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Synthesis and Characterization of Substituted Isoxazolidines through 1,3-Dipolar Cycloaddition and Evaluation of their Antibacterial, Antioxidant and Molecular Docking Studies

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A series of substituted isoxazolidine derivatives (**3a-g**) was synthesized through a 1,3-dipolar [3+2] cycloaddition of a glutaraldehyde-derived nitrone with substituted cinnamaldehyde-derived chalcones. The synthesized derivatives were evaluated for their *in vitro* antibacterial activity against selected Gram-positive and Gram-negative bacterial strains using the disk diffusion method and for DPPH radical-scavenging activity. All compounds exhibited measurable antibacterial activity, with inhibition zones of 13-28 mm. Compound **3a** gave the highest inhibition zones against *E. coli* (24 mm), *K. oxytoca* (20 mm), *P. aeruginosa* (28 mm) and *S. epidermidis* (21 mm). In the DPPH assay, the compounds displayed concentration-dependent radical-scavenging activity. Compound **3c** showed the lowest reported IC_{50} value (47.8 μg/mL) followed by **3e** (49.5 μg/mL) and **3b** (50.6 μg/mL), while **3a** showed 80.3% scavenging at 100 μg/mL. Molecular docking studies were performed against MRSA Sar2676 pantothenate synthetase, α-amylase and α-glucosidase. Compound **3a** showed favourable predicted interactions with all three targets, with docking scores of -7.1, -8.4 and -7.8 kcal/mol, respectively.

Keywords: Isoxazolidine compounds, 1,3-Dipolar cycloaddition, Nitrone, Cinnamaldehyde, Chalcone, Antioxidant activity.

INTRODUCTION

Saturated five-membered isoxazolidines have attracted sustained interest in synthetic and medicinal chemistry due to the presence of adjacent nitrogen and oxygen atoms within a versatile heterocyclic framework. This structural feature is associated with diverse chemical reactivity and has contributed to the reported biological relevance of isoxazolidine derivatives [1]. Compounds containing this scaffold have been investigated for antibacterial, antifungal, anticancer, antioxidant anti-inflammatory and antiviral properties [2]. The structural diversity accessible through substitution at different positions of the ring further supports the use of isoxazolidines as useful scaffolds in medicinal chemistry.

The 1,3-dipolar cycloaddition reaction represents an established method for constructing isoxazolidine rings. In this transformation, nitrones act as 1,3-dipoles and undergo [3+2] cycloaddition with alkenes or other suitable dipolarophiles. The reaction can provide highly functionalized five-membered heterocycles with useful regio- and stereochemical control [3]. Nitrones are particularly valuable in this chemistry since modification of their substituents permits structural variation in the resulting isoxazolidines [4,5]. The N–O bond of these heterocycles can undergo reductive cleavage under suitable conditions, which provide an access to β-amino alcohols, amino sugars, β-lactams, alkaloid derivatives and amino acid related structures [6-10]. Isoxazolidines consequently serve both as biologically relevant molecules and as versatile synthetic intermediates [4].

Glutaraldehyde-derived nitrones offer a useful route to structurally extended isoxazolidine systems. Glutaraldehyde is a five-carbon dialdehyde containing aldehyde groups at both terminal and has been widely used in chemical and industrial applications [11]. Its bifunctional structure provides a suitable precursor for the preparation of nitrone intermediates. Similarly, chalcones, characterized by an α,β-unsaturated carbonyl moiety, constitute another important class of substrates in synthetic and medicinal chemistry. Their conjugated double bond provides an appropriate site for 1,3-dipolar cycloaddition, while substitution on the aromatic rings allows modification of the electronic and steric properties of the products [12,13].

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The combination of substituted chalcones with glutaraldehyde-derived N-aryl nitrones was selected in the present work to access structurally diverse isoxazolidine derivatives. The approach permits variation of substituents on the reacting molecules and provides a framework for examining their influence on biological activity. A series of substituted isoxazolidines was synthesized through [3+2] cycloaddition of the nitrone intermediates with cinnamaldehyde-derived chalcones. The synthesized compounds were characterized by appropriate spectroscopic and analytical methods and evaluated for their antibacterial and antioxidant properties. Antibacterial activity was examined against selected Gram-positive and Gram-negative bacterial strains, while antioxidant activity was assessed using the DPPH radical-scavenging assay.

Selected compounds were also subjected to molecular docking studies to obtain structural information on their possible interactions with biologically relevant protein targets. The Sar2676 protein from methicillin-resistant *Staphylococcus aureus* (MRSA; PDB ID: 2X3F) was selected as an antibacterial target. α-Amylase (PDB ID: 2QV4) and α-glucosidase (PDB ID: 3W37) were selected as carbohydrate-metabolizing enzymes to examine the possible interaction of the synthesized compounds with targets associated with carbohydrate hydrolysis.

EXPERIMENTAL

Reagent grade chemicals and solvents were used throughout the experimental work. Ethanol, sodium hydroxide, ammonium chloride, acetophenone, glutaraldehyde, zinc powder and nitrobenzene were obtained from commercial sources and used directly without additional purification. Reaction progress was followed by thin-layer chromatography (TLC) on silica gel plates. FT-IR spectra were recorded using an FTIR-PRO-ONE spectrometer in the range of 4000-400 cm^{-1}. Nuclear magnetic resonance measurements were carried out using a Bruker spectrometer at 400 MHz for ^1H NMR and 100 MHz for ^{13}C NMR. Spectra were acquired in $CDCl_3$, with tetramethylsilane (TMS) used as the reference standard.

Synthesis of phenyl hydroxylamine: A conical flask equipped with a mechanical stirrer and thermometer was charged with ammonium chloride (10 g) dissolved in water (320 mL), followed by the addition of nitrobenzene (16.6 mL). The mixture was stirred thoroughly and zinc powder (23.6 g, 90% purity) was added gradually. The addition of zinc caused a rapid rise in temperature to approximately 60-65 ºC. The reaction mixture was maintained under stirring for 15 min and then stirred for a further 15 min while allowing the temperature to decrease. The hot mixture was filtered to remove the ZnO formed during the reaction. The solid residue was washed with hot water (100 mL) and the combined filtrate was transferred to a clean conical flask. Sodium chloride (60 g) was added to the filtrate, which was then cooled in an ice bath for at least 1 h to promote crystallization. The resulting pale-yellow crystals of phenylhydroxylamine were collected by suction filtration and thoroughly dried.

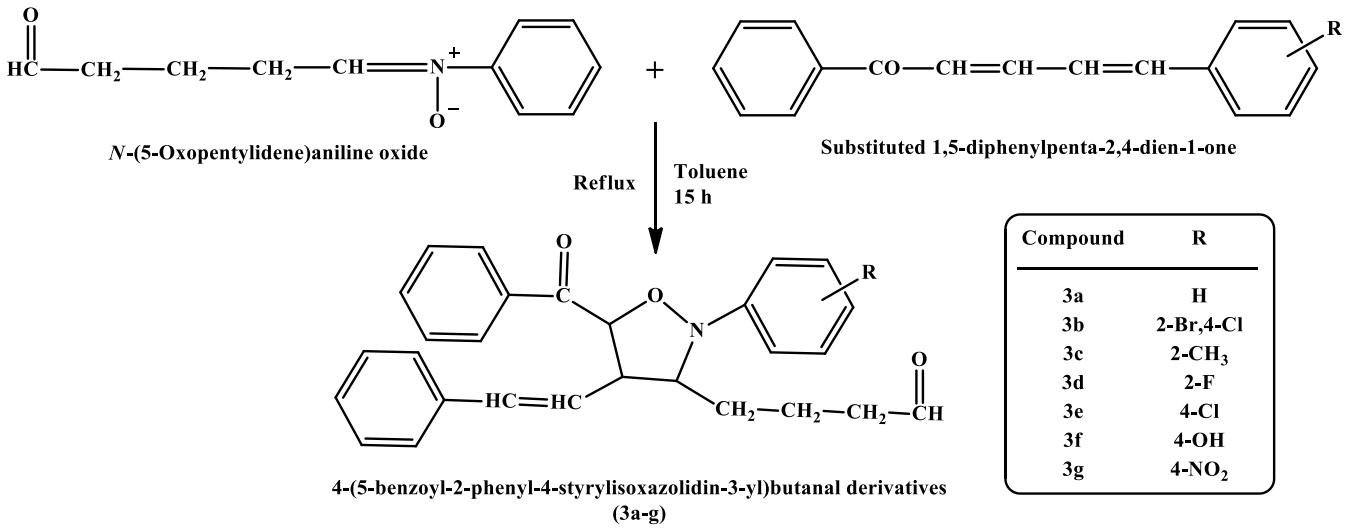
Synthesis of glutaraldehyde nitrone: Phenyl hydroxylamine was dissolved in ethanol followed by the addition of glutaraldehyde in a 1:1 molar ratio. The reaction mixture was heated under reflux for 1 h under dark conditions and subsequently cooled to room temperature. After completion of the reaction, the solvent was removed to afford a solid residue. The solid was separated by filtration and purified by recrystallization from ethanol, yielding the glutaraldehyde-derived nitrone as a crystalline product.

Synthesis of substituted 1,5-diphenylpenta-2,4-dien-1-one: Substituted cinnamaldehyde (7 mL) and acetophenone (6 mL) were dissolved in ethanol (20 mL) separately. Then, NaOH pellets (20 g) were added to the reaction mixture and the resulting suspension was stirred for 40 min at 40 ºC. After completion of the reaction, ethanol was further added to maintain a suitable reaction medium. The mixture was then allowed to cool followed by the addition of cold water for dilution. The resulting solid was separated and purified by an appropriate procedure to afford the corresponding chalcones.

Synthesis of isoxazolidine derivatives (3a-g): Glutaraldehyde-derived nitrone (**1**) and cinnamaldehyde-derived chalcone (**2**) were mixed in toluene (50 mL) and heated under reflux for the required reaction period. Reaction progress was monitored by thin-layer chromatography (TLC). After completion, the solvent was removed under reduced pressure and the resulting residue was purified by recrystallization from petroleum ether to afford 4-(5-benzoyl-2-phenyl-4-styrylisoxazolidin-3-yl)butanal (**3a**) (**Scheme-I**).

4-(5-Benzoyl-2-phenyl-4-styrylisoxazolidin-3-yl)-butanal (3a): Brown solid, yield: 95%, m.p.: 185-187 ºC; Anal. calcd. for $C_{28}H_{27}NO_3$: C, 79.03 (79.01); H, 6.40 (6.35); N, 3.29 (3.30); O, 11.28 (11.26); IR (KBr, ν_{max}, cm^{-1}): 3042 (arom. C–H), 2927, 2815 (aliph. C–H), 2816, 2724 (aldehydic C–H), 1718 (aldehydic C=O), 1682 (benzoyl C=O), 1598, 1517 (arom. C=C), 1242 (C–O), 1098 (N–O); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.51-1.70 (4H, 1.57 (quint, *J* = 7.5 Hz), 1.64 (td, *J* = 7.4, 6.8 Hz), 2.53 (2H, td, *J* = 7.5, 6.9 Hz), 3.30 (1H, ddd, *J* = 7.0, 4.4, 4.2 Hz), 3.82 (1H, dt, *J* = 7.0, 6.8 Hz), 5.34 (1H, d, *J* = 4.4 Hz), 5.82 (1H, dd, *J* = 10.2, 4.2 Hz), 6.80 (1H, d, *J* = 10.2 Hz), 6.99-7.48 (10H, 7.05 (tt, *J* = 8.1, 1.2 Hz), 7.08 (dtd, *J* = 8.2, 1.2, 0.5 Hz), 7.19 (tt, *J* = 7.7, 1.3 Hz), 7.32 (dddd, *J* = 8.2, 8.1, 1.4, 0.5 Hz), 7.32 (dddd, *J* = 7.7, 7.6, 1.8, 0.5 Hz), 7.42 (dddd, *J* = 7.6, 1.8, 1.3, 0.5 Hz), 7.49-7.73 (3H, 7.57 (dddd, *J* = 8.4, 7.4, 1.4, 0.5 Hz), 7.67 (tt, *J* = 7.4, 1.5 Hz), 7.97 (2H, dddd, *J* = 8.4, 1.9, 1.5, 0.5 Hz), 9.66 (1H, t, *J* = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 29.5 (1C, s), 34.4 (1C, s), 43.4 (1C, s), 54.2 (1C, s), 71.1 (1C, s), 83.1 (1C, s), 124.3 (2C, s), 127.3 (2C, s), 128.0-128.2 (3C, 128.1 (s), 128.1 (s), 128.1 (s), 128.5-128.7 (6C, 128.6 (s), 128.6 (s), 128.6 (s), 129.0 (2C, s), 131.9 (1C, s), 132.2 (1C, s), 134.8 (1C, s), 137.0 (1C, s), 148.0 (1C, s), 201.4-201.6 (2C, 201.5 (s), 201.5 (s); MS: *m/z*: 425 [M+].

4-(5-Benzoyl-4-(2-bromo-4-chlorostyryl)-2-phenylisoxazolidin-3-yl)butanal (3b): Brown solid, yield: 75%, m.p.: 160-162 ºC. Anal. calcd. (found) % for $C_{28}H_{25}BrClNO_3$: C, 62.41 (62.45); H, 4.68 (4.66); Br, 14.83 (14.79); Cl, 6.58 (6.60); N, 2.60 (2.65); O, 8.91 (8.94); IR (KBr, ν_{max}, cm^{-1}): 3066 (arom. C–H), 2945, 2862 (aliph. C–H), 2825, 2718 (aldehydic C–H), 1706 (aldehydic C=O), 1685 (benzoyl C=O), 1607, 1507 (arom. C=C), 1256 (C–O), 1112 (N–O), 1048 (C–N), 754, 698 (C–Br/C–Cl); ^1H NMR (400 MHz, $CDCl_3$, δ ppm): 1.51-1.71 (4H, 1.57 (quint, *J* = 7.5 Hz), 1.64



Scheme-I: Synthesis of isoxazolidine

(td, J = 7.4, 6.8 Hz), 2.53 (2H, td, J = 7.5, 6.9 Hz), 3.21 (1H, ddd, J = 7.0, 4.4, 4.1 Hz), 3.85 (1H, dt, J = 7.0, 6.8 Hz), 5.17 (1H, d, J = 4.4 Hz), 6.39-6.58 (2H, 6.46 (dd, J = 16.7, 4.1 Hz), 6.51 (d, J = 16.7 Hz), 6.97-7.39 (8H, 7.03 (dd, J = 8.3, 0.5 Hz), 7.05 (tt, J = 8.1, 1.2 Hz), 7.08 (dtd, J = 8.2, 1.2, 0.5 Hz), 7.20 (dd, J = 8.3, 1.7 Hz), 7.29 (dd, J = 1.7, 0.5 Hz), 7.32 (dddd, J = 8.2, 8.1, 1.4, 0.5 Hz), 7.49-7.73 (3H, 7.57 (dddd, J = 8.4, 7.4, 1.4, 0.5 Hz), 7.67 (tt, J = 7.4, 1.5 Hz), 7.97 (2H, dddd, J = 8.4, 1.9, 1.5, 0.5 Hz), 9.66 (1H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 29.5 (1C, s), 34.4 (1C, s), 43.4 (1C, s), 54.2 (1C, s), 71.1 (1C, s), 83.1 (1C, s), 122.6 (1C, s), 124.3 (2C, s), 128.0-128.2 (2C, 128.1 (s), 128.1 (s), 128.5-128.7 (4C, 128.6 (s), 128.6 (s), 128.8-129.1 (4C, 128.8 (s), 128.9 (s), 129.0 (s), 131.3 (1C, s), 131.9 (1C, s), 132.2 (1C, s), 133.1 (1C, s), 136.3 (1C, s), 137.0 (1C, s), 148.0 (1C, s), 201.4-201.6 (2C, 201.5 (s), 201.5 (s); MS: *m/z*: 538.86 [M+].

4-(5-Benzoyl-4-(2-methylstyryl)-2-phenylisoxazolidin-3-yl)butanal (3c): Brown solid, yield: 80%, m.p.: 150-152 ºC, Anal. calcd. (found) % for $C_{29}H_{29}NO_3$: C, 79.24 (79.29); H, 6.65 (6.60); N, 3.19 (3.17); O, 10.92 (10.96); IR (KBr, ν_{max}, cm^{-1}): 3062 (arom. C–H), 2928, 2858 (aliph. C–H), 2820, 2720 (aldehydic C–H), 1716 (aldehydic C=O), 1677 (benzoyl C=O), 1607, 1507 (arom. C=C), 1256 (C–O), 1112 (N–O), 1042 (C–N); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.51-1.71 (4H, 1.57 (quint, J = 7.5 Hz), 1.64 (td, J = 7.4, 6.8 Hz), 2.19 (3H, s), 2.53 (2H, td, J = 7.5, 6.9 Hz), 3.26 (1H, ddd, J = 7.0, 4.4, 4.1 Hz), 3.85 (1H, dt, J = 7.0, 6.8 Hz), 5.17 (1H, d, J = 4.4 Hz), 6.41-6.56 (2H, 6.48 (d, J = 16.9 Hz), 6.49 (dd, J = 16.9, 4.1 Hz), 6.99-7.39 (9H, 7.05 (tt, J = 8.1, 1.2 Hz), 7.08 (dtd, J = 8.2, 1.2, 0.5 Hz), 7.09 (td, J = 7.8, 1.2 Hz), 7.14 (td, J = 7.7, 2.2 Hz), 7.16 (ddd, J = 7.8, 2.2, 0.5 Hz), 7.26 (ddd, J = 7.7, 1.2, 0.5 Hz), 7.32 (dddd, J = 8.2, 8.1, 1.4, 0.5 Hz), 7.49-7.73 (3H, 7.57 (dddd, J = 8.4, 7.4, 1.4, 0.5 Hz), 7.67 (tt, J = 7.4, 1.5 Hz), 7.97 (2H, dddd, J = 8.4, 1.9, 1.5, 0.5 Hz), 9.66 (1H, t, J = 6.9 Hz); ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 19.4 (1C, s), 29.5 (1C, s), 34.4 (1C, s), 43.4 (1C, s), 54.2 (1C, s), 71.1 (1C, s), 83.1 (1C, s), 124.3 (2C, s), 128.0-128.2 (2C, 128.1 (s), 128.1 (s), 128.3 (1C, s), 128.5-128.7 (6C, 128.6 (s), 128.6 (s), 128.6 (s), 128.6 (s), 129.0 (2C, s), 129.8 (1C, s), 131.9 (1C, s), 132.2 (1C, s), 135.4 (1C, s), 136.6 (1C, s), 137.0 (1C, s), 148.0 (1C, s), 201.4-201.6 (2C, 201.5 (s), 201.5 (s); MS: *m/z*: 439.21 [M+].

4-(5-Benzoyl-4-(2-fluorostyryl)-2-phenylisoxazolidin-3-yl)butanal (3d): Brown solid, 78% m.p.: of 170-172 ºC, Anal. calcd. (found) % for $C_{28}H_{26}FNO_3$: C, 75.83 (75.85); H, 5.91 (5.93); F, 4.28 (4.26); N, 3.16 (3.18); O, 10.82 (10.85); IR (KBr, ν_{max}, cm^{-1}): 3060 (arom. C–H), 2925, 2855 (aliph. C–H), 2820, 2720 (aldehydic C–H), 1716 (aldehydic C=O), 1685 (benzoyl C=O), 1600, 1505 (arom. C=C), 1250 (C–O), 1222 (C–F), 1110 (N–O), 1047 (C–N); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.51-1.70 (4H, 1.57 (quint, J = 7.5 Hz), 1.64 (td, J = 7.4, 6.8 Hz), 2.53 (2H, td, J = 7.5, 6.9 Hz), 3.29 (1H, ddd, J = 7.0, 4.4, 4.1 Hz), 3.84 (1H, dt, J = 7.0, 6.8 Hz), 5.16 (1H, d, J = 4.4 Hz), 6.41-6.58 (2H, 6.49 (dd, J = 16.7, 4.1 Hz), 6.51 (d, J = 16.7 Hz), 6.93-7.14 (5H, 7.00 (ddd, J = 8.0, 7.4, 1.6 Hz), 7.05 (tt, J = 8.1, 1.2 Hz), 7.07 (ddd, J = 8.3, 1.6, 0.5 Hz), 7.08 (dtd, J = 8.2, 1.2, 0.5 Hz), 7.24-7.48 (3H, 7.32 (dddd, J = 8.2, 8.1, 1.4, 0.5 Hz), 7.41 (ddd, J = 8.3, 7.4, 1.4 Hz), 7.49-7.73 (4H, 7.57 (dddd, J = 8.4, 7.4, 1.4, 0.5 Hz), 7.57 (ddd, J = 8.0, 1.4, 0.5 Hz), 7.67 (tt, J = 7.4, 1.5 Hz), 7.97 (2H, dddd, J = 8.4, 1.9, 1.5, 0.5 Hz), 9.66 (1H, t, J = 6.9 Hz). ^{13}C NMR (100 MHz, $CDCl_3$, δ ppm): 29.5 (1C, s), 34.4 (1C, s), 43.4 (1C, s), 54.2 (1C, s), 71.1 (1C, s), 83.1 (1C, s), 115.4 (1C, s), 121.3 (1C, s), 124.3 (2C, s), 128.0-128.2 (2C, 128.1 (s), 128.1 (s), 128.3 (1C, s), 128.5-128.7 (6C, 128.6 (s), 128.6 (s), 128.6 (s), 128.6 (s), 129.0 (2C, s), 131.9 (1C, s), 132.2 (1C, s), 137.0 (1C, s), 148.0 (1C, s), 160.6 (1C, s), 201.4-201.6 (2C, 201.5 (s), 201.5 (s); MS: *m/z*: 443.19 [M+].

4-(5-Benzoyl-4-(4-chlorostyryl)-2-phenylisoxazolidin-3-yl)butanal (3e): Brown solid yield: 82%; m.p.: 190-192 ºC; Anal. calcd. (found) % for $C_{28}H_{26}ClNO_3$: C, 73.11 (73.15); H, 5.70 (5.73); Cl, 7.71 (7.75); N, 3.05 (3.01); O, 10.44 (10.47); IR (KBr, ν_{max}, cm^{-1}): 3060 (arom. C–H), 2925, 2855 (aliph. C–H), 2820, 2720 (aldehydic C–H), 1710 (aldehydic C=O), 1685 (benzoyl C=O), 1600, 1505 (arom. C=C), 1250 (C–O), 1100 (N–O), 1035 (C–N), 760, 700 (C–Cl); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.51-1.70 (4H, 1.57 (quint, J = 7.5 Hz),

1.64 (td, *J* = 7.4, 6.8 Hz), 2.53 (2H, td, *J* = 7.5, 6.9 Hz), 3.30 (1H, ddd, *J* = 7.0, 4.4, 4.1 Hz), 3.85 (1H, dt, *J* = 7.0, 6.8 Hz), 5.25 (1H, d, *J* = 4.4 Hz), 6.40-6.64 (2H, 6.48 (dd, *J* = 16.9, 4.1 Hz), 6.56 (d, *J* = 16.9 Hz), 6.99-7.14 (3H, 7.05 (tt, *J* = 8.1, 1.2 Hz), 7.08 (dtd, *J* = 8.2, 1.2, 0.5 Hz), 7.20-7.73 (9H, 7.26 (ddd, *J* = 8.0, 1.8, 0.5 Hz), 7.32 (dddd, *J* = 8.2, 8.1, 1.4, 0.5 Hz), 7.45 (ddd, *J* = 8.0, 1.5, 0.5 Hz), 7.57 (dddd, *J* = 8.4, 7.4, 1.4, 0.5 Hz), 7.67 (tt, *J* = 7.4, 1.5 Hz), 7.97 (2H, dddd, *J* = 8.4, 1.9, 1.5, 0.5 Hz), 9.66 (1H, t, *J* = 6.9 Hz); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 29.5 (1C, s), 34.4 (1C, s), 43.4 (1C, s), 54.2 (1C, s), 71.1 (1C, s), 83.1 (1C, s), 124.3 (2C, s), 128.0-128.2 (2C, 128.1 (s), 128.1 (s), 128.3 (2C, s), 128.5-128.7 (4C, 128.6 (s), 128.6 (s), 128.8-129.1 (4C, 128.9 (s), 129.0 (s), 131.9 (1C, s), 132.2 (1C, s), 134.1 (1C, s), 134.8 (1C, s), 137.0 (1C, s), 148.0 (1C, s), 201.4-201.6 (2C, 201.5 (s), 201.5 (s); MS: *m/z*: 459.96 [M+].

4-(5-Benzoyl-4-(4-hydroxystyryl)-2-phenylisoxazolidin-3-yl)butanal (3f): Brown solid, yield: 90%, m.p.: 150-152 ºC, Anal. calcd. (found) % for $C_{28}H_{27}NO_4$: C, 76.17 (76.19); H, 6.16 (6.13); N, 3.17 (3.19); O, 14.49 (14.43); IR (KBr, ν_{max}, cm^{-1}): 3410 (O–H), 1710 (aldehydic C=O), 1685 (benzoyl C=O), 2925, 2855 (aliph. C–H), 2820, 2720 (aldehydic C–H), 1600, 1510 (arom. C=C), 1250 (C–O), 1100 (N–O); ^{1}H NMR (400 MHz, $CDCl_3$, δ ppm): 1.51-1.70 (4H, 1.57 (quint, *J* = 7.5 Hz), 1.64 (td, *J* = 7.4, 6.8 Hz), 2.53 (2H, td, *J* = 7.5, 6.9 Hz), 3.12 (1H, ddd, *J* = 7.0, 4.4, 4.1 Hz), 3.81 (1H, dt, *J* = 7.0, 6.8 Hz), 5.07 (1H, d, *J* = 4.4 Hz), 6.32-6.57 (2H, 6.39 (dd, *J* = 17.0, 4.1 Hz), 6.49 (d, *J* = 17.0 Hz), 6.73 (2H, ddd, *J* = 8.3, 2.8, 0.4 Hz), 6.99-7.14 (3H, 7.05 (tt, *J* = 8.1, 1.2 Hz), 7.08 (dtd, *J* = 8.2, 1.2, 0.5 Hz), 7.19-7.39 (4H, 7.26 (ddd, *J* = 8.3, 1.9, 0.4 Hz), 7.32 (dddd, *J* = 8.2, 8.1, 1.4, 0.5 Hz), 7.49-7.73 (3H, 7.57 (dddd, *J* = 8.4, 7.4, 1.4, 0.5 Hz), 7.67 (tt, *J* = 7.4, 1.5 Hz), 7.97 (2H, dddd, *J* = 8.4, 1.9, 1.5, 0.5 Hz), 9.66 (1H, t, *J* = 6.9 Hz); ^{13}C NMR (400 MHz, $CDCl_3$, δ ppm): 29.5 (1C, s), 34.4 (1C, s), 43.4 (1C, s), 54.2 (1C, s), 71.1 (1C, s), 83.1 (1C, s), 115.5 (2C, s), 124.3 (2C, s), 128.0-128.2 (2C, 128.1 (s), 128.1 (s), 128.3 (2C, s), 128.5-128.7 (4C, 128.6 (s), 128.6 (s), 129.0 (2C, s), 131.9 (1C, s), 132.2 (1C, s), 134.8 (1C, s), 137.0 (1C, s), 148.0 (1C, s), 157.2 (1C, s), 201.4-201.6 (2C, 201.5 (s), 201.5 (s); MS: *m/z*: 441.52 [M+].

4-(5-Benzoyl-4-(4-nitrostyryl)-2-phenylisoxazolidin-3-yl)butanal (3g): Brown solid, yield: 85% m.p.: 165-167 ºC; Anal. calcd. (found) % for $C_{28}H_{26}N_2O_5$: C, 71.47 (71.43); H, 5.57 (5.55); N, 5.95 (5.55); O, 17.00 (17.03); IR (KBr, ν_{max}, cm^{-1}): 3060 (arom. C–H), 2925, 2855 (aliph. C–H), 1715 (aldehydic C=O), 1685 (benzoyl C=O), 1600, 1495 (arom. C=C), 1250 (C–O), 1100 (N–O), 1035 (C–N), 970 (C=C); ^{1}H NMR: (400 MHz, $CDCl_3$, δ ppm): 1.51-1.74 (4H, 1.57 (quint, *J* = 7.5 Hz), 1.68 (td, *J* = 7.4, 6.8 Hz), 2.53 (2H, td, *J* = 7.5, 6.9 Hz), 3.25 (1H, ddd, *J* = 7.0, 4.7, 4.4 Hz), 3.92 (1H, dt, *J* = 7.0, 6.8 Hz), 5.52 (1H, d, *J* = 4.4 Hz), 6.60 (1H, d, *J* = 15.8 Hz), 6.81 (1H, dd, *J* = 15.8, 4.7 Hz), 6.99-7.14 (3H, 7.05 (tt, *J* = 8.1, 1.2 Hz), 7.08 (dtd, *J* = 8.2, 1.2, 0.5 Hz), 7.32 (2H, dddd, *J* = 8.2, 8.1, 1.4, 0.5 Hz), 7.49-7.73 (5H, 7.57 (dddd, *J* = 8.4, 7.4, 1.4, 0.5 Hz), 7.56 (ddd, *J* = 8.6, 1.7, 0.5 Hz), 7.67 (tt, *J* = 7.4, 1.5 Hz), 7.90-8.13 (4H, 7.97 (dddd, *J* = 8.4, 1.9, 1.5, 0.5 Hz), 8.07 (ddd, *J* = 8.6, 1.9, 0.5 Hz), 9.66 (1H, t, *J* = 6.9 Hz); ^{13}C NMR: (400 MHz, $CDCl_3$, δ ppm): 29.5 (1C, s), 34.4 (1C, s), 43.4 (1C, s), 54.2 (1C, s), 71.1 (1C, s), 83.1 (1C, s), 123.8 (2C, s), 124.3 (2C, s), 128.0-128.2 (2C, 128.1 (s), 128.1 (s), 128.3 (2C, s), 128.5-128.7 (4C, 128.6 (s), 128.6 (s), 129.0 (2C, s), 131.9 (1C, s), 132.2 (1C, s), 134.8 (1C, s), 137.0 (1C, s), 147.3 (1C, s), 148.0 (1C, s), 201.4-201.6 (2C, 201.5 (s), 201.5 (s); MS: *m/z*: 470.18 [M+].

Antibacterial activity: The antibacterial activity of the synthesized isoxazolidine derivatives **3a-g** was evaluated by the Kirby-Bauer disk diffusion method [14] against selected Gram-negative and Gram-positive bacterial strains. The standardized bacterial suspensions were uniformly inoculated onto sterile Mueller-Hinton agar plates to obtain a confluent bacterial lawn. Sterile paper disks containing the test compounds were placed on the inoculated agar surface and the plates were incubated at 37 ºC for 18-24 h. Amikacin was used as the standard antibacterial agent, while DMSO was used as the vehicle control. After incubation, the diameter of the clear zone surrounding each disk was measured in millimetres and recorded as the zone of inhibition (ZOI).

Antioxidant activity: The DPPH radical-scavenging activity of compounds **3a-g** was evaluated using a previously reported spectrophotometric method [15]. A fresh 0.1 mM DPPH solution was prepared in 95% ethanol. The test compounds were dissolved in DMSO and diluted to concentrations of 10, 25, 50, 75 and 100 μg/mL. For each measurement, 40 μL of the test solution was mixed with 2.96 mL of DPPH solution and incubated in the dark at room temperature for 20 min. The absorbance was recorded at 517 nm using a UV-Visible spectrophotometer. DPPH solution without the test compound served as the control and ascorbic acid was used as the reference standard. The percentage of DPPH radical-scavenging activity (RSA) was calculated using the following equation:

$$\text{RSA (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

where $Abs_{control}$ is the absorbance of the DPPH solution without the test compound and Abs_{sample} is the absorbance of the DPPH solution containing the synthesized compound. The IC_{50} values, defined as the concentration of the test compound required to achieve 50% radical-scavenging activity, were determined from the concentration–response curves.

Molecular docking study: Molecular docking studies were performed to investigate the predicted binding affinities and interaction patterns of the synthesized cycloadducts with selected antibacterial and carbohydrate-metabolizing enzyme targets. The crystal structure of MRSA Sar2676 pantothenate synthetase (PDB ID: 2X3F; resolution 1.95 Å) was selected as the antibacterial target. Human pancreatic α-amylase (PDB ID: 2QV4; resolution 1.97 Å) and sugar beet α-glucosidase (PDB ID: 3W37; resolution 1.70 Å) were selected as carbohydrate-metabolizing enzyme targets to explore possible antidiabetic relevance. The protein structures were obtained from the Protein Data Bank and prepared for docking. Docking calculations were performed using AutoDock and the resulting protein–ligand complexes were visualized and analysed using Molegro Molecular Viewer. The docking results were assessed based on predicted binding energy, ligand orientation and hydrogen-bond interactions [16-19].

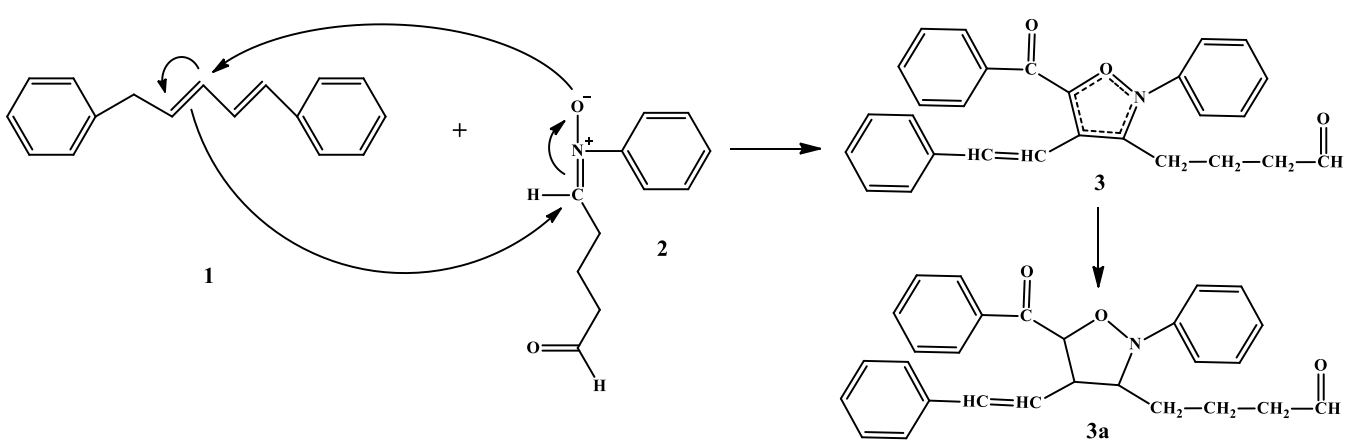
RESULTS AND DISCUSSION

In view of the reported biological relevance of isoxazolidine derivatives, the present study focuses on the synthesis of a series of substituted 4-(5-benzoyl-2-phenyl-4-styrylisoxazolidin-3-yl)butanal derivatives (**3a-g**) through a 1,3-dipolar cycloaddition reaction. Glutaraldehyde-derived nitrone was used as the 1,3-dipole, while substituted cinnamaldehyde derived chalcones served as dipolarophiles. The cycloaddition was conducted in refluxing toluene and the reaction progress was monitored by thin-layer chromatography (TLC). The resulting cycloadducts were isolated and purified by column chromatography using petroleum ether/ethyl acetate as the eluent.

The FT-IR spectrum of compound **3a** exhibited characteristic absorption bands consistent with the proposed isoxazolidine structure. The absorption at 3039 cm^{-1} was assigned to aromatic C–H stretching, while the band at 2848 cm^{-1} corresponded to aliphatic C–H stretching vibrations. A strong absorption attributable to the benzoyl carbonyl (C=O) stretching vibration was observed in the carbonyl region, which confirm the presence of the benzoyl group in the cycloadduct. The band at 1593 cm^{-1} was assigned mainly to aromatic C=C stretching vibrations. The absorption at 1265 cm^{-1} can be attributed to C–O and C–N stretching vibrations associated with the isoxazolidine ring. The band at 1004 cm^{-1} was assigned to N–O stretching, which further confirmed the formation of the isoxazolidine moiety. The absorption near 705 cm^{-1} was associated with aromatic C–H out-of-plane bending.

Mechanism: The proposed mechanism for the formation of 4-(5-benzoyl-2-phenyl-4-styrylisoxazolidin-3-yl)butanal (**3a**) is shown in **Scheme-II**. The cycloadduct is formed through a [3+2] 1,3-dipolar cycloaddition between the glutaraldehyde derived nitrone and the cinnamaldehyde derived chalcone. The nitrone serves as the 1,3-dipole, while the electron-deficient C=C bond of the chalcone acts as the dipolarophile. The reaction involves the interaction of the nitrone 1,3-dipolar system with the alkene π-bond, resulting in the formation of two new C–N and C–O σ-bonds and construction of the five-membered isoxazolidine ring.

Antibacterial activity: The synthesized isoxazolidine derivatives **3a-g** exhibited measurable antibacterial activity against all five tested bacterial strains, with inhibition zones ranging from 13 to 28 mm (Table-1). Compound **3a** showed the highest zones against *E. coli* (24 mm), *K. oxytoca* (20 mm), *P. aeruginosa* (28 mm) and *S. epidermidis* (21 mm), while an 18 mm zone was observed against *S. aureus*. *P. aeruginosa* showed comparatively higher susceptibility, with inhibition zones of 22-28 mm, whereas *S. aureus* showed lower responses (13-18 mm). Compounds **3c**, **3f** and **3g** exhibited lower inhibition across several strains. The variation in activity may be associated with structural differences in the styryl substituent, which can influence molecular properties and interactions with bacterial cells. Amikacin produced an 18 mm zone against all tested organisms; compound **3a** gave larger zones against four strains and an equivalent zone against *S. aureus*. DMSO showed no observable inhibition,



Scheme-II: Mechanism route of synthesis of isoxazolidine

TABLE-1
ANTIBACTERIAL ACTIVITY DATA OF THE SYNTHESIZED ISOXAZOLIDINE DERIVATIVES **3a-g** AGAINST SELECTED BACTERIAL STRAINS

Compound	Zone of inhibition (mm)				
	E. coli	*K. oxytoca*	*P. aeruginosa*	*S. aureus*	*S. epidermidis*
3a	24	20	28	18	21
3b	20	18	27	17	20
3c	18	16	25	15	18
3d	22	19	24	17	20
3e	21	16	23	16	16
3f	20	15	22	14	17
3g	19	16	25	13	18
Amikacin (Std.)	18	18	18	18	18

confirming that the solvent did not contribute to the measured activity. Based on the observed ZOI values, compound **3a** displayed the highest antibacterial response within the synthesized series.

Antioxidant activity: Synthesized isoxazolidines **3a-g** exhibited concentration-dependent DPPH radical-scavenging activity, with the response generally increasing from 10 to 100 μg/mL (Table-2). Among the compounds for which IC_{50} values were determined, compound **3c** showed the lowest IC_{50} (47.8 μg/mL), followed by compound **3e** (49.5 μg/mL) and compound **3b** (50.6 μg/mL). Compounds **3d**, **3f** and **3a** gave IC_{50} values of 58.0, 58.7 and 61.4 μg/mL, respectively. At 100 μg/mL, compound **3a** showed the highest reported RSA (80.3%) followed by compounds **3e** (79.5%) and **3d** (78.7%). The variation in radical-scavenging response may be related to the electronic and steric characteristics of the substituents, which can influence electron- or hydrogen-atom transfer to the DPPH radical. The observed IC_{50} values confirm measurable antioxidant activity within the synthesized series, although comparison with ascorbic acid should be based on the corresponding IC_{50} value rather than percentage inhibition at a single concentration.

Molecular docking studies: The synthesized cycloadducts were docked against MRSA Sar2676 pantothenate synthetase (2X3F) and the predicted binding poses are shown in Fig. 1, while docking scores and amino-acid interactions are shown in Table-3. The compounds showed docking scores reaching -7.1 kcal/mol. Compound **3a** exhibited a favourable docking score of -7.1 kcal/mol and formed a hydrogen bond with LEU113 at a calculated distance of 2.306 Å. Docking with human pancreatic α-amylase (2QV4) gave scores reaching -8.4 kcal/mol. Compound **3a** showed a docking score of -8.4 kcal/mol and formed a hydrogen-bond interaction with TYR151 at a distance of 2.274 Å. For sugar beet α-glucosidase (3W37), docking scores reached -7.8 kcal/mol, with

TABLE-2
ANTIOXIDANT ACTIVITY DATA OF THE SYNTHESIZED ISOXAZOLIDINE DERIVATIVES **3a-g** DETERMINED BY DPPH RADICAL-SCAVENGING ASSAY

Compound	IC_{50} (μg/mL)	DPPH radical scavenging activity at 100 μg/mL (%)
3a	61.4	80.3
3b	50.6	–
3c	47.8	–
3d	58.0	78.7
3e	49.5	79.5
3f	58.7	–
3g	–	–
Ascorbic acid	–	–

TABLE-3
MOLECULAR DOCKING RESULTS OF COMPOUND **3a** WITH SELECTED TARGET PROTEINS

PDB ID	Binding free energy (kcal/mol)	Amino acid residues	Bond distance (Å)
2X3F	-7.1	LEU113:HN	2.306
2QV4	-8.4	TYR151:HH	2.274
3W37	-7.8	ALA234:HN	1.875
		ASN237:HD21	2.632

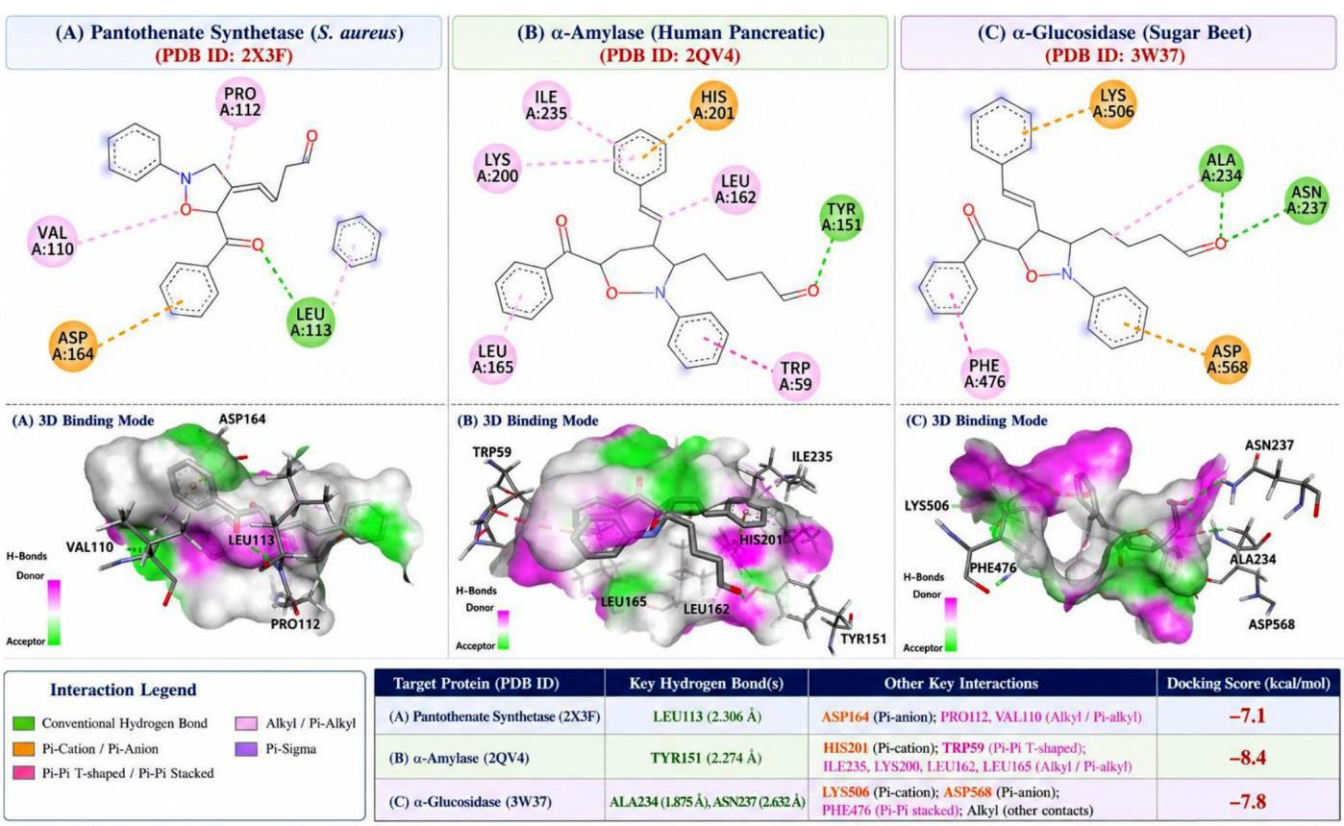


Fig. 1. Binding interaction between ligand (**3a**) and proteins(PDB code: 2X3F), (PDB code: 2QV4) and (PDB ID: 3W37)

compound **3a** displaying hydrogen-bond interactions with ALA234 and ASN237 at distances of 1.875 and 2.632 Å, respectively.

The docking profile of compound **3a** indicates favourable predicted interactions within the binding sites of the three selected targets and provides molecular-level hypotheses for further biological investigation. The α-amylase and α-glucosidase docking studies were conducted independently of the DPPH antioxidant assay and should not be interpreted as evidence of antioxidant activity or direct antidiabetic activity.

Conclusion

A series of substituted 4-(5-benzoyl-2-phenyl-4-styrylisoxazolidin-3-yl)butanal derivatives (**3a-g**) was synthesized through 1,3-dipolar cycloaddition of a glutaraldehyde-derived nitrone with substituted cinnamaldehyde-derived chalcones. All the compounds were characterized using FT-IR, ^{1}H NMR, ^{13}C NMR, mass spectrometry and elemental analysis. The antibacterial screening revealed measurable activity against the tested Gram-positive and Gram-negative bacteria, with compound **3a** showing the largest inhibition zones within the synthesized series. The derivatives exhibited concentration-dependent DPPH radical-scavenging activity, with compound **3c** providing the lowest reported IC_{50} value of 47.8 μg/mL. Molecular docking identified possible interactions of the synthesized compounds with MRSA Sar2676 pantothenate synthetase, α-amylase and α-glucosidase. Compound **3a** showed favourable predicted binding interactions. These results provide structural insights but do not establish antibacterial or enzyme-inhibitory mechanisms.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

DECLARATION OF AI-ASSISTED TECHNOLOGIES

During the preparation of this manuscript, the authors used an AI-assisted tool(s) to improve the language and no data, results or scientific conclusions were generated by AI. All analyses, interpretations and conclusions are the authors' own. The authors reviewed and edited the content and take full responsibility for the published work.

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