00 (N–CH_3), 128.57–132.00, (Aromat. C), 134.0 (CH = N).

Preparation of C-(4- N-Di methylphenyl)-N-methylnitrone(1b)

4- N-Dimethylamine benzaldehyde (1342mg) was used for the synthesis as described in the general procedure. Yield 72%; M.p.108–110 °C; R_f 0.72, IR(KBr): 3347(N–CH_3),3086(aromat. H),2889 (CH_3),1595 (C=N),1516–

1435 (aromatic. C=C), 1178(N^+-O^-), 1065(C–N), 887, 808 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.22–7.22 (m,5H, aromat.H),7.25 (CH = N), 3.87 (s, 3H, N–CH_3), 3.21 (s, 6H, N–CH_3),^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 39.87(H_3C–N–CH_3), 56.65 (N–CH_3), 120.44–145.06, (Aromat. C), 134.32 (CH = N).

Preparation of C-(2- nitrophenyl)-N-methylnitrone(1c)

2- Nitrobenzaldehyde (1360 mg) was used for the synthesis as described in the general procedure. Yield 84%; M.p.84–86 °C; R_f 0.60, IR(KBr): 3095(aromat. H),2976(CH_3), 1698(C=N), 1574(NO_2), 1507-1414 (aromatic. C=C), 1169 (N^+-O^-), 1054 (C–N), 935, 734, 663 (Ortho-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.02–7.20 (m,4H, aromat.H), 7.15 (CH = N), 3.72 (s, 3H, N–CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 56.33 (CH_3), 126.00–150.00, (Aromat. C), 135.03 (CH = N).

Preparation of C-(4- fluorophenyl)-N-methylnitrone(1d)

4- Florobenzaldehyde (1117 mg(was used for the synthesis as described in the general procedure. Yield 86%; M.p.99–102 °C; R_f 0.64, IR(KBr): 3015 (aromat. H), 2947(CH_3), 1597 (C=N), 1504-1412(aromatic.C=C), 1230(C–F), 1154(N^+-O^-), 1048(C–N), 937, 834, 792 (Para-disubstituted benzene) Cm^{-1}.^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 3.75 (s, 3H, N–CH_3), 7.15 (CH = N),7.20–7.28 (m, 2H, aromat.H. a,e), 8.02–8.08 (m,2H,aromat.H.b,d). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 56.65 (N–CH_3), 120.44–145.06, (Aromat. C), 134.07 (CH = N).

Preparation of C-(4- hydroxy-3-methoxyphenyl)-N-methyl nitrone (1e)

4- Hydroxy-3-methoxy benzaldehyde (1369mg (was used for the synthesis as described in the general procedure. Yield 90%; M.p.171–173 °C; R_f 0.33, IR(KBr): 3353(O–H), 3059(aromat. H),2959,2840 (CH_3),1670(C=N),1585, 1459 (aromatic –C=C-),1282(C–O), 1197(N^+-O^-),1023(C–N),910,879 (Para-substitutedbenzene)724,814(meta sub.) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 9.52 (s,1H, OH),7.37 (d,1H, aromat, Hd) 7.68 (d, 1H, aromat.He), 7.42 (s, 1H, aromat.Ha),7.15 (CH = N), 3.76 (S, 3H, N–CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 54.33(O–CH_3), 56.00 (N–CH_3), 120.05–152.23, (Aromat. C), 135.0 (CH = N)140,78(C–OH).

Preparation of C-(4-chlorophenyl)-N-methylnitrone(1f)

4-Chloro benzaldehyde (1265mg) was used for the synthesis as described in the general procedure. Yield 69%; M.p.114–117 °C; R_f 0.58, IR(KBr):3001 (aromat. H),2759 (CH_3), 1673(C=N),1584-1474(aromatic.C=C), 1158(N^+-O^-),1079(C–N), 939,849,819 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 8.15–8.20 (m, 2H, aromat.H. b,d), 7.35–7.40 (m, 2H, aromat.H. a,e),7.30 (CH = N),3.85–3.90 (s, 3H, N–CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 54.00 (N–CH_3), 127.00–134.74, (Aromat. C), 134.00 (CH = N).

Preparation of C-(2- hydroxyphenyl)-N-methylnitrone(1g)

2- Hydroxy benzaldehyde(1099mg) was used for the synthesis as described in the general procedure. Yield 65%; M.p.134–136 °C; R_f 0.39, IR(KBr): 3446(O–H),3039 (aromat. H),2876 (CH_3),1604(C=N),1580-1458(aromatic.C=C),1265(C–O), 1148(N^+-O^-), 1084(C–N),942,774 (ortho-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 9.73(OH),7.27–7.01 (m,4H, aromat.H),7.20 (CH = N), 3.82 (s, 3H, N–CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 56.33 (N–CH_3), 121.00–130.39, (Aromat. C), 135.24 (CH = N), 144.27(C–OH).

Preparation of C-(4-hydroxyphenyl)-N-methylnitrone(1h)

4- Hydroxy benzaldehyde (1099mg) was used for the synthesis as described in the general procedure. Yield 64%; M.p.178–180 °C; R_f 0.37, IR(KBr): 3446(O–H),3039(aromat. H), 2876 (CH_3), 1580(C=N),1516-1485(aromatic C=C), 1205 (C–O), 1149(N^+-O^-), 1084(C–N), 774 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 9.63(OH),7.29–7.33 (m,2H, aromat. H.a,e), 7.14–7.17 (m, 2H, aromat.H b,d), 7.18 (CH = N), 3.86- (s, 3H, N–CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 56.00 (N–CH_3), 123.20–145.05, (Aromat. C), 134.68(CH = N), 145.05(C–OH).

Preparation of C-(4-methoxyphenyl)-N-methylnitrone(1i)

4-Methoxy benzaldehyde (1225mg) was used for the synthesis as described in the general procedure. Yield 75%; M.p.60–63 °C; R_f 0.60, IR(KBr):3083 (aromat. H), 2946,2838 (CH_3), 1680(C=N), 1599-1413(aromatic.C=C), 1251 (C–O), 1155 (N^+-O^-), 1022(C–N),939,833, 774 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.38–7.06 (m,4H, aromat.H),7.46 (CH = N), 3.94 (S, 3H, CH_3) 3.86 (S, 3H, N–CH_3), ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 54.7(-CH_3)56.78 (N–CH_3), 129.00–132.05, (Aromat. C), 134.12 (CH = N).

Preparation of C-(4-bromophenyl)-N-methylnitrone(1j)

4-Bromo benzaldehyde (1665mg) was used for the synthesis as described in the general procedure. Yield 82%; M.p.124–126 °C; R_f 0.53, IR(KBr): 3373(aromat. H),3001 (CH_3),1676(C=N),1581-1466(aromatic. C=C), 1160 (N^+-O^-), 1057(C–N),998,940,849 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.19–7.35 (m, 4H, aromat. H), 7.28 (CH = N), 3.83 (s, 3H, N– CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 56.86 (N–CH_3), 123.10–152.06, (Aromat. C), 135.11 (CH = N).

Preparation of C-(4- Isopropylphenyl)-N-methylnitrone(1k)

4- Isopropyl benzaldehyde (1333mg) was used for the synthesis as described in the general procedure. Yield 85%; M.p. syrup; R_f 0.37, IR(KBr): 3046(aromat. H),2940,2886 (CH_3),1612(C=N),1580-1457(aromatic. C=C), 1160 (N^+-O^-), 1050(C–N), 876, 806 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.36–7.10 (m, 4H, aromat. H),7.10 (CH = N), 3.74 (s, 3H, N–CH_3), 2.98–3.03 (m, 1H, CH),2.07 (d,6H, CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 25.84(-CH_3),41.50(-CH_2) 57.00 (N–CH_3), 122.00–141.18, (Aromat. C), 134.28 (CH = N).

Preparation of C-(4- methylphenyl)-N-methylnitrone(1l)

4- Methyl benzaldehyde (1081mg) was used for the synthesis as described in the general procedure. Yield 72%; M.p.106–109 °C; R_f 0.34, IR(KBr): 3001(aromat. H), 2993, 2945 (CH_3),1605(C=N),1500-1406 (aromatic. C=C), 1153(N^+-O^-),1043 (C–N),934,834,757 (Para-disubstituted benzene) Cm^{-1}.^{1}H-NMR (400 MHz, $CDCl_3$): δ (ppm) = 7.95–7.10 (m,4H, aromat.H), 7.09 (CH = N), 3.95 (s,3H, N–CH_3), 2.06 (s, 3H, Ar-CH_3). ^{13}C-NMR (400 MHz, $CDCl_3$): δ (ppm) = 24.00 (Ar-CH_3), 54.00(N–CH_3), 126.50,128.02 (Aromat. C), 135.06 (C=N).

Preparation of isoxazolidine derivatives 3,4 (a-l)

The isoxazolidine derivatives were synthesized following the procedures reported in references [29, 30] with slight modifications. In a 100 mL round-bottom flask, the appropriate nitrone was dissolved in 25 mL of toluene with continuous stirring. To this solution, 3,9-divinyl-2,4,8,10-tetraoxaspiro(5.5)undecane (200 mg, 0.94 mmol) was added. The reaction mixture was refluxed at 110 °C for 21–72 hours, and the progress was monitored by TLC until completion. Upon completion, the reaction mixture was allowed to cool, and the solvent was removed under reduced pressure. The residue was treated with 20 mL of water, followed by extraction with 30 mL of chloroform. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated. The crude product was purified by recrystallization from hot ethanol to yield the target isoxazolidine derivatives.

Preparation of 2-methyl-5-(9-(2-methyl-3-phenylisoxazolidin-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-3-phenylisoxazolidine (4a)

Nitrone **(1a)** (254mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (48hr); yield 88%; M.p.77–79 °C; R_f 0.49, IR(KBr): 3087 (CH_3),1588-1435(aromatic. C=C),1248-1203(C–O), 1163(N–O),1024(C–N), 985,850,755 (Mono-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 7.36–6.96 (m, 10H,H-aromatic), 5.75 (ddd, *J* = 15.4, 10.7, 4.3 Hz, 2H,Hd), 5.36 (d, *J* = 11.1, 2H,Hh), 5.28 (d, *J* = 11.3, 2H,Hf), 4.85 (d, *J* = 13.9, Hz, 2H,He), 4.28 (dd, *J* = 11.3, 2.5 Hz, 2H,Ha), 3.78 (s, 6H,2CH_3), 3.65–3.59 (m, 4H,2Hg,2Hf), 3.52 (ddd, *J* = 11.6, 5.2, 2.3 Hz, 2H,Hb), 3.42 (ddd, *J* = 11.6,6.2, 4.3 Hz, 2H,Hc). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 138.81, 129.42, 129.37, 128.16, 127.16, 127.10 (Ar–C), 119.45 (C4,4\), 111.28(C5,5\), 100.45(C7,7\), 69.96(C3,3\), 69.45(C2,2\), 53.45(C6,6\), 41.23(2CH_3), 32.35(C1). Anal calc for $C_{26}H_{29}F_2N_2O_6$: C, 62.02; H, 5.81; N, 5.56. Found: C, 62.12; H, 5.92; N, 5.66.

Preparation of 4-(5-(9-(3-(4-(dimethylamino) phenyl)-2-methyl isoxazolidin-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-2-methylisoxazolidin-3-yl)-N, N-dimethylaniline(3b)

Nitrone (**1b**) (335mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (30hrs); yield 76%; M.p.85–87 °C; Rf 0.76, IR(KBr): 3000(N–CH_3),2950 (CH_3),1555-1420(aromatic.C=C),1245, 1203(C–O), 1162(N–O), 1073, 1040(C–N),899,817,720 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 8.05 (d, *J* = 8.7 Hz, 2H), 6.78 (m,1H,H3),6.67 (d, *J* = 9.2 Hz, 2H), 6.26(m, 2H, H2,H4), 6.20 (dd, *J* = 17.3, 6.4 Hz, 1H, H1) 5.74 (ddd, *J* = 15.5, 10.8, 4.5 Hz, 1H, Hd), 5.35 (d, *J* = 13.7 Hz 2H,Hh), 5.25 (d, *J* = 13.5 Hz, 2H,Hf), 4.86 (d, *J* = 13.6Hz, 1H,He), 4.27 (dd, *J* = 11.3, 2.4 Hz, 1H, Ha), 3.63 (s, 3H, N–CH_3), 3.60 (m, 2H,Hg), 3.56 (m, 2H,Hf), 3.43 (dt, *J* = 11.5, 2.5 Hz, 1H,Hc), 3.40 (dt, *J* = 11.6,4.5 Hz, 1H,Hb), 3.33 (s, 3H, N–CH_3), 3.33 (s, 3H, N–CH_3). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 151.39, 135.42, 129.97, 119.60(ArC), 132.09(C9), 125.05(C10), 119.60(C8), 118.98(C4,4\), 111.60(C5,5\), 100.87(C7,7\), 69.96(C3,3\), 69.44(C2,2\), 53.67(C6,6\), 42.13(2CH_3),40.16 (4CH_3), 32.44(C1). Anal calc for $C_{21}H_{30}N_2O_5$: C, 64.60; H, 7.74; N, 7.17. Found: C, 64.69; H, 7.82; N, 7.25.

Preparation of 2-methyl-5-(9-(2-methyl-3-(2-nitrophenyl) isoxazolidin-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecane-3-yl)-3-(2-nitrophenyl) isoxazolidine(4c)

Nitrone **(1c)** ((339mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (16hrs); yield 74%; M.p.133–135 °C; R_f 0.69, IR(KBr): 2954 (CH_3),1607, 1575(NO_2), 1523-1436 (aromatic C=C),1247, 1203(C–O), 1162(N–O),1072(C–N),935,899,720(orth-o-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D6) δ = 8.40 (d, *J* = 7.9 Hz,2H, aromatic H), 8.00–7.63 (m,2H, aromatic-H),7.62–7.60 (m, 2H, aromatic H), 7.59 (t, *J* = 7.7 Hz, 2H, aromatic-H), 5.81–5.67 (m, 2H,Hd), 5.34 (d, *J* = 11.1 Hz, 2H,Hh), 5.21 (d, *J* = 11.4 Hz, 2H,Hf), 4.85 (m, 2H,He), 4.28 (m, 2H,Ha), 3.78 (s, 6H,2CH_3), 3.58 (m, 4H, Hg,Hi), 3.45 (m, 2H,Hb), 3.33 (m, 2H,Hc). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 148.19, 135.40, 133.61, 130.74, 129.90, 124.76(ArC), 118.96(C4,4\), 101.89(C5,5\), 100.89(C7,7\) 69.96(C3,3\), 69.45(C2,2\), 54.62(C6,6\), 44.09(2CH_3), 32.44(C1). Anal calc for $C_{27}H_{32}N_4O_{10}$: C, 56.64; H, 5.63; N, 9.79. Found: C, 56.71; H, 5.70; N, 9.90.

Preparation of 3-(4-fluorophenyl)-5-(9-(3-(4-fluorophenyl)-2-methylisoxazolidin-4-yl)-1, 5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-2-methylisoxazolidine(4d)

Nitrone **(1d)** (288mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (17hrs); yield 87%; M.p. syrup; R_f 0.78, IR(KBr): 3085 (CH_3),1504-1420(aromatic. C=C),1294-1247 (C–O), 1162(N–O),1048(C–N),937,834,792 (para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 8.32–8.24 (m, 4H,aromatic H), 7.27–7.18 (m, 4H,aromatic-H), 5.75 (ddd, *J* = 15.3, 10.4, 4.6 Hz, 2H,Hd), 5.34 (d, *J* = 11.6 Hz, 2H,Hh), 5.22 (d, *J* = 11.8 Hz, 2H,Hf), 4.86 (d, *J* = 13.8 Hz, 2H,He), 4.25 (dd, *J* = 11.3, 2.5 Hz, 2H,Ha), 3.72 (s, 6H,2CH_3), 3.67–3 (m, 4H,2Hg,2Hf), 3.56 (ddd, *J* = 11.5, 5.1, 2.5 Hz, 2H,Hb), 3.35 (ddd, *J* = 11.8,6.5, 4.5 Hz, 2H, Hc). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 160.23, 139.32, 128.65, 127.98, (Ar–C), 119.13 (C4,4\), 113.45 (C5,5\), 100.82(C7,7\), 69.98 (C3,3\), 69.45(C2,2\), 53.49(C6,6\), 42.09(2CH_3), 32.67(C1). Anal calc for $C_{26}H_{29}F_2N_2O_6$: C, 62.02; H, 5.81; N, 5.56. Found: C, 62.09; H, 5.88; N, 5.64.

Preparation of 4-(5-(9-(3-(4-hydroxy-3-methoxyphenyl)-2-methylisoxazolidin-4-yl)-1,5, 7, 11-tetraoxaspiro [5.5] undecan-3-yl)-2-methylisoxazolidin-3-yl)-2-methoxyphenol(3e)

Nitrone **(1e)** (340mg,1.88mmol) was used for the synthesis as described in the general procedure The mixture was stirred for (30hrs); yield 79%; M.p.124–126 °C; R_f 0.55, IR(KBr): 3079(OH),3028,2960 (CH_3),1513-1436 (aromatic.C=C),1288, 1261,1249(C–O), 1161(N–O),1073(C–N),985,916(para-sub. benzene),720, 778 (meta-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 9.5 (s,1H,OH), 8.08 (d, *J* = 1.8 Hz, 1H, aromatic H), 7.62 (s, 1H, aromatic H), 7.50 (dd, *J* = 8.3, 2.0 Hz, 1H, aromatic H),6.7 (m,1H,H3), 6.26(m, 2H, H2,H4), 6.20 (dd, *J* = 17.4, 6.2 Hz, 1H, H1) 5.75 (ddd, *J* = 15.2, 10.6, 3.9 Hz, 1H, Hd), 5.35 (d, *J* = 13.5 Hz 2H,Hh), 5.21 (d, *J* = 13.7 Hz, 2H, Hf), 4.86 (d, *J* = 13.5 Hz, 1H,He), 4.28 (dd, *J* = 11.4, 2.5 Hz, 1H, Ha), 3.72 (s, 3H, O–CH_3)), 3.65 (m, 2H,Hg), 3.59 (m, 2H,Hi), 3.57 (s,3H, N–CH_3),3.62 (dt, *J* = 11.4, 2.7 Hz, 1H,Hc), 3.42 (dt, *J* = 11.4,4.5 Hz, 1H,Hb). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 148.80, 135.41, 134.32, 123.49, 122.88, 119.87 (Ar–C), 132.45(C9),119.00(C10),118.60(C8), 115.74(C4,4\), 111.97(C5,5\), 100.87(C7,7\) 69.95(C3,3\), 69.43(C2,2\), 55.75(C6,6\)54.00(O–CH_3), 42.68(N–CH_3), 32.45(C1). Anal calc for $C_{20}H_{27}NO_7$: C, 61.06; H, 6.92; N, 3.56. Found: C, 61.18; H, 7.01; N, 3.65.

Preparation of 3-(4-chlorophenyl)-5-(9-(3-(4-chlorophenyl)-2-methylisoxazolidin-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-2-methylisoxazolidine(4f)

Nitrone **(1f)** (319mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (18hrs); yield 95%; m.p.101–103 °C; R_f 0.58, IR(KBr): 3031(CH_3),1591-1435(aromatic.C=C),1285-1203(C–O), 1162(N–O),1073(C–N),935,838 (para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 8.24–8.02 (m, 4H, aromatic H),7.15–7.00 (m, 4H, aromatic-H), 5.71 (ddd, *J* = 15.1, 10.2, 4.1 Hz, 2H, Hd), 5.35 (d, *J* = 11.1, 2H, Hh), 5.24 (d, *J* = 11.3, 2H, Hf), 4.84 (d, *J* = 13.2, Hz, 2H, He), 4.28 (dd, *J* = 11.6, 2.2 Hz, 2H, Ha), 3.71 (s, 6H,2CH_3), 3.63–3.58 (m, 4H,2Hg,2Hf), 3.54 (ddd, *J* = 11 .6, 5.5, 2.8 Hz, 2H, Hb), 3.32 (ddd, *J* = 11.9,6.3, 4.1 Hz, 2H,Hc). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 140.32, 138.96, 135.42, 129.97, (Ar–C), 118.60 (C4,4\), 111.42(C5,5\), 100.89(C7,7\), 69.97(C3,3\), 69.46(C2,2\), 53.69(C6,6\), 40.22(2CH_3), 32.49(C1). Anal calc for $C_{27}H_{32}Cl_2N_2O_6$: C, 58.81; H, 5.85; N, 5.08. Found: C, 58.87; H, 5.91; N, 5.14.

Preparation of 2-(5-(9-(3-(2-hydroxyphenyl)-2-methylisoxazol-idin-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-2-methylisoxa-zolidin-3-yl) phenol(4g)

Nitrone (**1g**) (284mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (15hrs); yield 69%; m.p.112–114 °C; R_f 0.74, IR(KBr): 3036 (OH),2948(CH_3),1584-1454(aromatic. C=C),1388,1259, 1203(C–O),1145(N–O),1072(C–N),932,812,765 (orthodisubstitutedbenzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D6) δ = 8.09 (s, 2H, OH), 7.41–7.28 (m, 4H,aromatic H), 6.86–6.74 (m, 4H,aromatic-H), 5.74 (ddd, *J* = 15.3, 10.3, 4.1 Hz, 2H,Hd), 5.39 (d, *J* = 11.4, 2H,Hh), 5.21 (d, *J* = 11.6 Hz, 2H,Hf), 4.86 (d, *J* = 13.8, Hz, 2H,He), 4.27 (dd, *J* = 11.2, 2.4 Hz, 2H,Ha), 3.77 (s, 6H,2CH_3), 3.67 (m, 2H,Hg), 3.57 (ddd, *J* = 11.5,4.2, 2.4 Hz, 2H,Hb), 3.43 (ddd, *J* = 11.6,5.6,4.4 Hz, 2H,Hc). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 159.03 (C–OH), 141.33, 135.41, 133.90, 132.71, 132.60 (Ar–C), 119.23(C4,4\), 117.79(C5,5\), 100.89(C7,7\), 69.96 (C3,3\), 69.44 (C2,2\), 52.2.7 (C6,6\), 39.46 (2CH_3), 32.44(C1). Anal calc for $C_{27}H_{34}N_2O_8$: C, 63.02; H, 6.66; N, 5.44. Found: C, 63.20; H, 6.86; N, 5.62.

Preparation of 4-(5-(9-(3-(4-hydroxyphenyl)-2-methylisoxazoli-din-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-2-methylisoxaz lidin-3-yl) phenol(4h)

Nitrone **(1h)** (284mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (20hrs); yield 82%; M.p. syrup; R_f 0.77, IR(KBr): 3246(OH), 2976(CH_3),1508-1430(aromatic.C=C),1274, 12.45 (C–O), 1158(N–O),1077(C–N),933, 854, 774 (Para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D6) δ = 9.16 (s, 2H,OH), 7.84–7.64 (m, 4H,aromatic H), 7.38–7.12 (m, 4H,aromatic-H), 5.76 (ddd, *J* = 15.5, 10.2, 4.4 Hz, 2H,Hd), 5.35 (d, *J* = 11.7, 2H,Hh), 5.26 (d, *J* = 11.5 Hz, 2H,Hf), 4.87 (d, *J* = 13.8, Hz, 2H,He), 4.25 (dd, *J* = 11.1, 2.3 Hz, 2H,Ha), 3.75 (s, 6H,2CH_3), 3.67–3.61 (m, 4H,2Hg,2Hf), 3.56 (ddd, *J* = 11.3, 4.6, 2.5 Hz, 2H,Hb), 3.42 (dd, *J* = 11.0,5.5 4.8 Hz, 2H,Hc). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 156.66 (C–OH), 140.12, 130.96, 129.81, (Ar–C), 118.03(C4,4\), 117.27(C5,5\), 100.36(C7,7\), 69.95(C3,3\), 69.43(C2,2\), 53.25(C6,6\), 40.48(2CH_3), 32.51(C1). Anal calc for $C_{27}H_{34}N_2O_8$: C, 63.02; H, 6.66; N, 5.44. Found: C, 63.19; H, 6.80; N, 5.56.

Preparation of 3-(4-methoxyphenyl)-5-(9-(3-(4-methoxyphenyl)-2-methylisoxazolidin-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-2-methylisoxazolidine(4i)

Nitrone **(1i)** (311mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (30hr); yield 65%; M.p. syrup; R_f 0.52, IR(KBr): 3395 (O–CH_3)3089 (CH_3),1507-1420(aromatic. C=C), 1319, 1252 (C–O), 1161 (N–O),1073 (C–N),936, 840, 719 (para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 7.37–7.20 (m, 4H, aromatic H), 6.88–6.76 (m, 4H, aromatic H) 5.73(ddd, *J* = 15.6, 10.5, 3.4 Hz, 1H, Hd), 5.32 (d, *J* = 13.5 Hz 2H,Hh), 5.22 (d, *J* = 13.4 Hz, 2H,Hf), 4.83 (d, *J* = 13.7 Hz, 1H,He), 4.24 (dd, *J* = 11.2, 2.6 Hz, 1H, Ha), 3.85 (s, 3H, O–CH_3), 3.62 (m, 2H,Hg), 3.58 (m, 2H,Hf), 3.54 (s,3H, N–CH_3),3.63 (dt, *J* = 11.4, 2.4 Hz, 1H,Hc), 3.43 (dt, *J* = 11.6,4.5 Hz, 1H,Hb). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 155.70, 135.67, 123.49, 122.88, (Ar–C), 117.74 (C4,4\), 111.63(C5,5\), 100.38(C7,7\), 69.95(C3,3\), 69.43(C2,2\), 57.73(C6,6\),56.00(O–CH_3), 52.11 (N–CH_3), 32.42(C1). Anal calc for $C_{29}H_{38}N_2O_8$: C, 64.19; H, 7.06; N, 5.16. Found: C, 64.24; H, 7.10; N, 5.21.

Preparation of 3-(4-bromophenyl)-5-(9-(3-(4-bromophenyl)-2-methylisoxazolidin-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-2-methylisoxazolidine(4j)

Nitrone **(1j)** (402mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (24hrs); yield 94%; M.p. semi-solid; R_f 0.56**,** IR(KBr): 3088 (CH_3),1587-1435(aromatic. C=C),1330-1247(C–O), 1163(N–O), 1071(C–N),984,953,821 (para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 8.20–8.02 (m, 4H,aromatic H), 7.14–7.01 (m, 4H,aromatic-H), 5.72 (ddd, *J* = 15.1, 10.4, 4.2 Hz, 2H,Hd),5.3 5 (d, *J* = 11.2, 2H,Hh), 5.22 (d, *J* = 11.3 Hz, 2H,Hf), 4.86 (d, *J* = 13.3, Hz, 2H,He), 4.24 (dd, *J* = 11.5, 2.2 Hz,2H,Ha), 3.72 (s, 6H,2CH_3), 3.65–3.59 (m, 4H,2Hg,2Hf), 3.55 (ddd *J* = 11.5, 5.4, 2.8 Hz, 2H,Hb), 3.35 (ddd, *J* = 11.7,6.1, 4.1 Hz, 2H,Hc). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 137.48, 132.34, 128.12, 122.98, (Ar–C), 117.12(C4,4\), 105.35(C5,5\), 102.74(C7,7\), 69.97(C3,3\), 69.42(C2,2\), 52.49(C6,6\), 42.01(2CH_3), 32.62(C1). Anal calc for $C_{27}H_{32}Br_2N_2O_6$: C, 50.64; H, 5.04; N, 4.37. Found: C, 50.72; H, 5.11; N, 4.44.

Preparation of 3-(4-isopropylphenyl)-5-(9-(3-(4-isopropylph-enyl)-2-methylisoxazolidin-4-yl)-1,5,7,11-tetraoxaspiro [5.5] unde-can-3-yl)-2-methylisoxazolidine(3k)

Nitrone **(1k)** (333mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (26hrs); yield 78%; M.p. syrup; R_f 0.63, IR(KBr): 3373,2959 (CH_3),1508-1417(aromatic.C=C),1286,1246(C–O), 1152(N–O),1060(C–N),984, 899, 847 (para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 7.19 (m, 2H, aromatic H), 7.30 (d, *J* = 8.7,1.8 Hz, 2H, aromatic H), 6.75 (m,1H,H3), 6.24(m, 2H, H2,H4), 6.19 (dd, *J* = 17.2, 6.5 Hz, 1H, H1), 5.72 (ddd, *J* = 15.5, 10.2, 3.9 Hz, 1H, Hd), 5.34 (d, *J* = 13.3 Hz 2H,Hh), 5.22 (d, *J* = 13.7 Hz, 2H,Hf), 4.87 (d, *J* = 13.6 Hz, 1H,He), 4.26 (dd, *J* = 11.1, 2.7 Hz, 1H, Ha), 3.53 (m, 1H, CH)), 3.69 (m, 2H,Hg), 3.62 (m, 2H,Hf), 3.59 (s,3H, N–CH_3),3.60 (dt, *J* = 11.3, 2.6 Hz, 1H,Hc), 3.44 (dt, *J* = 11.6,4.3 Hz, 1H,Hb)2.77 (d, *J* = 6.8 Hz, 6H,2CH_3). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 148.77, 138.20, 123.49, 122.88, 119.00 (Ar–C), 134.10(C9), 118.76 (C10), 117.60(C8), 111.50(C4,4\), 109.66(C5,5\), 101.04(C7,7\) 69.9.5 (C3,3\), 69.44(C2,2\), 57.70(C6,6\), 54.38(N–CH_3), 34.68(C–H),32.42(C1) 29.08(CH_3). Anal calc for $C_{22}H_{31}NO_5$: C, 67.84; H, 8.02; N, 3.60; O, 20.54 Found: C, 67.98; H, 8.16; N, 3.74.

Preparation of 2-methyl-5-(9-(2-methyl-3-(p-tolyl) isoxazole-din-4-yl)-1,5,7,11-tetraoxaspiro [5.5] undecan-3-yl)-3-(p-tolyl) isoxazolidine(4l)

Nitrone **(1l)** (280mg,1.88mmol) was used for the synthesis as described in the general procedure. The mixture was stirred for (35hrs); yield 95%; M.p.99–103 °C; R_f 0.60, IR(KBr): 2954, 2849(CH_3),1510-1421(aromatic.C=C),1343, 1247(C–O), 1163(N–O),1072(C–N), 937 (para-disubstituted benzene) Cm^{-1}. ^{1}H-NMR (400 MHz, DMSO-D_6) δ = 7.34–7.23 (m, 4H, aromatic H), 7.12–7.03 (m, 4H, aromatic H), 5.74 (ddd, *J* = 15.2, 10.6, 3.9 Hz, 1H, Hd), 5.36 (d, *J* = 13.5 Hz 2H,Hh), 5.23 (d, *J* = 13.7 Hz, 2H,Hf), 4.88 (d, *J* = 13.5Hz, 1H,He), 4.25 (dd, *J* = 11.4, 2.5 Hz, 1H, Ha), 3.65 (m, 2H,Hg), 3.59 (m, 2H,Hf), 3.56.(s,3H, N–CH_3),3.62 (dt, *J* = 11.4, 2.7 Hz, 1H, Hc), 3.42 (dt, *J* = 11.4,4.5 Hz, 1H, Hb), 2.88 (s,3H, CH_3). ^{13}C-NMR (400 MHz, DMSO-D_6) δ = 135.80, 134.41, 123.49, 122.88 (Ar–C), 113.44(C4,4\), 110.45(C5,5\), 102.12(C7,7\) 69.97 (C3,3\), 69.42(C2,2\), 57.77(C6,6\), 54.20(N–CH_3), 32.45(C1), 26.13(CH_3). Anal calc for $C_{29}H_{38}N_2O_6$: C, 68.21; H, 7.50; N, 5.49. Found: C, 68.27; H, 7.56; N, 5.56.

Results and Discussion

Chemistry

The study commenced with the synthesis of C-aryl-N-methyl nitrones (1a–l) via the condensation of benzaldehyde derivatives with N-methylhydroxylamine hydrochloride. The resulting nitrones were isolated, purified, and fully characterized. Subsequently, a [3 + 2] cycloaddition was performed between the nitrones (2 equivalents of 1a–l) and the bis-dipolarophile 3,9-divinyl-2,4,8,10-tetraoxaspiro(5.5)undecane (1 equivalent) in dry toluene under reflux at 110 °C for 20–40 hours, affording the bicyclic isoxazolidine cycloadducts 3 and 4 in good yields, as illustrated in **Figure 1**. The reactions proceeded with high regio- and diastereoselectivity, delivering the target isoxazolidine derivatives (e.g., 3b, 3e, 3k, and 4a–l) in moderate to excellent yields.

The newly synthesized isoxazolidines, which are novel and have not been previously reported, were characterized using ^{1}H-NMR, ^{13}C-NMR, and IR spectroscopy. Their biological activities were evaluated, demonstrating significant efficacy, including potential as orally active drug candidates.

Spectral analysis

FTIR analysis

The infrared spectra of the synthesized nitrones exhibited two characteristic absorption bands: one in the range of 1500–1660 cm^{-1} corresponding to the C=N stretching vibration, and another between 1100–1196 cm^{-1} assigned to the $N^{+}–O^{-}$ stretching vibration. These bands provide clear evidence for the formation of the nitrone functionality. As a representative example, compound 1g showed the C=N and $N^{+}–O^{-}$ bands at 1604 cm^{-1} and 1169 cm^{-1}, respectively.

Upon cycloaddition, the IR spectrum of the isoxazolidine derivative 4g displayed new absorption bands at 1072, 1145, and 1388 cm^{-1}, corresponding to C–N, N–O, and C–O stretching vibrations within the isoxazolidine ring. Notably, the disappearance of the C=N stretching band observed in the nitrone confirms the successful formation of the isoxazolidine ring.

NMR calculations

To better understand the regio- and stereochemical outcomes of the [3 + 2] cycloaddition and the nature of the transition states, the structures of both the nitrones and alkenes were considered. Acyclic nitrones can undergo E/Z isomerization. Analysis of the ^{1}H-NMR spectra of the nitrones indicated that the reaction produced a mixture of E and Z isomers in an approximate 5:1 ratio, respectively. The choice of solvent strongly influenced the dominant isomer: non

polar solvents such as dichloromethane favored the E-isomer, whereas polar solvents (e.g., ethanol/water mixtures) led to a predominance of the Z-isomer. Temperature also affected reaction rates: at room temperature, the Z-isomer reacted faster and was more stable, while at elevated temperatures in non-polar solvents, the E-isomer underwent cycloaddition more rapidly and became the major product. Under the conditions employed in this study, the nitrone equilibrium was shifted toward the E-isomer.

Using nitrone 1g as a model, the methyl protons of the Z-isomer appeared at δ ~3.85 ppm, while those of the E-isomer resonated at δ ~3.90 ppm. Similarly, the CH=N proton of the E-isomer appeared at δ ~7.30 ppm, slightly upfield (~0.05 ppm) relative to the Z-isomer at δ ~7.25 ppm. These chemical shift differences reflect the stereochemical environment of the nitrone and highlight the influence of the oxygen-substituted olefinic bond on the cycloaddition stereochemistry.

[3 + 2] cycloaddition of nitrones to alkenes can generate two regioisomeric products: 4- or 5-substituted isoxazolidines. The regiochemistry is largely governed by the electronic properties of the alkene substituents. Electron-rich alkenes favor formation of 5-substituted isoxazolidines, whereas alkenes bearing electron-withdrawing groups (e.g., carbonyl or cyano) promote 4-substituted products due to the interaction of the HOMO of the dipole with the LUMO of the dipolarophile, which is lowered in energy by the electron-withdrawing substituents **(Figure 2)**.

In the present study, the alkene employed—3,9-divinyl-2,4,8,10-tetraoxaspiro(5.5)undecane—can be considered a monosubstituted alkene with electron-donating substituents, favoring interaction between the LUMO of the dipole (nitrone) and the HOMO of the alkene. Consequently, the reaction proceeded with excellent regioselectivity, exclusively producing 5-substituted isoxazolidines. The electron-withdrawing effect of the benzene ring in nitrones 1a–l enhanced their electrophilicity, ensuring that the LUMO of the nitrone interacted predominantly with the HOMO of the alkene. The cycloaddition occurs via polar interactions between the more nucleophilic oxygen of the nitrone and the more electrophilic C5 carbon of the alkene, fully accounting for the exclusive formation of 5-isoxazolidine adducts.

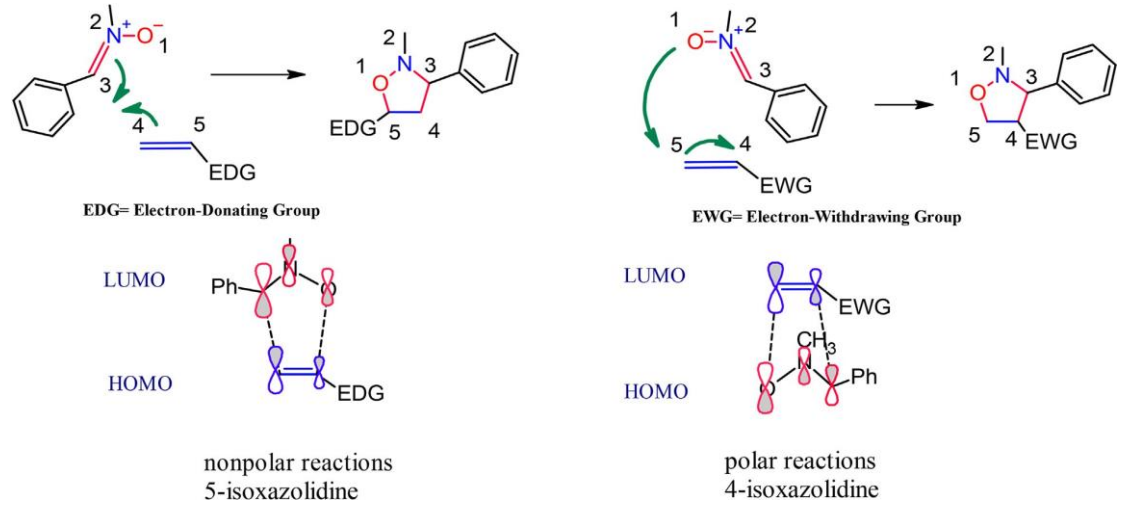

Figure 2. Regioselectivity of 1,3-dipolar cycloaddition.

The product yields indicate that the cycloaddition reaction proceeded with high chemoselectivity, predominantly affording bis-5-isoxazolidines. Mono-isoxazolidine products were also detected in some cases, as confirmed by spectroscopic analysis. The reaction is (i) regioselective, as the addition occurs via the oxygen of the nitrone to the C5 carbon of the alkene, producing isomers 3 and 4. The reaction mechanism can be divided into ten distinct phases [31], grouped into three stages **(Figure 3)**:

Group A (Phases i–iv): Initiated by the cleavage of the N2–C3 and C4–C5 double bonds of the reactants, generating a lone pair on the N2 nitrogen of the nitrone.

Group B (Phases v–vi): Involves the formation of two pseudoradical centers, first at C3 and then at C4, facilitating the creation of a new C3–C4 single bond.

Group C (Phases vii–x): Features the formation of two additional single bonds—C3–C4 (finalizing the first bond) and O1–C5—resulting in either bis-5-isoxazolidines (4) or mono-5-isoxazolidines (3).

The reaction is also stereoselective. The spatial relationship between the aryl ring and the C5 moiety in the isoxazolidine ring can adopt either a cis or trans configuration. Analysis of ^{1}H NMR spectra confirmed that, in most cycloadducts (4a–l), the aryl ring and C5 substituent are in a cis configuration.

Theoretical studies indicate that the regioselectivity of Exo/Endo pathways in 1,3-dipolar cycloaddition is influenced by the electronic properties of the alkene and the size of the reactants [32]. An endo approach is favored in polar reactions involving electron-withdrawing alkene substituents due to favorable electrostatic interactions between the polarized reactants, which contribute to endo stereoselectivity.

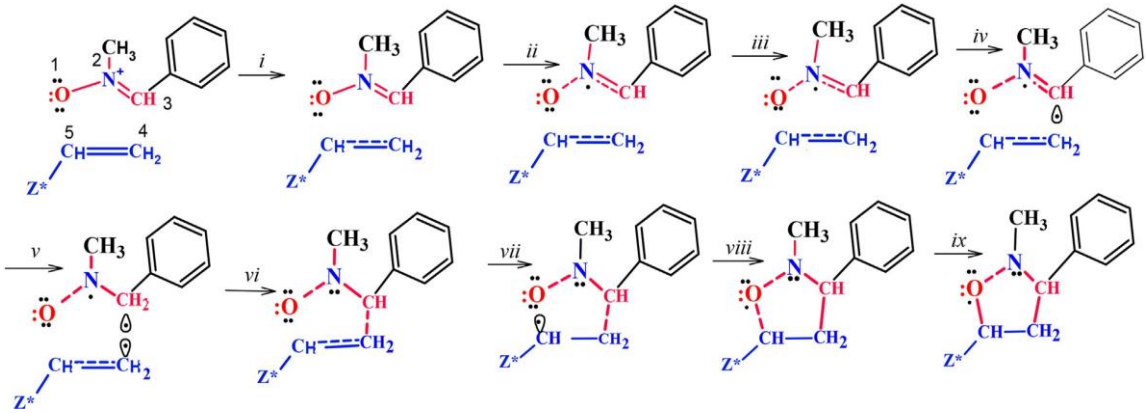

Figure 3. Exo reaction pathway for the nonpolar 1,3-dipolar cycloaddition between nitrone 1a and the dipolarophile.

In nonpolar cycloaddition reactions involving alkenes with electron-donating substituents, steric hindrance along the endo approach often makes the exo pathway more favorable, leading to the arrangement of bulky substituents in the exo orientation [31]. Liu *et al*. [33] performed DFT calculations on the 1,3-dipolar cycloaddition of simple

nitrones with electron-rich dipolarophiles and found that the endo approach is kinetically favored due to stabilizing secondary orbital interactions. This observation

aligns with our results, which indicate that the E-nitrone predominantly undergoes an endo attack. Although exo attacks of the Z-nitrone may also occur, their contribution is minor, consistent with the low proportion of Z-nitrone present under the reaction conditions, as illustrated in **Figure 4**.

The regiochemistry of the cycloadducts was confirmed through standard spectroscopic analyses. For instance, the ^{1}H-NMR spectrum of compound 4g **(Figure 6)** exhibited a symmetric pattern with two distinct ddd signals at δ 3.60–3.57 ppm (Hb, J = 11.5, 8.2, 6.3 Hz) and δ 3.43–3.40 ppm (Hc, J = 11.6, 5.6, 2.4 Hz), characteristic of the methylene protons in the isoxazolidine ring. The appearance of two ddd signals, rather than two doublets, further confirms the stereochemical environment of the ring protons.

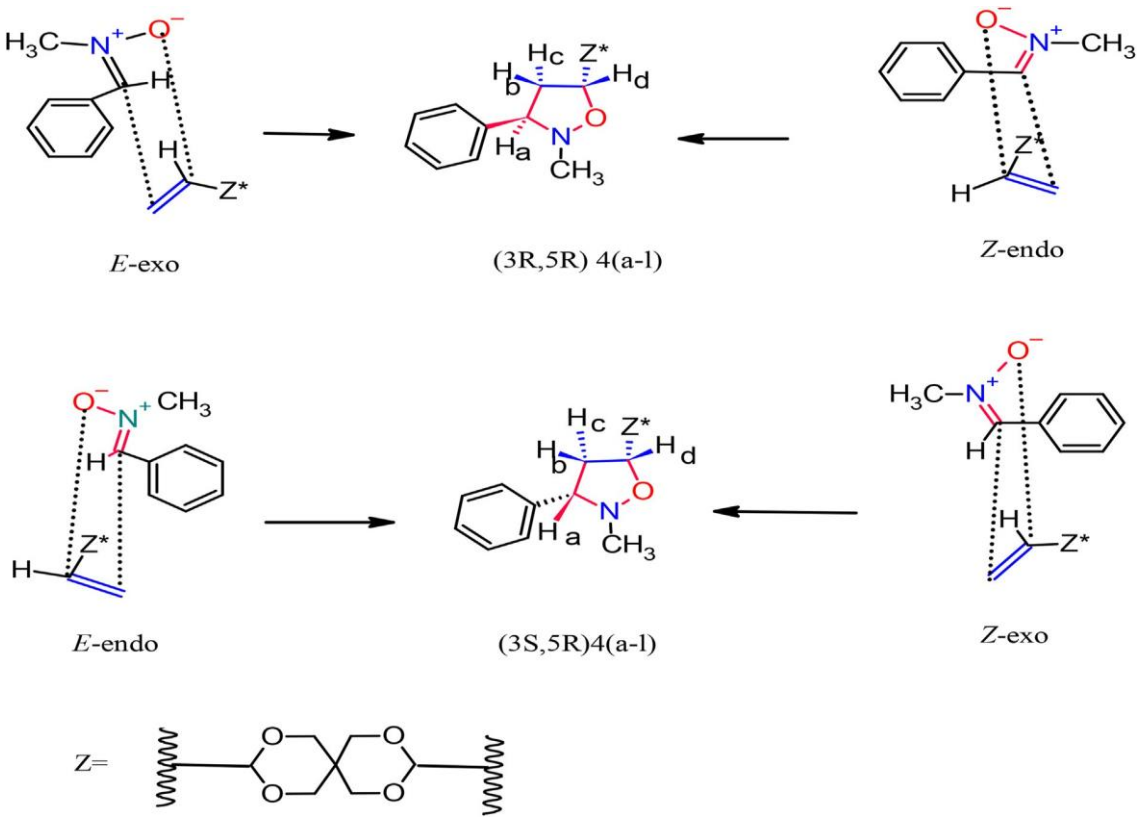

Figure 4. Four possible attack pathways on the nitrone.

The ^{1}H-NMR analysis of the isoxazolidine cycloadducts provides strong evidence for the formation of the bis-5-isoxazolidine regioisomer (4g), while excluding other potential regioisomers such as bis-4,5-isoxazolidine (5) or bis-4-isoxazolidine (6). The methylene protons Hb and Hc in the five-membered isoxazolidine ring are diastereotopic and therefore non-equivalent, resulting in distinct chemical shifts. The rigid ring structure restricts rotation, which is reflected in the observed chemical shifts of δ 3.60–3.40 ppm for Hb and Hc (ddd), consistent with the expected bis-5-isoxazolidine structure. The H-a proton appears as a doublet of doublets at δ 4.27 ppm ($J_{a,\beta}$ = 2.4 Hz, $J_{a,c}$ = 11.2 Hz), indicating that H-a is in a cis relationship with H-b and a trans relationship with H-c. For most cycloadducts, the relative configuration of H-b, H-a, and H-d protons is cis, supporting a predominant E-endo or Z-exo transition state geometry, as confirmed by the coupling constant JHb,Hd = 4.2 Hz. Additionally, the absence of an Hg proton in the ^{1}H-NMR spectrum of 4g suggests significant electrostatic interactions between the reacting species, consistent with the proposed cycloaddition mechanism **(Figure 5)**.

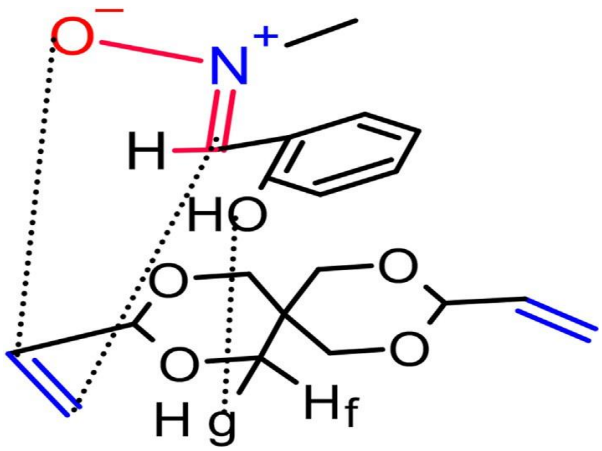

Figure 5. Electrostatic interactions in isoxazolidine 4g showing the OH proton on the aryl ring of the nitrone interacting with the Hg proton of the alkene via an E-endo transition state.

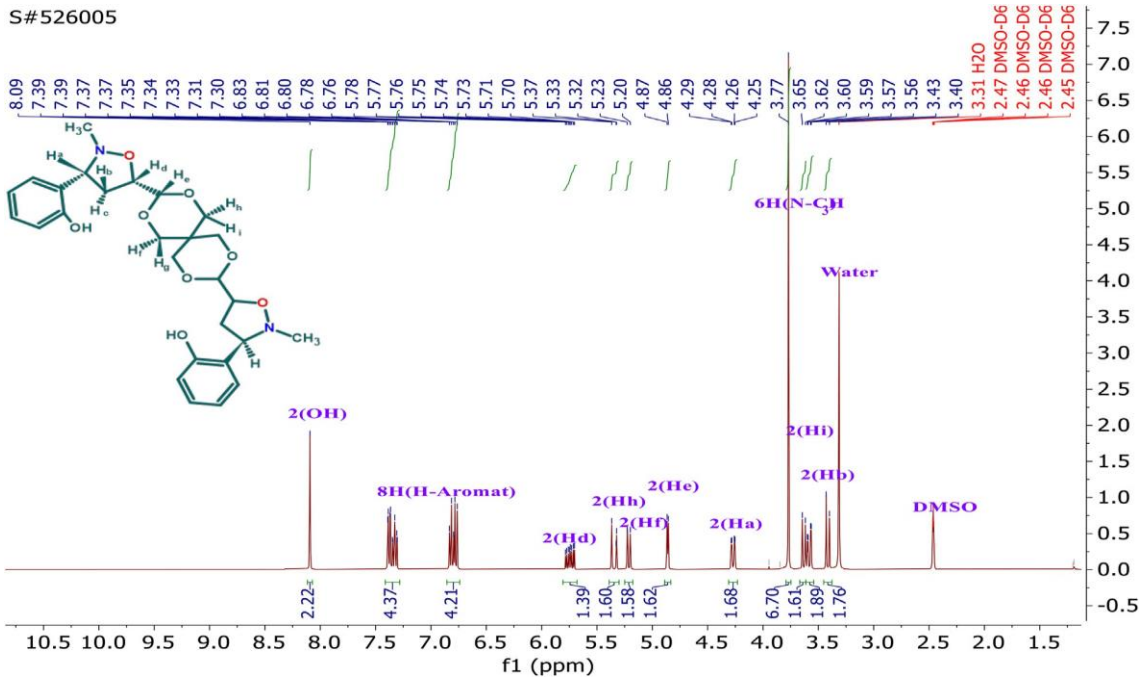

Figure 6. [1]H-NMR Spectrum of isoxazolidine 4g.

The interaction between the OH proton on the aryl ring of the nitrone and the Hg proton of the alkene via intermolecular hydrogen bonding lowers the energy of the LUMO of the dipolarophile and reduces the energy gap between the LUMO of the nitrone and the HOMO of the alkene [32]. During the cycloaddition, this interaction influences the regioselectivity, favoring the formation of the endo (cis) geometry in the E-nitrone pathway. The [13]C-NMR spectrum of 4g **(Figure 7)** displayed signals at δ 117.79, 100.89, and 52.27 ppm, corresponding to C5, C7, and C6 of the isoxazolidine ring, respectively. Detailed [1]H-NMR analysis of the cycloadducts confirmed the absence of diastereomers, indicating formation of a single symmetric product. As shown in **Table 1**, most compounds underwent complete cycloaddition to yield the bis-5-isoxazolidine derivatives, except for 3b, 3e, and 3k, which were isolated as mono-5-isoxazolidines due to steric hindrance preventing a second cycloaddition or through retro-cycloaddition.

For example, in compound 3b **(Figure 8)**, the isoxazolidine ring methylene protons appear at δ 3.33 ppm (Hb, dt, J = 11.6, 2.4 Hz) and δ 3.41 ppm (Hc, dt, J = 11.5, 8.3 Hz). The Ha proton resonates at δ 4.27 ppm as a doublet of doublets (J = 11.3, 2.4 Hz), while Hd appears at δ 5.74 ppm as a ddd (J = 15.5, 10.4, 4.4 Hz). Protons of the residual double bond appear as H1, H2 doublets in the range δ 6.20–6.26 ppm, with H4 overlapping H2, and H3 appearing as a multiplet at δ 6.78 ppm. These chemical shifts confirm the cis relationship between Ha and Hc and

between Hb and Hd, consistent with literature data [34]. Trans adducts (3R,5R) can arise from the exo attack of the E-nitrone or endo attack of the Z-nitrone **(Figure 4)**.

The ^{13}C-NMR spectrum of 3b shows peaks at δ 111.60, 100.87, and 53.67 ppm corresponding to C5, C7, and C6 of the isoxazolidine ring, respectively. Additional signals at δ 119.60, 125.05, and 132.09 ppm correspond to C8, C10, and C9 of the aryl moiety. The high symmetry of derivative 4c was evidenced in its ^{1}H-NMR spectrum, where Hb, Hc, and Hd appeared as multiplets, while Ha appeared as two isolated peaks, reflecting cis and trans orientations. These spectral features indicate that cycloaddition occurred via an E-endo pathway at one double bond and an E-exo pathway at the other, confirming the proposed stereochemical outcomes.

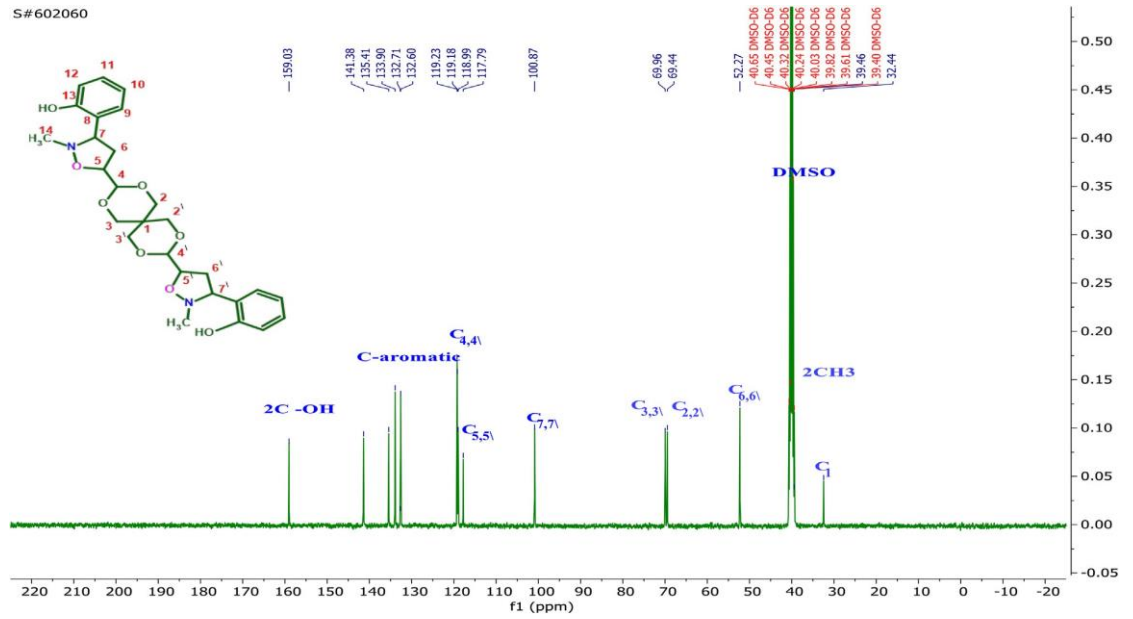

Figure 7. ^{13}C-NMR Spectrum for isoxazolidine (4g).

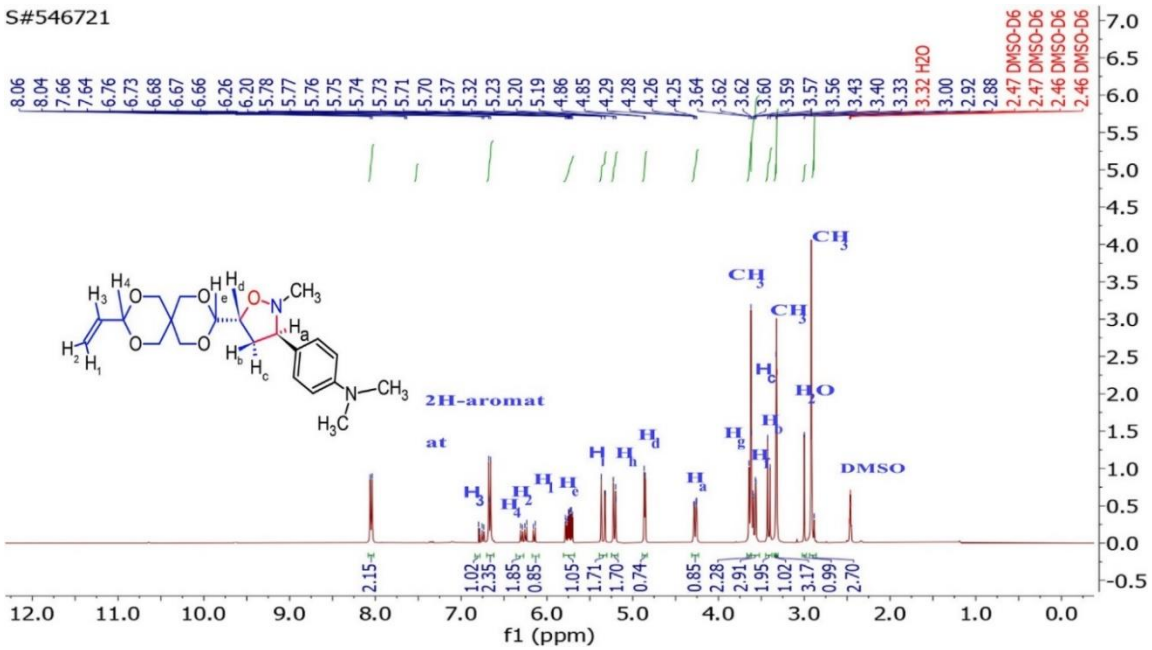

Figure 8. ^{1}H-NMR Spectrum for isoxazolidin (3b).

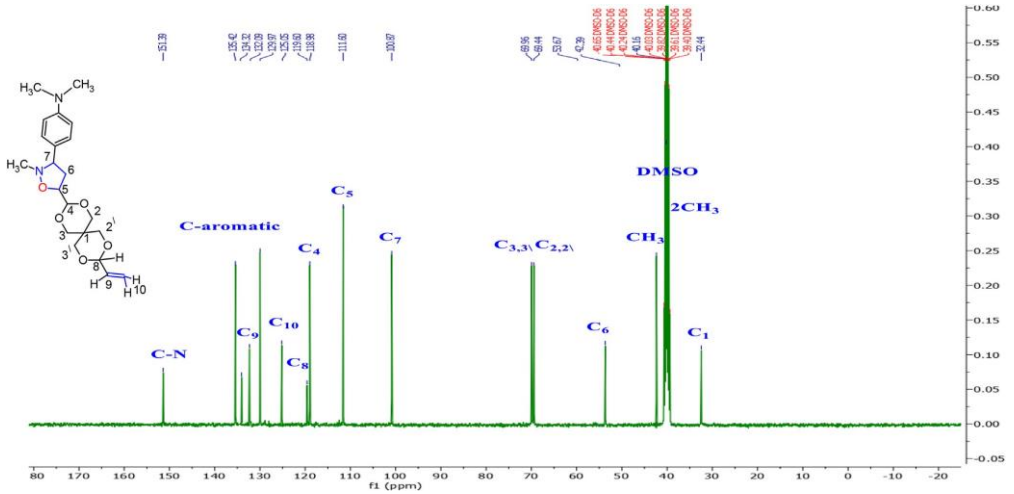

Figure 9. ^{13}C-NMR Spectrum for isoxazolidin (3b).

Figure 10. (a) Bioavailability radar and (b) BOILED-Egg plot for compounds 3 and 4(a–l) predicted using SwissADME. In the BOILED-Egg diagram, the white region indicates optimal human intestinal absorption, while the yellow region represents blood–brain barrier permeation.

Table 1. Yield of isoxazolidines.

	Comp.	a	b	c	d	e	f	g	h	i	j	k	l
The isolated yield of cycloadducts (%)	3	-	76	-	-	79	-	-	-	-	-	78	-
	4	88	-	74	87	-	95	69	82	65	94	-	95
	5	-	-	-	-	-	-	-	-	-	-	-	-
	6	-	-	-	-	-	-	-	-	-	-	-	-

The ^{1}H-NMR spectral data for the compounds are provided in the Experimental section. The trans product 4i exhibited a highly symmetrical structure, as confirmed by its ^{1}H-NMR spectrum. The methylene protons of the isoxazolidine ring were observed at δ 3.43 ppm (Hb, dt, J = 11.6, 2.4 Hz) and δ 3.63 ppm (Hc, dt, J = 11.4, 4.3 Hz), while the Ha proton appeared at δ 4.24 ppm as a doublet of doublets (dd, J = 11.3, 2.4 Hz). The Hd proton was detected at δ 5.73 ppm as a doublet of doublet of doublets (ddd, J = 15.5, 10.4, 4.4 Hz). These chemical shifts indicate a cis relationship between Ha and Hc, and between Hb and Hd. Accordingly, the cycloaddition likely proceeded via either an exo approach on the E-nitrone or an endo approach on the Z-nitrone.

Antimicrobial screening

Many heterocyclic compounds containing an N-bridge, such as isoxazolidines, have been reported to exhibit antiviral and antimicrobial activities. In this study, the MIC values of the newly synthesized analogs revealed that all compounds displayed varying degrees of antimicrobial activity against the tested microorganisms compared to the positive controls. Some compounds demonstrated strong activity, while others were moderately active **(Table 2)**.

Notably, compound 4l exhibited superior antibacterial activity relative to standard Ampicillin, showing higher potency against S. aureus and S. pyogenes, and comparable activity against E. coli. However, its activity against P. aeruginosa was weaker than that of Chloramphenicol. Compounds 3b and 4c displayed greater activity than Ampicillin against S. aureus and comparable effects to Ampicillin against S. pyogenes. Compound 3e showed slightly lower activity than Ampicillin against E. coli and S. pyogenes, but was approximately equipotent to Ampicillin against S. aureus.

Compound 4g demonstrated remarkable activity, surpassing Ampicillin against E. coli and S. pyogenes, and matching Chloramphenicol in potency. It also exhibited strong activity against S. aureus, comparable to Ampicillin. Similarly, 4h showed activity against P. aeruginosa equivalent to Chloramphenicol. Compound 4i had lower activity against E. coli and S. pyogenes compared to Ampicillin, but exhibited higher activity against S. aureus.

In comparison with Chloramphenicol, most compounds displayed moderate to weak activity. Exceptions included 4g, which showed activity close to the MIC of Chloramphenicol (62.5 µg/mL) against E. coli and equipotent activity against S. pyogenes (MIC 50 µg/mL). Compound 4h exhibited activity comparable to Chloramphenicol against P. aeruginosa (MIC 50 µg/mL), while 4l displayed activity close to Chloramphenicol against S. aureus (MIC 62.5 µg/mL) and equipotent effects against S. pyogenes (MIC 50 µg/mL). In addition to the bacterial strains, antifungal activity was evaluated against C. albicans, A. niger, and A. clavatus, with detailed results summarized in **Table 2**.

Table 2. MIC values (µg/mL) of some of the synthesized compounds.

	ANTIBACTERIAL ACTIVITY				**ANTIFUNGAL ACTIVITY**		
	minimum inhibition concentration				**minimal fungicidal concentration**		
Compound	***E. COLI***	***P. AERUGINOSA***	***S. AUREUS***	***S. PYOGENUS***	***C.ALBICANS***	***A. NIGER***	***A. CLAVATUS***
3b	125	100	**125**	**100**	**500**	250	250
4c	125	250	**125**	**100**	1000	500	500
3e	125	100	**250**	125	**500**	1000	1000
4g	**62.5**	100	**250**	**50**	1000	500	1000
4h	125	**50**	500	125	**500**	500	1000
4i	125	250	**125**	250	**250**	>1000	500

4l	**100**	250	**62.5**	**50**	**250**	500	>1000
Ampicillin	100	--	250	100	-	-	-
Chloramphenicol	50	50	50	50	-	-	-
Nystatin	-	-	-	-	100	100	100
Greseofulvin	-	-	-	-	500	100	100

Table 3. DPPH evaluate the antioxidant activity of studies compounds.

Entry	**Concentrations (µg/mL)**								
	1	**2**	**5**	**10**	**25**	**50**	**100**	**200**	**IC $_{50}$ µg/ml**
3b	3.00	4.43	14.94	29.40	33.63	34.96	42.12	50.22	19.66 ± 0.68
4c	3.12	5.06	15.51	29.87	34.08	35.40	42.50	50.55	40.77 ± 1.41
3e	3.22	4.75	15.22	29.64	33.86	35.18	42.31	50.38	18.12 ± 0.63
4g	3.04	8.86	18.88	32.67	36.71	37.98	44.80	52.53	14.05 ± 0.49
4h	4.46	10.20	20.07	33.66	37.64	38.89	45.61	53.22	11.15 ± 0.39
4i	8.56	14.05	23.50	36.51	40.32	41.51	47.94	55.23	7.86 ± 0.27
4l	14.02	19.18	28.07	40.30	43.88	45.00	51.05	57.90	5.90 ± 0.20
Ascorbic acid									5.31 ± 0.18

The data are expressed as mean ± S.D. of three independent experiments. IC_{50} values (µg/mL) represent the concentration required to scavenge 50% of the radicals in the reaction mixture.

The results indicate that compound 3b exhibited strong activity against C. albicans, comparable to the reference drug Greseofulvin, while showing moderate activity against the other tested fungal strains. Derivatives 4c and 4g demonstrated weak antifungal activity across all tested fungi. Compounds 3e and 4h displayed activity against C. albicans similar to Greseofulvin, but weak effects against other fungal strains. Notably, derivatives 4i and 4l showed higher antifungal activity against C. albicans than Greseofulvin, with moderate activity against the remaining fungi. In comparison to Nystatin, all tested compounds exhibited weak antifungal activity against the examined strains.

Antioxidant activity

The in vitro antioxidant activity of compounds 3b, 4c, 3e, 4g, 4h, 4i, and 4l was evaluated using the synthetic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), following the methodology reported in reference [35]. Ascorbic acid was employed as a positive control for comparison. The DPPH radical scavenging activity, expressed as IC_{50} values, is summarized in **Table 3**. The results demonstrated that all compounds exhibited dose-dependent inhibitory effects on DPPH radical absorbance.

The radical scavenging capacity of the tested compounds increased in the following order: 4c < 3b < 3e < 4g < 4h < 4i < 4l < ascorbic acid. The IC_{50} value, representing the concentration required to inhibit 50% of DPPH radicals, indicates that lower values correspond to higher antioxidant activity. Among the series, derivative 4l displayed the highest antioxidant activity with an IC_{50} of 5.90 ± 0.20 µg/mL, comparable to ascorbic acid (IC_{50} = 5.31 ± 0.18 µg/mL), whereas 4c exhibited the lowest activity (IC_{50} = 40.77 ± 1.41 µg/mL). The radical scavenging activity (RSA, %) was calculated using the equation:

RSA (%)=ADPPH−AADPPH×100\text{RSA (\%)} = \frac{A_{\text{DPPH}} - A}{A_{\text{DPPH}}} \times 100RSA (%)=ADPPHADPPH−A×100

where ADPPHA_{\text{DPPH}}ADPPH and AAA are the absorbances of DPPH alone and in the presence of the sample, respectively. The RSA values for all compounds at eight concentrations (1, 2, 5, 10, 25, 50, 100, and 200 µg/mL) are presented in **Table 2**, with absorbance measured at 517 nm. **Figure 8** illustrates the percentage of antioxidant activity and corresponding IC_{50} values.

Structure–activity relationship analysis revealed that the electronic nature of substituents on the phenyl ring significantly influenced the antioxidant potential of these isoxazolidine derivatives. The observed antioxidant activity followed the trend: 4-CH_3 > 4-OCH_3 > 4-OH > 2-OH > 4-OH-3-OCH_3 > 4-$N(CH_3)_2$ > 2-N_2O. Compounds with electron-donating groups such as methyl, methoxy, amine, and hydroxyl at the para position of the aromatic ring enhanced the electron density of the ring, thereby increasing radical scavenging activity.

Physicochemical properties

To correlate the in vitro biological activity with drug-likeness, an in silico ADME analysis was performed using the SwissADME predictor, a widely used tool for assessing the oral bioavailability and pharmacokinetic potential of drug candidates. Key parameters, including Lipinski's rule of five, were applied to evaluate drug-likeness: molecular weight ≤500 Da, hydrogen bond acceptors ≤10, hydrogen bond donors ≤5, topological polar surface area (TPSA) ≤140 Å², and Log P ≤5. Compounds violating two or more criteria were considered poor candidates for oral drugs [36, 37].

Most of the synthesized derivatives satisfied Lipinski's rule, except compound 4c, which displayed two violations and a bioavailability score of 0.17. The majority of compounds exhibited a bioavailability score of ~0.55. The number of rotatable bonds ranged from 4 to 6, suggesting low structural flexibility conducive to oral bioavailability. Hydrogen bond acceptors (6–12) and donors (0–2) also fell within acceptable ranges, supporting favorable drug-like properties (except compound 3c). TPSA values ranged from 49.39 to 153.50 Å², with most compounds around 82.32 Å², indicative of high intestinal absorption and blood-brain barrier (BBB) permeability, suggesting that compounds 4a, 3b, 4d, 3e, 4f, 4j, 3k, and 4l are promising pharmacological candidates.

Predicted gastrointestinal (GI) absorption was high for all compounds, with %ABS >80%, consistent with good oral absorption. XLogP values were <5 for all derivatives, reflecting good lipid solubility and membrane permeability. None of the compounds were predicted as P-glycoprotein (P-gp) substrates, supporting favorable intestinal absorption and bioavailability. Several derivatives (4a, 3b, 4d, 4f, 4j, 3k, 4l) were predicted to cross the BBB, suggesting potential central nervous system activity, whereas 3e, 4g, 4h, and 4i showed high intestinal absorption but limited BBB penetration.

The Boiled-Egg model **(Figure 10)** confirmed these predictions, with compounds located inside the white ellipse expected to be well absorbed in the GI tract, and those in the yellow ellipse likely to penetrate the BBB. No inhibitory effects were predicted against major CYP enzymes (CYP1A2, CYP2D6, CYP2C19, CYP3A4, CYP2C9), indicating low risk of metabolic toxicity. Negative skin permeability values (-8.04 ≤ log Kp ≤ -6.43) suggested minimal dermal absorption.

Medicinal chemistry analysis indicated synthetic accessibility scores between 5.24 and 6.20, suggesting practical feasibility for laboratory synthesis. Bioavailability radar plots **(pink regions, Figure 10)** demonstrated that all compounds fell within the optimal ranges for lipophilicity, size, solubility, polarity, flexibility, and saturation, confirming superior oral bioavailability. Overall, the physicochemical and pharmacokinetic profiles suggest that the designed isoxazolidines are promising candidates for oral drug development.

Table 4. Physicochemical-Pharmacokinetic/ADME properties and drug-likeness predictions and of tested compounds. Here, MW = Molecular Weight, HBA = Hydrogen Bond Acceptors (O and N atoms <10), RB = Rotatable Bond <10, TPSA = Total Polar Surface Area, HBD = Hydrogen Bond Donors (OH and NH group <5), GIA = GI Absorption, Topological polar surface area. c %ABS = 109 – (0.3345 × TPSA), BBBP = Blood Brain Barrier Permeation, PgPS = P-Glycoprotein Substrate, LV = Lipinski Violation, LLV = Lead likeness Violations, BS = Bioavailability Score, SA = Synthetic Accessibility, S = Ampicillin.

Entry	4a	3b	4c	4d	3e	4f	4g	4h	4i	4j	3k	4l	S
Pharmacokinetics													
MW (< 500 Da)	482.57 g/mol	390.47 g/mol	572.56 g/mol	518.55 g/mol	393.43 g/mol	551.46 g/mol	514.57 g/mol	514.57 g/mol	542.62 g/mol	640.36 g/mol	389.48 g/mol	510.62 g/mol	349.40 g/mol
RP	4	4	6	4	4	4	4	4	6	4	4	4	5
HBA (< 10)	8	6	12	10	8	8	10	10	10	8	6	8	5
HBD (< 5)	0	0	0	0	1	0	2	2	0	0	0	0	3
TPSA	61.86 A^2	52.63 A2	153.50 A^2	61.86 A^2	78.85 A^2	61.86 A^2	102.32A^2	102.32A^2	80.32 A^2	61.86 A^2	49.39 A^2	61.86 A^2	138.03 $Å^2$

ABS%	88.30	91.39	57.65	88.30	82.62	88.30	74.77	74.77	82.13	88.30	92.47	88.30	62.82
Fraction Csp3	0.56	0.62	0.56	0.56	0.60	0.56	0.56	0.56	0.59	0.56	0.64	0.59	0.44
XLOGP3 (LogP < 5)	3.24	2.17	2.90	3.45	1.66	4.50	2.54	2.54	3.19	4.63	3.17	3.98	-1.13
GIA	High	High	Low	High	High	High	High	High	High	High	High	High	Low
BBBP	Yes	Yes	No	Yes	No	Yes	No	No	No	Yes	Yes	Yes	No
PgPS	No	No	Yes	No	No	No	No	No	No	No	No	No	No
CYP1A2 INHIBITION	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2C19 INHIBITION	No	No	No	No	No	No	No	No	No	No	No	No	No
CYP2C9 INHIBITION	No	No	Yes	No	No	No	No	No	No	No	No	No	No
CYP2D6 INHIBITION	Yes	Yes	No	Yes	Yes	No	Yes	Yes	Yes	No	Yes	No	No
CYP3A4 INHIBITION	No	No	No	No	No	No	No	No	No	No	No	Yes	No
Log Kp (cm/s)	-6.94	-7.14	-8.04	-7.01	-7.52	-6.47	-7.64	-7.64	-7.35	-6.92	-6.43	-6.59	-9.23
LV	0	0	2	1	0	1	1	1	1	1	0	1	0
BS	0.55	0.55	0.17	0.55	0.55	0.55	0.55	o.55	0.55	0.55	0.55	0.55	0.55
LLV	1	1	1	1	1	2	1	1	1	1	1	2	No
SA	5.97	5.26	6.20	5.99	5.24	5.32	6.06	5.98	6.18	6.02	5.39	6.18	4.16

Docking studies

The in vitro antimicrobial evaluation of the synthesized isoxazolidine derivatives revealed that several compounds (3b, 4c, 3e, 4g, 4h, 4i, 4l) exhibited significant activity against selected bacterial strains, with some showing potency comparable to or exceeding that of standard antibiotics such as Ampicillin. Variations in activity among the derivatives can be attributed to differences in the core scaffold geometry, the nature and position of substituents, and the presence of heteroatoms.

Molecular docking studies were conducted to support these experimental observations and to investigate the binding interactions of the synthesized compounds with Methicillin-Resistant Staphylococcus aureus Pantothenate Synthetase (PDB ID: 2X3F). The docking results, including docking scores, hydrogen bonding interactions, and RMSD values (≤1.9 Å), are summarized in **Table 5**. Compared to Ampicillin and the native ligand, all studied compounds exhibited favorable binding energies, with most derivatives showing higher docking scores than the standard drug, except for compound 4h.

For detailed docking analysis, the most active compounds (4l, 4i, 3b) were selected. The binding affinities ranged from −5.71 kcal/mol (4h) to −8.32 kcal/mol (4l), compared to −8.69 kcal/mol for the native ligand and −7.01 kcal/mol for Ampicillin. The ranking based on docking score was: native ligand > 4l > 4i > 3b > 4g > 4c > 3e > Ampicillin > 4h, which correlates well with the in vitro activity against S. aureus, particularly for the top three compounds.

Compound 4l, the best-docked derivative, displayed a binding score of −8.32 kcal/mol and formed multiple interactions with the active site, including two hydrogen bonds with Gln62 (2.93 Å) and Arg122 (3.31 Å), one π-H bond with Arg188, and one π-cation stacking interaction with Arg188. Compound 4i showed three hydrogen bond interactions: Gln154 (2.98 Å), Gln62 (2.69 Å), and Thr30 (3.29 Å), corresponding to a docking score of

−8.21 kcal/mol. Compound 3b formed two key interactions with the protein: a hydrogen bond between the dipolarophile oxygen and Lys150 (2.88 Å) and a π-H interaction with Thr30 (2.82 Å).

These docking results are consistent with the observed in vitro antimicrobial activity, confirming that the synthesized cycloadducts, particularly 4l, 4i, and 3b, exhibit strong binding to the Pantothenate Synthetase active site, thereby supporting their potential as potent antibacterial agents.

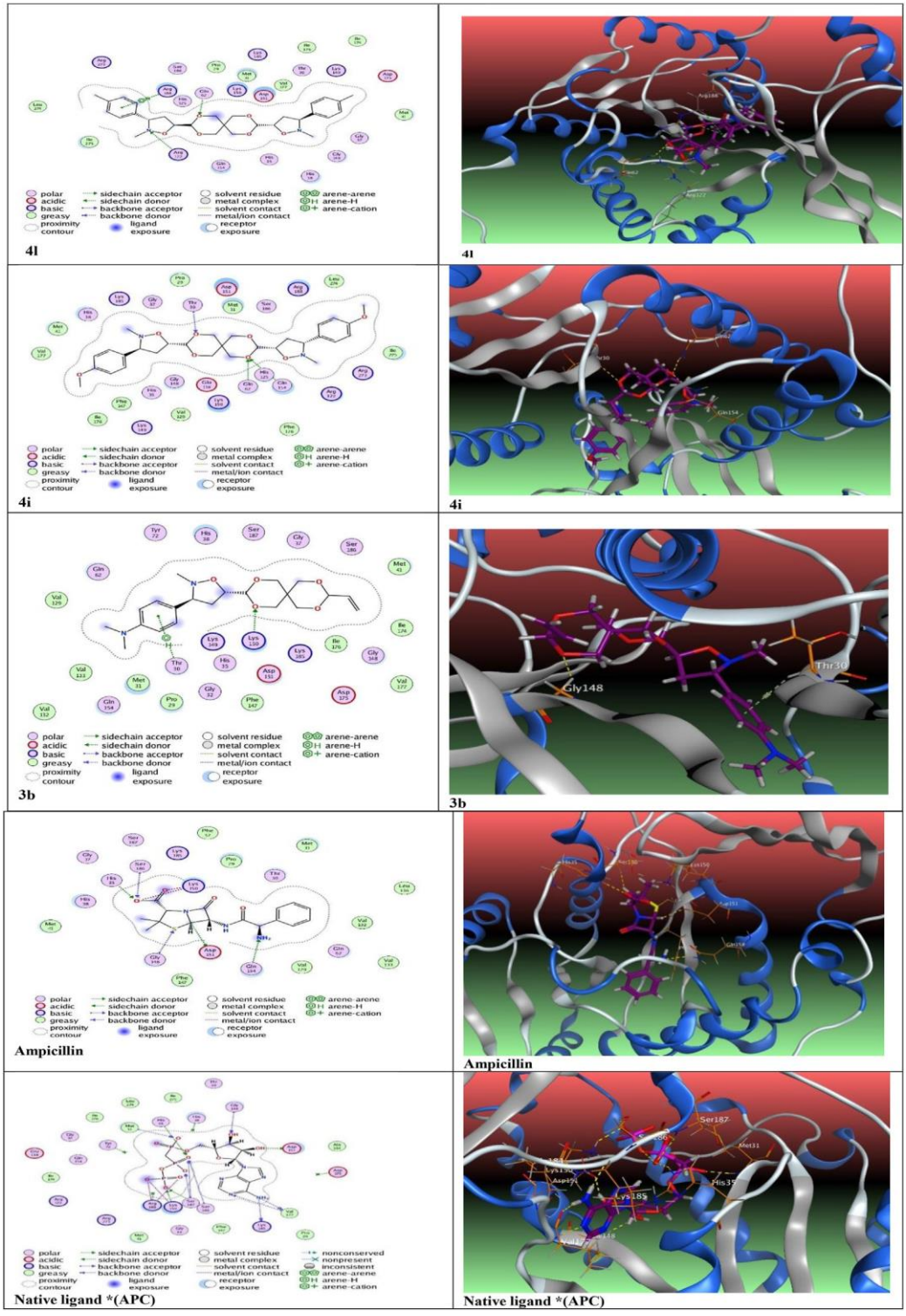

Figure 11. Interaction of selected compounds 4l, 4i, 3b, ampicillin, and Native ligand with 2 × 3F, both 2D (left) and 3D (right) diagrams, is given below with yellow dotted line in the 3D figure represents H-bond.

Table 5. Docking studies for compounds 3b,4c,3e,4g,4h,4i,4l with PDB ID: 2X3F.

Comp.	Docking score	RMSD (Kcal/mol)	Residue	No. of interactions
3b	-8.20	1.23	LYS150, THR30	1 H-acceptor 1 pi- H
4c	-7.5	0.97	GLU118, LYS150, SER186, LYS150, ARG273GLN154	One H-donor, 4 H-acceptor
3e	-7.31	1.02	MET31, MET31, SER187	2 H-acceptor 1 pi- H
4g	-7.42	1.54	GLU118, ARG188	1 H-donor, 1 H-acceptor
4h	-5.71	1.79	HIS38, TYR72, SER187	3 H-acceptor
4i	8.21	1.61	GLN62, GLN154, THR30	3 H-acceptor
4l	-8.32	1.39	GLN62, ARG122, ARG188, ARG188	2 H-acceptor 1 pi- H 1 pi-cation
Ampicillin	-7.01	1.92	ASP151, GLN154, HIS35, SER186, GLY148, LYS150, LYS150	One H-donor, 4 H-acceptor 2 ionic
Chloramph-enicol	-5.94	1.6	GLN148, ARG188, LYS150	3 H-acceptor
Native ligand (APC)	-8.69	1.75	ASP151, VAL127, LYS185, LYS150, SER186, HIS35, SER186, SER187, SER187, MET31, GLY148, VAL177, ARG188, LYS150, LYS150, ARG188,	3 H-donor, 9 H-acceptor 3 ionic

∗Native ligand: DIPHOSPHOMETHYLPHOSPHONIC ACID ADENOSYL ESTER.

Conclusion

In conclusion, we successfully designed and synthesized a series of novel isoxazolidine derivatives via 1,3-dipolar cycloaddition of readily accessible chiral C-aryl-N-phenylnitrones with 3,9-Divinyl-2,4,8,10 tetraoxaspiro(5-5)undecane, yielding new candidates as antioxidant and antibacterial agents. The cycloaddition proceeded with high regio- and stereoselectivity, predominantly forming mono-5- and bis-5-isoxazolidines, with the nitrone oxygen preferentially linking to the 5-carbon of the alkene. Sterically bulky substituents, such as 4-N-dimethylamine, 4-isopropyl, and 3-methoxy-4-hydroxy groups on the phenyl ring of the nitrone, led exclusively to mono-5-isoxazolidine formation. The cycloaddition exhibited facial diastereoselectivity, attributable to steric effects.

Biological evaluation revealed that compound 4l displayed the highest antibacterial and antioxidant activity among the series, making it a promising hit for further medicinal chemistry investigations. ADME and physicochemical analyses indicated favorable oral bioavailability for the synthesized derivatives, supporting their potential as drug candidates. Molecular docking studies against Methicillin-Resistant Staphylococcus aureus Pantothenate Synthetase (2×3F) demonstrated strong binding affinities, consistent with the in vitro antibacterial results.

Although this study focused on the antimicrobial and antioxidant properties of the synthesized isoxazolidines, their structural features suggest potential for additional therapeutic applications, including anti-inflammatory, anticancer, and antidiabetic activities. Overall, the synthesized derivatives, particularly 4l, represent promising scaffolds for further drug discovery and development following systematic in vivo evaluations.

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Conflict of Interest: None

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Ethics Statement: None

References

1. A. Kadri, K. Aouadi, In vitro antimicrobial and α-glucosidase inhibitory potential of enantiopure cycloalkylglycine derivatives: insights into their in silico pharmacokinetic, druglikeness, and medicinal chemistry properties, J. Appl. Pharmaceut. Sci. 10 (2020) 107–115.
2. S. Ghannay, M. Snoussi, S. Messaoudi, A. Kadri, K. Aouadi, Bioorganic Chemistry Novel enantiopure isoxazolidine and C -alkyl imine oxide derivatives as potential hypoglycemic agents : design , synthesis , dual inhibitors of α- amylase and α-glucosidase , ADMET and molecular docking study, Bioorg. Chem. 104 (2020), 104270.
3. S. Ghannay, S. Bakari, M. Msaddek, S. Vidal, A. Kadri, K. Aouadi, Design, synthesis, molecular properties and in vitro antioxidant and antibacterial potential of novel enantiopure isoxazolidine derivatives, Arab. J. Chem. 13 (2020) 2121–2131.
4. A. Ghabi, J. Brahmi, F. Alminderej, S. Messaoudi, S. Vidal, Bioorganic Chemistry Multifunctional isoxazolidine derivatives as α-amylase and α-glucosidase inhibitors, Bioorg. Chem. 98 (2020), 103713.
5. K.R.R. Kumar, H. Mallesha, K.S. Rangappa, Synthesis and Characterization of 5- Substituted Novel Isoxazolidines Derived from 1 , 3-Dipolar Cycloaddition of Nitrones with Olefins : Studies of Antibacterial and Antifungal Activities Cycloaddition of Nitrones with Olefins, Studies of Antibacterial, 2006, p. 7911.
6. V. Sharma, R. Kalia, T. Raj, V.K. Gupta, N. Suri, A.K. Saxena, D. Sharma, S.S. Bhella, G. Singh, M.P.S. Ishar, Synthesis and cytotoxic evaluation of substituted 3-(3'- indolyl-/3'-pyridyl)-isoxazolidines and bis-indoles, Acta Pharm. Sin. B. 2 (2012) 32–41.
7. J.K. Gallos, S.C. Demeroudi, C.C. Stathopoulou, C.C. Dellios, Expeditious synthesis of seven-membered iminocyclitols, Tetrahedron Lett. 42 (2001) 7497–7499.
8. V.B. Ganga, E. Suresh, R.L. Varma, Synthesis of isoxazolidine derived macrocycles 49, 2008, pp. 1750–1752.
9. M. Frederickson, School, Optically Active Isoxazolidines via Asymmetric Cycloaddition Reactions of Nib-Ones with Alkenes: Applications in Organic Synthesis, 1997, pp. 403–425.
10. A.B. Andrea Goti, Valentina Fedi, Luca Nannelli, Francesco De Sarlo, Cycloaddition of an enantiopure cyclic nitrone to maleate: straightforward synthesis of the necine base (-)-Hastanecine, Synlett 5 (1997) 577–579.
11. A.B. Madiha Djahieche, Hamza BOUFENAYA, REGIO-AND STEREOSELECTIVITY OF [3+2] CYCLOADDITION REACTION.pdf, 2019, pp. 973–980.
12. T. Cheviet, G. Dujardin, I. Parrot, J. Martinez, M. Mousseron, I.U. Cnrs, U. De Montpellier, P.E. Bataillon, Isoxazolidine : A Privileged Sca Ff Old for Organic and Medicinal Chemistry Mathe O, 2016.
13. J.K. Gallos, K.C. Damianou, C.C. Dellios, A new total synthesis of pentenomycin, Tetrahedron Lett. 42 (2001) 5769–5771, 42.
14. P.C.A.E. Huie, THE [3+2] NITRONE-OLEFIN CYCLOADDITION REACTION, 3th ed., John Wiley & Sons, New York, 2002.
15. M. Carda, R. Portoles, J. Murga, S. Uriel, J.A. Marco, L.R. Domingo, R.J. Zaragoza, H. Roper, Stereoselective 1,3-dipolar cycloadditions of a chiral nitrone derived from erythrulose. An experimental and DFT theoretical study, J. Org. Chem. 65 (2000) 7000–7009.
16. N. Paul, S. Kaladevi, S. Muthusubramanian, Microwave-Assisted Stereoselective 1 , 3-Dipolar Cycloaddition of C , N - diarylnitrone (i. e., N - (Arylmethylidene) benzenamine N -Oxide) and Crystal Analysis of Diastereoisomeric Bis (isoxazolidines) 95, 2012, pp. 173–184.
17. D. Goyard, S.M. Telligmann, C. Goux-Henry, M.M.K. Boysen, E. Framery, D. Gueyrard, S. Vidal, Carbohydrate-based spiro bis(isoxazolines): synthesis and evaluation in asymmetric catalysis, Tetrahedron Lett. 51 (2010) 374–377.
18. D. Kuriakose, R.K. Thumpakara, A. Jesna, J.P. Jacob, Substituent effects in the formation of a few acenaphthenone-2-ylidene ketones and their molecular docking studies and in silico ADME profile, J. Mol. Struct. 1224 (2021), 129209.
19. K.R.A. Abdellatif, E.K.A. Abdelall, H.A.H. Elshemy, P.F. Lamie, E. Elnahaas, D.M.E. Amin, Design , synthesis of new anti-inflammatory agents with a pyrazole core : COX-1/COX-2 inhibition assays , anti-

inflammatory , ulcerogenic , histopathological , molecular Modeling , and ADME studies, J. Mol. Struct. 1240 (2021), 130554.

20. V.K. Singh, H. Chaurasia, R. Mishra, R. Srivastava, F. Naaz, P. Kumar, R.K. Singh, Docking, ADMET prediction, DFT analysis, synthesis, cytotoxicity, antibacterial screening and QSAR analysis of diarylpyrimidine derivatives, J. Mol. Struct. 1247 (2021), 131400.
21. A. AL Adhreai, M. ALSaeedy, M. Farooqui, A. Alrabie, I. Al-Qadsy, U. Al-Timari, Stereoselective synthesis of novel chiral open-chain D-ribose and D-glucose-derived nitrones through 1,3-dipolar cycloaddition of maleimide and maleic acid and investigation of their antimicrobial activity via molecular docking and ADMET studies, J. Mol. Struct. 1256 (2022), 132481.
22. A. Daina, O. Michielin, V. Zoete, SwissADME: a free web tool to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of small molecules, Sci. Rep. 7 (2017) 1–13.
23. C.I. Owuama, Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a novel dilution tube method 11, 2017, pp. 977–980.
24. A.O. Kshetry, N.D. Pant, R. Bhandari, S. Khatri, K.L. Shrestha, S.K. Upadhaya, A. Poudel, B. Lekhak, B.R. Raghubanshi, Minimum inhibitory concentration of vancomycin to methicillin resistant Staphylococcus aureus isolated from different clinical samples at a tertiary care hospital in Nepal, Antimicrob. Resist. Infect. Control 5 (2016) 4–9.
25. E. Yuanita, I.M. Sudarma, N.M. Sudewiningsih, J. Syahri, N.K.T. Dharmayani, Sudirman, M. Ulfa, I. Sumarlan, Antibacterial activity and molecular docking studies of series hydroxyxanthone, AIP Conf. Proc. 2243 (2020).
26. M.K. Ibrahim, M.S. Taghour, A.M. Metwaly, A. Belal, A.B.M. Mehany, M.A. Elhendawy, M.M. Radwan, A.M. Yassin, N.M. El-Deeb, E.E. Hafez, M.A. ElSohly, I.H. Eissa, Design, synthesis, molecular modeling and anti-proliferative evaluation of novel quinoxaline derivatives as potential DNA intercalators and topoisomerase II inhibitors, Eur. J. Med. Chem. 155 (2018) 117–134.
27. I. Althagafi, N. El-Metwaly, T.A. Farghaly, New Series of Thiazole Derivatives: Synthesis, Structural Elucidation, Antimicrobial Activity, Molecular Modeling and MOE Docking, Molecules 24 (2019).
28. W.K.S.R. Sandler, Organic Functional Group Preparations, 2013, p. 435.
29. V. Yanmaz, A. Disli, S. Yavuz, H. Ogutcu, G. Dilek, Synthesis of some novel isoxazolidine derivatives via 1,3-dipolar cycloaddition and their biological evaluation, Gazi Univ. J. Sci. 32 (2019) 78–89.
30. F.M., A.-T.U. Al Adhreai Arwa, Alsaeedy Mohammed, REGIO-AND STEREOSELECTIVITY OF 1,3-DIPOLAR CYCLOADDITION REACTION OF CINNARIZINE DRUG WITH CHIRAL NITRONES, AND THEIR ANTIMICROBIAL ACTIVITY, 2021, pp. |2728–2738.
31. L.R. Domingo, M. Ríos-Guti´errez, P. P´erez, A molecular electron density theory study of the reactivity and selectivities in [3 + 2] cycloaddition reactions of C,N- dialkyl nitrones with ethylene derivatives, J. Org. Chem. 83 (2018) 2182–2197.
32. G. Periyasami, N. Arumugam, A. Aldalbahi, Inexpensive ionic liquid mediated green synthetic approach of multi- functionalized regioselective ββββ-lactam fused isoxazolidine heterocyclic hybrids, Tetrahedron 73 (2017) 322–330.
33. J. Liu, S. Niwayama, Y. You, K.N. Houk, Theoretical prediction and experimental tests of conformational switches in transition states of diels-alder and 1,3-dipolar cycloadditions to enol ethers, J. Org. Chem. 63 (1998) 1064–1073.
34. P. Merino, J. Revuelta, T. Tejero, U. Chiacchio, A. Rescifina, A. Piperno, G. Romeo, Enantioselective synthesis of 4-hydroxy-D-pyroglutamic acid derivatives by an asymmetric 1,3-dipolar cycloaddition, Tetrahedron Asymmetry 13 (2002) 167–172.
35. S. Ghannay, S. Bakari, A. Ghabi, A. Kadri, M. Msaddek, K. Aouadi, Stereoselective synthesis of enantiopure N-substituted pyrrolidin-2,5-dione derivatives by 1,3- dipolar cycloaddition and assessment of their in vitro antioxidant and antibacterial activities, Bioorg. Med. Chem. Lett 27 (2017) 2302–2307.
36. S. Garg, A. Anand, Y. Lamba, A. Roy, Molecular docking analysis of selected phytochemicals against SARS - CoV - 2 - M pro receptor, Vegetos 33 (2020) 766–781.
37. C.A. Lipinski, Lead- and drug-like compounds: the rule-of-five revolution, Drug Discov. Today Technol. 1 (2004) 337–341.