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Synthesis, *in vitro* Antimicrobial Evaluation, Molecular Docking and Phytotoxicity Profiling of *N*-(Substituted benzylidene)-5-(substituted phenyl)-1,3,4-oxadiazol-2-amines

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A series of *N*-(substituted benzylidene)-5-(substituted phenyl)-1,3,4-oxadiazol-2-amines (**2AC07-2AC13**) was synthesized through a three-step sequence involving hydrazone formation, oxidative cyclization to the 1,3,4-oxadiazol-2-amine scaffold and final Schiff-base condensation. The products were characterized using FT-IR, ^{1}H and ^{13}C NMR and HRMS. *In vitro* antimicrobial screening was conducted against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacterial strains and fungal strains (*Candida albicans*, *Aspergillus niger* and *Trichoderma* sp.) using the agar well diffusion method, with ampicillin and fluconazole as respective reference standards. The series exhibited selective antimicrobial activity however compound **2AC11** displayed concentration-dependent antibacterial activity against *B. subtilis* and *P. aeruginosa* while compounds **2AC07-2AC09** demonstrated antifungal activity against all three tested fungal strains. Compound **2AC09** showed the highest antifungal profile against *A. niger*, *Trichoderma* sp. and *C. albicans* at 100 μg mL^{-1} and was selected for molecular docking against *A. niger* EstA (PDB: 1UKC) and *C. albicans* CYP51 (PDB: 5TZ1). Their phytotoxic effects were assessed in a Petri-dish assay using tomato (*Solanum lycopersicum*) seeds exposed to 10, 50, 100 and 300 ppm. The germination speed index (GSI), root elongation and shoot elongation were normalized against the water control and integrated into a phytotoxicity index (PTI). The biological activity profile identifies compound **2AC11** as a potential selective antibacterial candidate and compound **2AC09** as a promising antifungal scaffold for further structural modification and optimization.

Keywords: 1,3,4-Oxadiazoles, Structure–activity relationship, Phytotoxicity, Tomato, Antimicrobial activity, Molecular docking.

INTRODUCTION

Heterocyclic compounds play a pivotal role in medicinal chemistry and agricultural sciences due to their structural diversity and wide-ranging biological activities [1]. Among them, oxadiazoles are important five-membered heterocycles containing oxygen and nitrogen atoms, known for their stability, strong electronic effects and broad biological properties. 1,3,4-Oxadiazole is an important pharmacophore and has variety of important biological roles, including anticancer [2], anti-inflammatory [3], antibacterial [4], antimalarial [5], antitubercular [6], anticonvulsant [7], analgesic [8], anti-allergic agent [9] and vasodilator activities [10], herbicidal and pesticidal activities [11]. The antibacterial activity of oxadiazoles has been extensively reported, with several studies exhibiting inhibitory effects against both Gram-positive *B. subtilis*, *S. aureus* and Gram-negative *P. aeruginosa* and *E. coli* bacterial strains [12,13]. However, recent investigations have shown that while many oxadiazole derivatives are effective against Gram-positive bacteria, their efficacy against Gram-negative strains often remains poor compared to standard antibiotics such as ampicillin [14-16].

Many reports have also highlighted new structural modifications of oxadiazoles with improved antibacterial and pharmacological properties [17-19]. Oxadiazoles continue to attract interest in medicinal chemistry, although their biological activity and selectivity depend strongly on molecular structure and require careful optimization [20]. Their potential extends beyond pharmaceutical applications to the agrochemical field. Despite this broader relevance, the effects of

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oxadiazole derivatives on plant germination and early growth remain relatively unexplored. Such investigations are important for assessing their environmental impact and possible use in herbicidal applications [21]. Germination speed index (GSI), root and shoot elongation and phytotoxicity index (PTI) provide useful measures for evaluating their effects on early plant development [22,23].

Although 1,3,4-oxadiazole derivatives have been widely investigated for their biological properties [24], *N*-(benzylidene) substituted 1,3,4-oxadiazol-2-amines containing a Schiff base imine linkage at the N-2 position remain relatively less explored in terms of their antibacterial, antifungal, molecular docking and phytotoxic properties. Incorporation of the 1,3,4-oxadiazole ring and the imine (C=N) linkage within the same molecular framework may influence biological interactions through hydrogen bonding and π–π interactions. To examine their possible antifungal interactions at different molecular targets, *Aspergillus niger* EstA, a serine esterase involved in fungal metabolism (PDB: 1UKC) [25] and *Candida albicans* CYP51, a sterol 14α-demethylase involved in ergosterol biosynthesis (PDB: 5TZ1) [26], were selected for molecular docking studies. These targets provide different molecular environments for examining the potential binding behaviour of the synthesized derivatives.

The present study focuses on the synthesis of a series of *N*-(substituted benzylidene)-5-(substituted phenyl)-1,3,4-oxadiazol-2-amine derivatives (**2AC07-2AC13**) and their *in vitro* antibacterial and antifungal evaluation using the agar well diffusion method. The study examines the influence of the substituted aromatic groups and the oxadiazole-imine framework on the observed biological activity. Molecular docking was performed for the most active derivative against EstA and CYP51 to examine its binding interactions and possible affinity towards these fungal targets. The phytotoxic response of all synthesized compounds was examined using tomato (*Solanum lycopersicum*) seeds exposed to four different concentrations. Germination speed index, root elongation and shoot elongation were also measured to assess the effects of the compounds on early plant growth.

EXPERIMENTAL

All chemicals and solvents were of analytical grade, procured from AVRA Chemicals (India) and used without further purification. Reaction progress was routinely monitored by analytical TLC on pre-coated Merck silica gel 60 aluminium plates using hexane:ethyl acetate (8:2, v/v) as the mobile phase; spots were visualized under UV light. Melting points were recorded in open glass capillary tubes and are uncorrected. Infrared spectra were recorded as KBr pellets on a Perkin-Elmer FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were acquired on a Bruker Avance Neo spectrometer at 500 and 125 MHz, respectively, in DMSO-d_6 or $CDCl_3$ as specified. Mass spectra were recorded using a Waters Micromass Q-Tof high-resolution mass spectrometer. The spectroscopic data were used to confirm the structures of the synthesized compounds.

Synthesis of title compounds: The target compounds were synthesized through a multistep reaction sequence using substituted benzaldehydes as starting materials. Initially, intermediates (**1a-d**) were synthesized and subsequently converted into the corresponding 1,3,4-oxadiazole intermediates (**2a-d**). The final derivatives, *N*-(substituted benzylidene)-5-(substituted phenyl)-1,3,4-oxadiazol-2-amines (**2AC07-2AC13**), were obtained by reacting the respective intermediates (**1a-d**) and (**2a-d**) under suitable reaction conditions.

General synthesis of 2-(substituted benzylidene)hydrazine-1-carboxamide (1a-d): Equimolar quantities (0.1 mol each) of semicarbazide hydrochloride and sodium acetate were dissolved in hot ethanol (20 mL). Separately, the appropriate aromatic aldehyde (0.1 mol) was dissolved in hot ethanol (20 mL). The aldehyde solution was added to the semicarbazide solution with continuous shaking. The mixture was allowed to stand and precipitates were formed, which were collected by filtration, washed with cold ethanol and dried. Completion of the reaction was confirmed by TLC (hexane:ethyl acetate, 8:2, v/v).

2-(2-Chlorobenzylidene)hydrazine-1-carboxamide (1a): White solid; yield: 71.33%, m.p.: 202-204 ºC; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.26 (s, 1H), 8.20 (t, 1H), 7.47 (t, 1H), 7.37 (t, 2H), 6.58 (brs, 2H, NH_2), 5.81 (brs, 1H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.46, 136.40, 131.96, 130.66, 129.83, 127.60, 126.31.

2-(4-Hydroxybenzylidene)hydrazine-1-carboxamide (1b): Light brown solid, yield: 74.49%, m.p.: 267-269 ºC; ^{1}H NMR (500 MHz, DMSO-d_6): δ 9.82 (brs, 1H), 7.78 (s, 1H), 7.53 (d, 2H), 6.82 (d, 2H), 6.39 (brs,2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.61, 157.34, 140.17, 128.04, 125.50, 115.55.

2-(3-Nitrobenzylidene)hydrazine-1-carboxamide (1c): Yellow solid, yield: 78.30%, m.p.: 260-262 ºC; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.54 (s, 1H), 8.19 (d, 2H), 7.98 (s, 1H), 7.67 (d, 1H), 6.66 (brs, 2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 161.26, 156.53, 147.90, 137.11, 132.86, 129.91, 123.40, 120.42.

2-(3,4-Dimethoxybenzylidene)hydrazine-1-carboxamide (1d): Off-white solid, yield: 72.73%, m.p.: 258-260 ºC; ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.78(s, 1H), 7.42 (s, 1H), 7.08 (d, 1H), 6.92 (d,1H), 6.49 (brs, NH_2), 3.80 (s, 3H), 3.76 (s, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.47, 149.60, 148.76, 139.68, 127.58, 120.75, 111.20, 107.82, 55.79, 54.02.

General synthesis of 5-(substituted phenyl)-1,3,4-oxadiazol-2-amine (2a-d): A mixture of compound **1a-d** (0.1 mol), sodium acetate (0.2 mol) and glacial acetic acid (30 mL) was placed in a round-bottom flask. A solution of bromine (1.6 mL) in glacial acetic acid (8 mL) was added dropwise with continuous stirring over 1 h at room temperature. The completion of the reaction was confirmed by TLC (hexane:ethyl acetate, 8:2, v/v). The resulting solid was filtered, washed with water, dried and recrystallized from ethanol to give compounds **2a-d**.

5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-amine (2a): White solid, yield: 62.23%, m.p.: 270-272 ºC; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.20 (d, 1H), 7.46 (d, 1H), 7.36-7.33 (m, 2H), 6.56 (brs, 2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.38, 134.91, 132.09, 131.83, 130.23, 129.51, 127.16, 126.64.

4-(5-Amino-1,3,4-oxadiazol-2-yl)phenol (2b): Light brown solid, yield: 70.21%, m.p.: 340-342 ºC; ^{1}H NMR (500

MHz, DMSO-d_6): δ 9.73(s, 1H), 7.74 (s, 1H), 7.54 (d, 1H), 6.78 (d, 2H), 6.37 (brs, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 158.63, 156.48, 139.67, 128.06, 125.96, 115.56.

5-(3-Nitrophenyl)-1,3,4-oxadiazol-2-amine (2c): Light yellow solid, yield: 60.40%, m.p.: 236-238 ºC; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.54 (s, 1H), 8.17 (d, 1H), 7.96 (s, 1H), 7.68 (t, 1H), 6.70 (brs, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 156.53, 148.29, 136.90, 136.77, 132.67, 130.00, 123.12, 120.59.

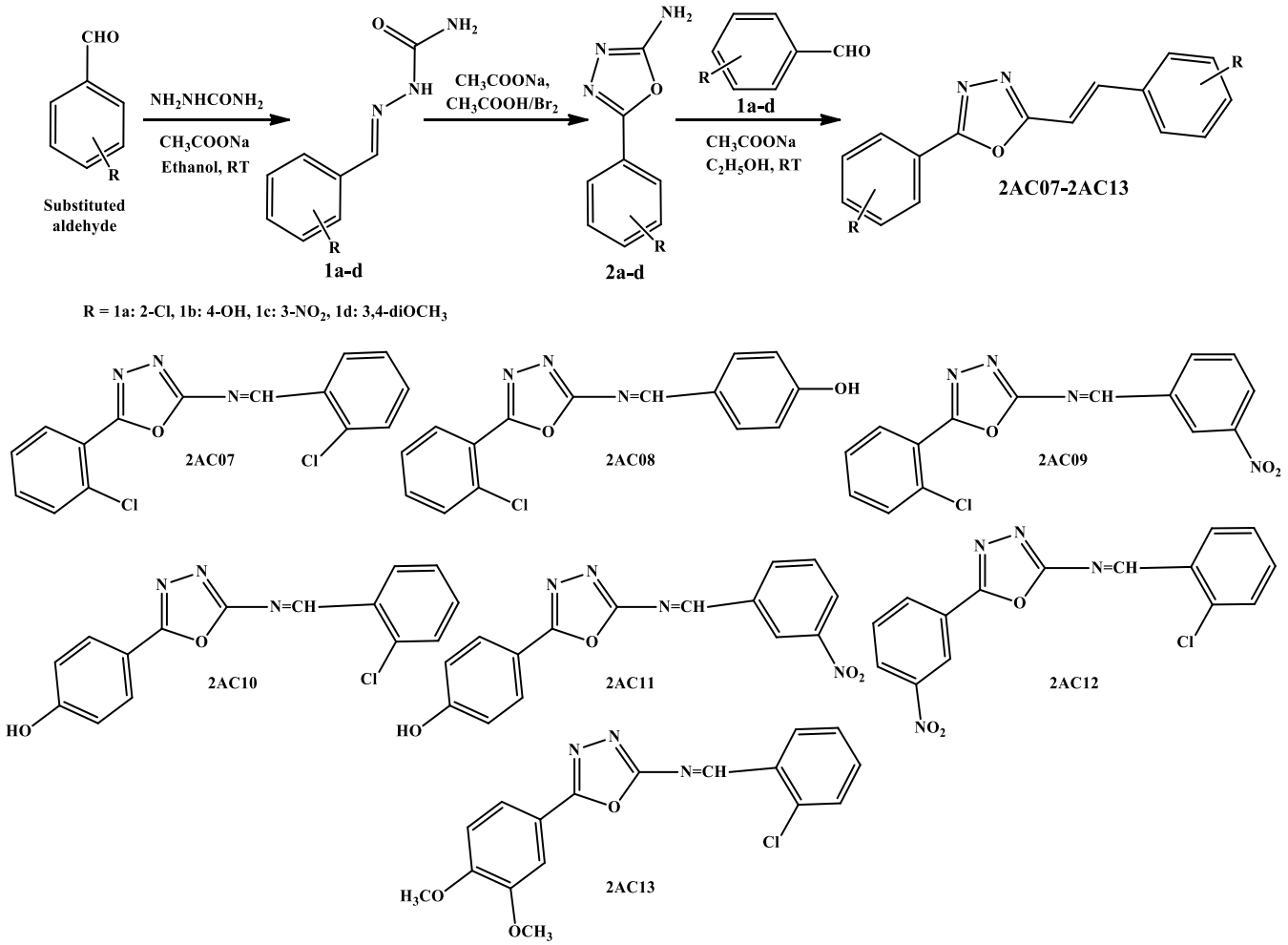
5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-amine (2d): Off-white solid, yield: 70.71%, m.p.: 320-322 ºC; ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.54 (s, 1H), 8.17 (d, 1H), 7.96 (s, 1H), 7.68 (t, 1H) 6.70 (brs, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6): δ 157.00, 149.86, 149.23, 139.82, 127.65, 120.87, 111.29, 108.19, 55.58.

Synthesis of *N*-(substituted benzylidene)-5-(substituted phenyl)-1,3,4-oxadiazol-2-amines (2AC07-2AC13): Equimolar amounts (0.01 mol each) of compound **2a-d** and the substituted aromatic aldehyde were dissolved in hot ethanol (20 mL each). The two solutions were combined and shaken vigorously until precipitates formed (**Scheme-I**). Final products were collected by filtration, washed with cold ethanol, dried and characterized. Completion of the reaction was confirmed by TLC (hexane:ethyl acetate, 8:2, v/v).

1-(2-Chlorophenyl)-*N*-(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl)methanimine (2AC07): White solid; yield: 63.42%; m.p.: 288-290 ºC; IR (KBr, ν_{max}, cm^{-1}): 3155.89 (arom. C–H), 1585.52 (C=N), 1220.38 (C–O–C), 737.99 (C–Cl); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.24 (s, 1H), 8.18-8.17 (m, 3H), 7.46-7.44 (m, 2H), 7.37-7.33 (m, 3H); ^{13}C NMR (125 MHz, DMSO-d_6): δ 160.42, 156.41, 134.92, 132.08, 131.89, 130.16, 129.51, 128.69, 128.22, 127.15, 126.82, 126.13; HRMS: (M+H_2O) = 336.

4-(((5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)imino)-methyl)phenol (2AC08): Light purple solid; yield: 56.58%; m.p.: 223-225 ºC; IR (KBr, ν_{max}, cm^{-1}): 3157 (–OH), 3052.86 (arom. C–H), 1586.39 (C=N), 1220.81 (C–O–C), 738.12 (C–Cl); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.47 (brs, OH), 8.22 (s, 1H), 8.19-8.17 (m, 3H), 7.46-7.45 (m, 3H), 7.37-7.33 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 164.04, 156.44, 155.61, 148.28, 135.37, 134.42, 131.95, 130.19, 129.35, 127.19, 126.73; HR-MS: (M+Na+3) = 325.

***N*-(5-(2-Chlorophenyl)-1,3,4-oxadiazol-2-yl)-1-(3-nitrophenyl)methanimine (2AC09):** Off-white solid; yield: 54.63%; m.p.: 222-224 ºC; IR (KBr, ν_{max}, cm^{-1}): 3150.25 (arom. C–H), 1585.88 (C=N), 1345.99 (N–O, asym.), 1220.48 (C–O–C), 738.45 (C–Cl); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.31 (s, 1H), 8.23-8.17 (m, 4H), 7.47-7.40 (m, 2H), 7.37-7.32



Scheme-I: Synthesis of N-(substituted benzylidene)-5-(substituted phenyl)-1,3,4-oxadiazol-2-amine (**2AC07-2AC13**)

(t, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 156.65, 156.44, 134.91, 132.21, 131.71, 130.42, 129.95, 129.44, 127.15, 126.93, 126.43; HR-MS: (M-2) = 326.

4-(5-((2-Chlorobenzylidene)amino)-1,3,4-oxadiazol-2-yl)phenol (2AC10): White solid; yield: 70.33%; m.p.: 250-252 ºC; IR (KBr, ν_{max}, cm^{-1}): 3350.64 (brs, O–H), 3159.35 (Ar C–H), 2981.31 (aliphatic C–H), 1637.57 (C=N, imine), 1054 (C–O–C), 739.38 (C–Cl); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.68 (brs, OH), 8.26 (d, 1H), 8.17 (d, 1H), 7.48 (d, 3H), 7.34 (d, 2H), 6.60 (brs, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.02, 174.10, 156.46, 155.64, 134.95, 131.97, 130.67, 130.23, 129.84, 127.61, 127.23, 126.78. HR-MS: (M+2) = 301.

4-(5-((3-Nitrobenzylidene)amino)-1,3,4-oxadiazol-2-yl)phenol (2AC11): Yellow solid; yield: 64.45%; m.p.: 248-250 ºC; IR (KBr, ν_{max}, cm^{-1}): 3384.26 (brs, O–H), 2973.92 (Ar C–H), 1650.11 (C=N, imine), 1535.82 (NO_2 asym.), 1340.31 (NO_2 sym.), 1179.56 (C–O–C); ^{1}H NMR (500 MHz, DMSO-d_6): δ 10.08 (brs, OH), 8.53 (s, 1H), 8.16 (d, 2H), 7.68 (d, 2H), 7.49 (d, 2H), 6.78 (d, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 175.04, 156.11, 149.60, 148.31, 137.54, 137.07, 136.41, 129.85, 127.61, 122.89, 115.55. HR-MS: (M+H_2O) = 328.

1-(2-Chlorophenyl)-*N*-(5-(3-nitrophenyl)-1,3,4-oxadiazol-2-yl)methanimine (2AC12): White solid; yield: 67.33%; m.p.: 240-242 ºC; IR (KBr, ν_{max}, cm^{-1}): 2981.26 (Ar C–H), 1661.11 (C=N, imine), 1578.06 (NO_2 asym.), 1331.65 (NO_2 sym.), 1023.86 (C–O–C), 758.20 (C–Cl); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.71 (s, 1H), 8.43 (d, 1H), 8.34-8.30 (m, 2H), 7.83 (d, 1H), 7.67 (t, 1H), 7.57-7.47 (m, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 169.36, 166.38, 164.63, 144.01, 143.56, 128.89, 128.53, 128.05, 127.59, 127.23, 126.29, 125.09; HR-MS: (M-3) = 325.

1-(2-Chlorophenyl)-*N*-(5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl)methanimine (2AC13): White solid; yield: 71.49%; m.p.: 230-232 ºC; IR (KBr, ν_{max}, cm^{-1}): 2981.50 (Ar C–H), 2844.22 (OCH_3 C–H), 1636 (C=N, imine), 1052.19 (C–O–C), 755.23 (C–Cl); ^{1}H NMR (500 MHz, DMSO-d_6): δ 8.34 (d, 2H), 7.74 (s, 1H), 7.43 (d, 2H), 7.14 (d, 1H), 6.92 (s, 1H), 3.87 (s, 3H, OCH_3), 3.78 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 174.52, 156.79, 149.76, 148.9, 139.38, 127.63, 120.72, 111.21, 108.21, 79.09, 55.50, 55.41; HR-MS: (M+H_2O) = 362.

Antimicrobial activities: The synthesized compounds (**2AC07-2AC13**) were screened for *in vitro* antimicrobial activity against 4 bacterial strains Gram-positive *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative *Pseudomonas aeruginosa* and *Escherichia coli* and 3 fungal strains *Candida albicans*, *Aspergillus niger* and *Trichoderma* sp., using the agar well diffusion method [27]. Ampicillin and fluconazole served as the respective antibacterial and antifungal reference standards. Each compound was tested at four concentrations (25, 50, 75 and 100 μg mL^{-1}) in triplicate and antimicrobial activity was expressed as the diameter (mm) of the zone of growth inhibition (ZOI) surrounding each well.

Bacterial strains were subcultured in nutrient broth and incubated at 35-37 ºC for 18-24 h to achieve a standardized inoculum of 0.5 McFarland turbidity. Mueller-Hinton agar (MHA) was freshly prepared, poured under aseptic conditions into sterile Petri dishes and allowed to solidify. The agar surface was uniformly swabbed with the standardized suspension. Wells of 6 mm diameter were bored using a sterile cork borer and 100 μL of each test solution (prepared in DMSO) was loaded per well using a calibrated micropipette. Bacterial plates were incubated at 37 ºC for 24 h and fungal plates at 28-30 ºC for 48-72 h. The ZOI diameter was measured in mm using a calibrated transparent ruler [27]. All assays were performed in triplicate and mean ZOI values are reported.

In silico studies

Molecular docking: Molecular docking studies were performed for compound **2AC09** the most potent antifungal derivative against two well-characterized fungal target proteins: *Aspergillus niger* esterase A (EstA; PDB ID: 1UKC) [25] and *Candida albicans* sterol 14α-demethylase (CYP51; PDB ID: 5TZ1) [26]. EstA is a serine esterase of the α/β-hydrolase fold superfamily whose hydrolytic activity is implicated in fungal metabolism and nutrient acquisition; it was selected to probe non-CYP51-mediated antifungal mechanisms. CYP51 is the established and clinically validated primary target of azole antifungals, including fluconazole, which acts by coordinating to the heme iron and blocking ergosterol biosynthesis [26,28].

Protein and ligand preparation, grid definition and docking calculations were performed using ArgusLab 4.0.1 [29]. The ArgusDock engine was used with the AScore scoring function in flexible ligand mode. The binding site grid was defined around the co-crystallized ligand or known active site residues with a 60 × 60 × 60 Å bounding box at 0.4 Å resolution. Ligand 3D geometries were built in Chem3D Pro, whereas hydrogen atoms were added and Clean Geometry was applied in Arguslab before docking. The reference antifungal drug fluconazole was docked under identical conditions for direct comparison. Ligand–protein interaction maps (2D and 3D) were generated and analyzed using BIOVIA Discovery Studio 2021.

Phytotoxicity bioassay: Phytotoxicity was evaluated on tomato (*Solanum lycopersicum*) seeds under laboratory conditions using Petri dish assays. Prior to testing, seeds were surface sterilized with 1% sodium hypochlorite (5 min), rinsed with sterile distilled water and subsequently soaked for 12 h in treatment solutions of synthesized oxadiazole derivatives at concentrations of 10, 50, 100 and 300 ppm. Seeds soaked in distilled water served as the negative control. For each treatment, two replicates of 15 seeds (n = 30) were placed in sterile Petri dishes (9 cm) lined with Whatman No. 1 filter paper and moistened with 3-4 mL of the corresponding treatment solution. The experiment was conducted at room temperature and filter papers were replenished with respective solutions as needed [30]. Germination was monitored for 14 days, with the first count recorded on day 5. A seed was considered germinated when the radicle length reached ≥ 1 mm [31]. At Day 14, three readings each of root length and shoot length were measured for normally germinated seedlings and average root length was used to measure the mean root length and shoot length. The germination speed index (GSI) was calculated using the method described by Maguire [22] to assess the rate of seed germination according to the following expression:

$$GSI = \sum\left(\frac{G_t}{D_t}\right)$$

where G_t = no of germinated seeds on day t, D_t = no of days counted from the start of experiment.

The phytotoxicity index (PTI) was calculated separately for GSI, root length and shoot length relative to the water control [32,33], according to the following expression:

$$PTI\ (\%) = 100 \times \left(\frac{1 - \text{Mean value of treatment}}{\text{Mean value of control}}\right)$$

(a) $$GSI\ PTI\ (\%) = 100 \times \left(\frac{1 - \text{GSI of treatment}}{\text{GSI of control}}\right)$$

(b) $$\text{Root PTI}\ (\%) = 100 \times \left(\frac{1 - \text{Mean root length of treatment}}{\text{Mean root length of control}}\right)$$

(c) $$\text{Shoot PTI}\ (\%) = 100 \times \left(\frac{1 - \text{Mean shoot length of treatment}}{\text{Mean shoot length of control}}\right)$$

A PTI value of 0% indicates no inhibitory effect relative to the water control, whereas a PTI value approaching 100% indicates near-complete inhibition of the respective growth parameter.

RESULTS AND DISCUSSION

The target series of *N*-(substituted benzylidene)-5-(substituted phenyl)-1,3,4-oxadiazol-2-amines (**2AC07-2AC13**) was synthesized by a straightforward three-step sequence from commercially available starting materials (**Scheme-I**). In the first step, condensation of semicarbazide hydrochloride with substituted aromatic aldehydes in hot ethanol/sodium acetate gave the acylhydrazone intermediates (**1a-d**). The oxidative cyclization with bromine in glacial acetic acid then delivered the 5-substituted 1,3,4-oxadiazol-2-amines (**2a-d**). In the final step, Schiff-base condensation of the oxadiazol-2-amines with a second substituted aromatic aldehyde in hot ethanol afforded the target *N*-(benzylidene) derivatives (**2AC07-2AC13**) in moderate-to-good yields (54-71%). All products were obtained as crystalline solids with sharp, reproducible melting points. Structural assignments were confirmed by FT-IR, ^{1}H NMR and ^{13}C NMR and HRMS. In the IR spectra, the characteristic C=N imine stretch appeared in the range 1661-1636 cm^{-1} and the C–O–C oxadiazole stretch appeared at ~1220-1023 cm^{-1} region, which is consistent with the ring for-mation. ^{1}H NMR spectra showed the imine singlet (H-C=N) in in the range δ 8.22-8.71 ppm for all the derivatives.

Biological evaluation

Antibacterial activity: All the synthesized derivatives were screened for antibacterial activity at four different concentrations (25-100 μg mL^{-1}) against two Gram-positive and two Gram-negative bacterial strains (Table-1). The majority of derivatives (**2AC07-2AC10**, **2AC12**, **2AC13**) were inactive at all tested concentrations, producing no measurable zone of inhibition (NZ) against any of the four strains. This general inactivity may reflect insufficient membrane permeability for these derivatives and the known resistance of Gram-negative organisms to compounds unable to traverse the outer membrane lipopolysaccharide barrier.

Compound **2AC11** was the sole antibacterial derivative, displaying selective, concentration-dependent activity against *B. subtilis* (ZOI = 24.78, 29.67 and 38.67 mm at 50, 75 and 100 μg mL^{-1}) and *P. aeruginosa* (ZOI = 12.45, 20.56 and 28.12 mm at 50, 75 and 100 μg mL^{-1}), with no activity at 25 μg mL^{-1} against either strain. No inhibition was observed for **2AC11** against *S. aureus* or *E. coli* at any concentration tested. Compound **2AC11** exhibited higher antibacterial activity against *B. subtilis* and *P. aeruginosa* than against *S. aureus*

TABLE-1
In vitro ANTIBACTERIAL ACTIVITY (ZONE OF INHIBITION, mm) OF SYNTHESIZED COMPOUNDS (**2AC07-2AC13**) AGAINST FOUR BACTERIAL STRAINS AT FOUR CONCENTRATIONS

Compound	*S. aureus*				*B. subtilis*			
	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL
2AC07	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC08	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC09	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC10	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC11	NZ	NZ	NZ	NZ	NZ	24.78	29.67	38.67
2AC12	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC13	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Ampicillin	16.78	28.50	30.70	31.69	23.65	28.78	32.30	39.74
Compound	*E. coli*				*P. aeruginosa*			
	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL
2AC07	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC08	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC09	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC10	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC11	NZ	NZ	NZ	NZ	NZ	12.45	20.56	28.12
2AC12	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC13	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Ampicillin	12.34	15.02	17.20	21.78	18.50	24.75	30.20	34.78

NZ = no zone of inhibition. Ampicillin = reference standard.

and *E. coli*. This strain-dependent response is consistent with variations in antibacterial susceptibility reported for other oxadiazole derivatives [15,17].

The antibacterial response of the synthesized derivatives varied with the nature and position of the aromatic substituents. Compound **2AC11** contains a 4-hydroxyphenyl group on the oxadiazole ring and a 3-nitrobenzylidene moiety. The presence of the same 4-hydroxyphenyl group in inactive compound **2AC10** points to the 3-nitrobenzylidene group as a possible contributor to the activity of **2AC11**. The electron-withdrawing nitro substituent may alter the electronic distribution across the Schiff-base linkage and influence its interaction with bacterial targets. In comparison, compounds **2AC07** and **2AC12** bearing chlorobenzylidene groups and **2AC08** containing a 4-hydroxybenzylidene group did not exhibit comparable activity. These findings suggest that the nature of the benzylidene substituent plays an important role in the antibacterial response of this oxadiazole series.

Antifungal activity: Antifungal screening against *A. niger*, *Trichoderma* sp. and *C. albicans* revealed distinct differences in the activity of the synthesized derivatives (Table-2). Compounds **2AC07-2AC09** exhibited antifungal activity whereas **2AC10-2AC13** remained inactive under the tested conditions. All three active derivatives consist of a 2-chlorophenyl group attached to the oxadiazole ring. In contrast the inactive compounds contain either a 4-hydroxyphenyl or a 3-nitrophenyl substituent at this position. This pattern points to the importance of the 2-chlorophenyl group in the antifungal response of the series. Its lipophilic and electron-withdrawing character may favour interactions with hydrophobic regions of fungal target proteins.

Compound **2AC09** exhibited the highest antifungal activity among the tested derivatives with inhibition zones of 28.90 mm against *A. niger* and 24.75 mm against *Trichoderma* sp. and 20.56 mm against *C. albicans* at 100 μg mL^{-1}. Although these values were lower than those obtained with fluconazole (37.78 mm, 28.45 mm and 39.80 mm respectively at the same concentration) **2AC09** showed activity against all three fungal strains. Compound **2AC08** exhibited a similar broad-spectrum response whereas **2AC07** was active against *A. niger* and *C. albicans* but remained inactive against *Trichoderma* sp. The activity followed the order **2AC09** > **2AC08** > **2AC07**. This variation may be related to differences in the electronic and physicochemical properties of the N-2 benzylidene substituents. The 3-nitrobenzylidene group in **2AC09** and the 4-hydroxybenzylidene group in **2AC08** may influence polarity and molecular interactions differently from the 2-chlorobenzylidene group in **2AC07**. These observations provide a basis for further structural modification of the oxadiazole scaffold to improve antifungal activity.

Phytotoxicity studies: The phytotoxic effects of the synthesized oxadiazole derivatives were examined using a tomato seed germination assay. The raw observations for seed germination and seedling growth are shown in Table-3. Germination was monitored throughout the experimental period while root and shoot lengths were measured at the end of the assay for normally germinated seedlings. These measurements were used to calculate the germination speed index (GSI) and the corresponding phytotoxicity indices (PTI) relative to the water control. All seven derivatives exhibited a concentration-dependent effect on tomato seed germination (Table-4). At 10 ppm the GSI-based PTI values ranged from 17.78% for **2AC11** to 48.07% for **2AC13**. At 300 ppm these values increased to 54.53% for **2AC11** and 75.42% for **2AC09** and **2AC13**. Root elongation exhibited the highest sensitivity among the three growth parameters. At 10 ppm the root PTI values ranged from 59.48% for **2AC11** to 67.42% for **2AC09**. These values increased to 76.16–84.31% at 300 ppm with 2AC07 showing the highest root growth inhibition. Shoot PTI values ranged from 24.51% for **2AC13** to 41.56% for **2AC07** at 10 ppm. At 300 ppm, the values increased to 65.89-79.08% with **2AC07** again exhibiting the strongest inhibitory effect. A slight reduction in shoot PTI was observed for **2AC09** and **2AC13** at 50 ppm compared with their respective values at 10 ppm. Their PTI values increased at higher concentrations. The higher sensitivity of root growth may be related to direct exposure of the emerging roots to the test compounds and their subsequent uptake [34,35]. Similar phytotoxic responses have been reported for certain oxadiazole derivatives which raises concerns regarding their potential effects on plant growth and environmental safety [36,37].

SAR based on phytotoxicity: The phytotoxic response varied across the synthesized derivatives and the growth parameters examined. At 300 ppm, the root PTI ranged from 76.16% for **2AC10** to 84.31% for **2AC07**. The highest shoot

TABLE-2
In vitro ANTIFUNGAL ACTIVITY (ZONE OF INHIBITION, mm) OF SYNTHESIZED COMPOUNDS (**2AC07-2AC13**) AGAINST THREE FUNGAL STRAINS AT FOUR DIFFERENT CONCENTRATIONS

Compound	*A. niger*				*Trichoderma* sp.				*C. albicans*			
	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL	25 μg/mL	50 μg/mL	75 μg/mL	100 μg/mL
2AC07	14.00	18.12	21.23	26.89	NZ	NZ	NZ	NZ	12.10	14.19	16.21	19.89
2AC08	14.67	17.10	20.20	25.80	12.20	14.12	16.23	18.89	14.62	16.65	18.20	20.34
2AC09	18.12	19.80	22.23	28.90	14.46	15.64	18.34	24.75	15.34	18.10	19.56	20.56
2AC10	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC11	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC12	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
2AC13	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ	NZ
Fluconazole	23.67	26.67	32.45	37.78	16.23	21.45	25.56	28.45	23.56	29.67	32.21	39.80

NZ = no zone of inhibition. Fluconazole = reference standard.

TABLE-3
PRIMARY PHYTOTOXICITY DATA FOR TOMATO (*Solanum lycopersicum*) SEEDS EXPOSED TO SYNTHESIZED 1,3,4-OXADIAZOLE DERIVATIVES AT DIFFERENT TREATMENT CONCENTRATIONS

Compound	Conc. (ppm)	Replicate	GSI	GSI mean	GSI PTI	Average root length (from three readings) (cm)	Root mean	Root PTI	Average shoot length (from three readings) (cm)	Shoot mean	Shoot PTI
2AC07	10	R1	5.12	5.02	26.02	3.10	3.00	64.24	4.27	4.28	41.56
		R2	4.92			2.90			4.30		
	50	R1	4.58	3.93	41.99	2.10	2.25	73.18	2.33	2.67	63.62
		R2	3.28			2.40			3.00		
	100	R1	3.08	2.96	56.37	2.10	2.00	76.16	2.63	2.57	64.98
		R2	2.83			1.90			2.50		
	300	R1	1.83	1.96	71.12	1.23	1.32	84.31	1.30	1.53	79.08
		R2	2.08			1.40			1.77		
2AC08	10	R1	5.12	4.95	27.00	3.03	2.87	65.83	4.60	4.45	39.29
		R2	4.78			2.70			4.30		
	50	R1	3.58	3.33	50.84	2.40	2.30	72.59	4.10	3.95	46.11
		R2	3.08			2.20			3.80		
	100	R1	3.08	2.79	58.83	2.00	1.90	77.35	3.77	3.58	51.11
		R2	2.50			1.80			3.40		
	300	R1	1.83	1.83	72.96	1.60	1.50	82.12	2.17	2.15	70.67
		R2	1.83			1.40			2.13		
2AC09	10	R1	4.92	4.38	35.48	2.87	2.73	67.42	4.40	4.30	41.34
		R2	3.83			2.60			4.20		
	50	R1	3.08	3.08	54.53	2.30	2.20	73.78	4.40	4.55	37.93
		R2	3.08			2.10			4.70		
	100	R1	2.83	2.83	58.22	1.90	1.80	78.55	2.50	2.72	62.94
		R2	2.83			1.70			2.93		
	300	R1	1.83	1.67	75.42	1.43	1.37	83.71	1.50	1.70	76.81
		R2	1.50			1.30			1.90		
2AC10	10	R1	5.87	5.49	19.01	3.37	3.25	61.26	5.27	5.43	25.88
		R2	5.12			3.13			5.60		
	50	R1	5.12	4.45	34.38	2.90	2.80	66.63	3.40	3.37	54.07
		R2	3.78			2.70			3.33		
	100	R1	3.92	3.50	48.38	2.50	2.40	71.39	4.00	3.90	46.79
		R2	3.08			2.30			3.80		
	300	R1	3.08	2.46	63.75	2.10	2.00	76.16	2.60	2.50	65.89
		R2	1.83			1.90			2.40		
2AC11	10	R1	5.87	5.58	17.78	3.57	3.40	59.48	5.13	4.98	32.01
		R2	5.28			3.23			4.83		
	50	R1	5.28	5.20	23.31	3.00	2.90	65.44	4.60	4.50	38.61
		R2	5.12			2.80			4.40		
	100	R1	4.78	4.18	38.31	2.60	2.50	70.20	3.20	3.30	54.98
		R2	3.58			2.40			3.40		
	300	R1	3.08	3.08	54.53	1.20	1.48	82.32	1.80	1.82	75.22
		R2	3.08			1.77			1.83		
2AC12	10	R1	5.87	5.46	19.43	3.47	3.28	60.87	5.00	4.85	33.83
		R2	5.06			3.10			4.70		
	50	R1	5.12	4.45	34.38	2.90	2.80	66.63	3.70	3.60	50.89
		R2	3.78			2.70			3.50		
	100	R1	3.95	3.12	54.04	2.50	2.40	71.39	3.23	3.57	51.34
		R2	2.28			2.30			3.90		
	300	R1	2.92	2.50	63.13	1.10	1.37	83.71	1.57	1.62	77.94
		R2	2.08			1.63			1.67		
2AC13	10	R1	3.12	3.52	48.07	3.03	2.87	65.83	5.77	5.53	24.51
		R2	3.93			2.70			5.30		
	50	R1	4.58	3.83	43.47	2.50	2.40	71.39	3.27	3.38	53.84
		R2	3.08			2.30			3.50		
	100	R1	3.08	2.79	58.83	2.10	2.00	76.16	3.70	3.80	48.16
		R2	2.50			1.90			3.90		
	300	R1	1.83	1.67	75.42	1.70	1.60	80.93	2.17	2.13	70.90
		R2	1.50			1.50			2.10		
Control water		15	7.38	6.78	0.00	9.72	8.39	0.00	7.20	7.33	0.00
		15	6.19			7.06			7.47		

TABLE-4
PHYTOTOXICITY INDEX (PTI, %) OF SEVEN SYNTHESIZED 1,3,4-OXADIAZOLE DERIVATIVES ON GERMINATION SPEED INDEX (GSI), ROOT LENGTH AND SHOOT LENGTH OF TOMATO (*Solanum lycopersicum*) SEEDLINGS AT FOUR TREATMENT CONCENTRATIONS

Compound	Conc. (ppm)	GSI PTI (%)	Root PTI (%)	Shoot PTI (%)	Compound	Conc. (ppm)	GSI PTI (%)	Root PTI (%)	Shoot PTI (%)
2AC07	10	26.02	64.24	41.56	**2AC11**	10	17.78	59.48	32.01
	50	41.99	73.18	63.62		50	23.31	65.44	38.61
	100	56.37	76.16	64.98		100	38.31	70.20	54.98
	300	71.12	84.31	79.08		300	54.53	82.32	75.22
2AC08	10	27.00	65.83	39.29	**2AC12**	10	19.43	60.87	33.83
	50	50.84	72.59	46.11		50	34.38	66.63	50.89
	100	58.83	77.35	51.11		100	54.04	71.39	51.34
	300	72.96	82.12	70.67		300	63.13	83.71	77.94
2AC09	10	35.48	67.42	41.34	**2AC13**	10	48.07	65.83	24.51
	50	54.53	73.78	37.93		50	43.47	71.39	53.84
	100	58.22	78.55	62.94		100	58.83	76.16	48.16
	300	75.42	83.71	76.81		300	75.42	80.93	70.90
2AC10	10	19.01	61.26	25.88	Water (control)	–	0.00	0.00	0.00
	50	34.38	66.63	54.07					
	100	48.38	71.39	46.79					
	300	63.75	76.16	65.89					

Values are PTI (%) relative to the water control (n = 30 seeds per treatment; two replicates of 15 seeds each).

PTI was recorded for **2AC07** (79.08%) while **2AC09** and **2AC13** exhibited the highest GSI PTI (75.42%). Compound **2AC11** showed comparatively lower effects on germination with the lowest GSI PTI at both 10 ppm (17.78%) and 300 ppm (54.53%). Notably **2AC11** was the only derivative with measurable antibacterial activity. This difference suggests that antibacterial activity and phytotoxic response are not directly related within the present series.

The influence of individual substituents on phytotoxicity remains difficult to establish as the derivatives differ in their

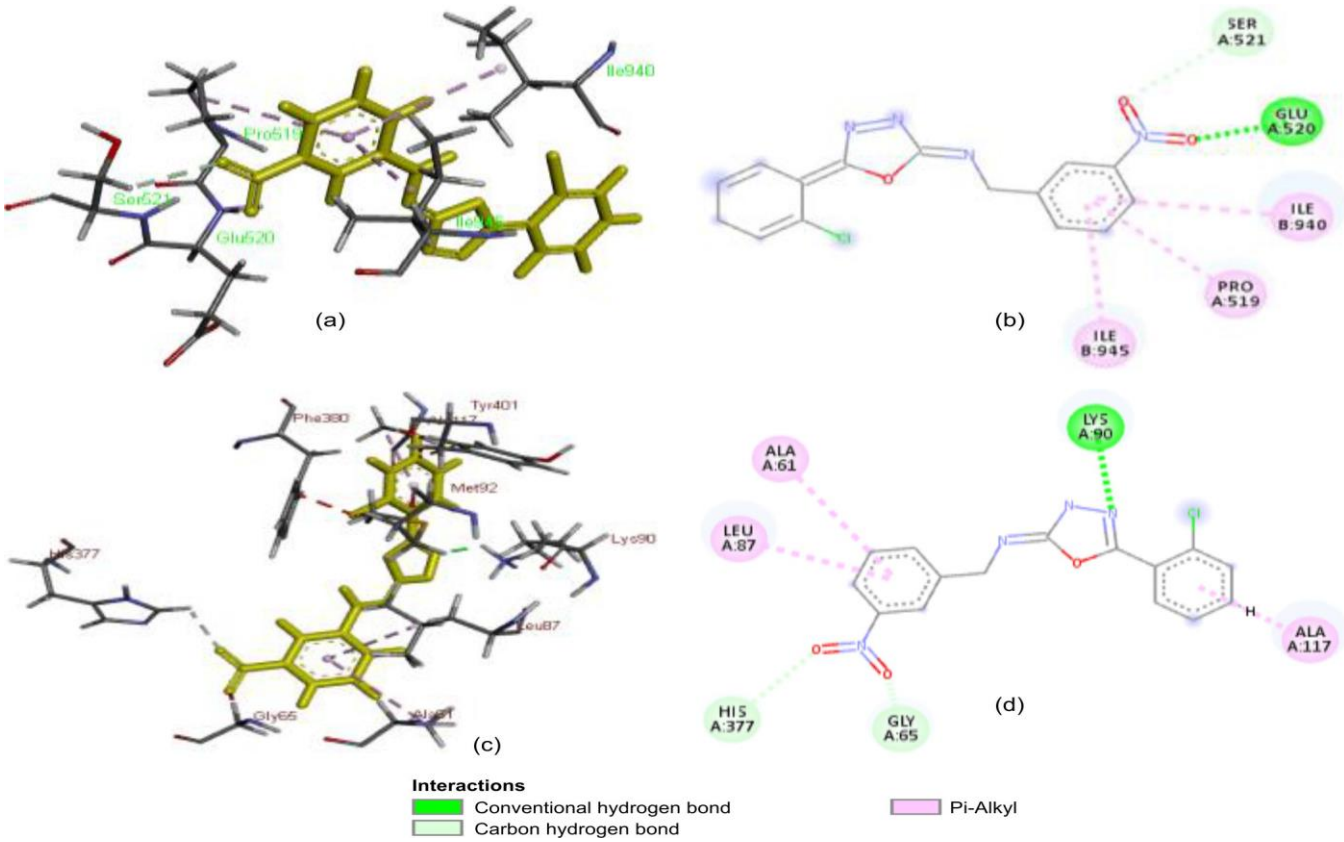


Fig. 1. Ligand–receptor interactions of compound **2AC09** with *A. niger* EstA in 3D (a) and 2D (b) modes and with *C. albicans* sterol 14α-demethylase (CYP51) in 3D (c) and 2D (d) modes

aromatic substitution patterns and their effects vary across growth parameters. Compound **2AC07** bearing chlorophenyl groups at both aromatic positions exhibited the highest root and shoot inhibition at 300 ppm. This may point to a relationship between its substitution pattern and the suppression of early seedling growth. The responses of **2AC08** and **2AC09** which share the 2-chlorophenyl group on the oxadiazole ring differed across the measured endpoints. This variation points to a possible contribution from the benzylidene substituent and other physicochemical properties of the compounds.

Molecular docking: Molecular docking analysis was carried out for the most active antifungal derivative **2AC09** against *A. niger* EstA (PDB: 1UKC) and *C. albicans* CYP51 (PDB: 5TZ1). The predicted binding energies and ligand-protein interactions are Figs. 1 and 2, respectively. Compound **2AC09** exhibited more favourable docking scores than fluconazole at both targets, with differences of 1.51 kcal/mol for EstA and 2.15 kcal/mol for CYP51 (Table-5).

TABLE-5
PREDICTED DOCKING BINDING ENERGIES (kcal/mol) OF COMPOUND **3c** AND FLUCONAZOLE AGAINST TWO FUNGAL TARGET RECEPTORS

Target protein	Compd. **3c** (kcal/mol)	Fluconazole (kcal/mol)	ΔE (kcal/mol)
C. albicans CYP51 (5TZ1)	-11.99	-9.84	-2.15
A. niger EstA (1UKC)	-9.81	-8.30	-1.51

Docking interaction with *A. niger* EstA (PDB: 1UKC): EstA belongs to the α/β hydrolase superfamily and functions as a serine esterase [38]. The docked pose of 2AC09 showed a carbon–hydrogen bond with SER521 (2.92 Å) and π–alkyl interactions with PRO519 (4.90 Å), ILE940 (4.72 Å) and ILE945 (3.99 Å). These contacts suggest the involvement of both polar and hydrophobic interactions in stabilizing the

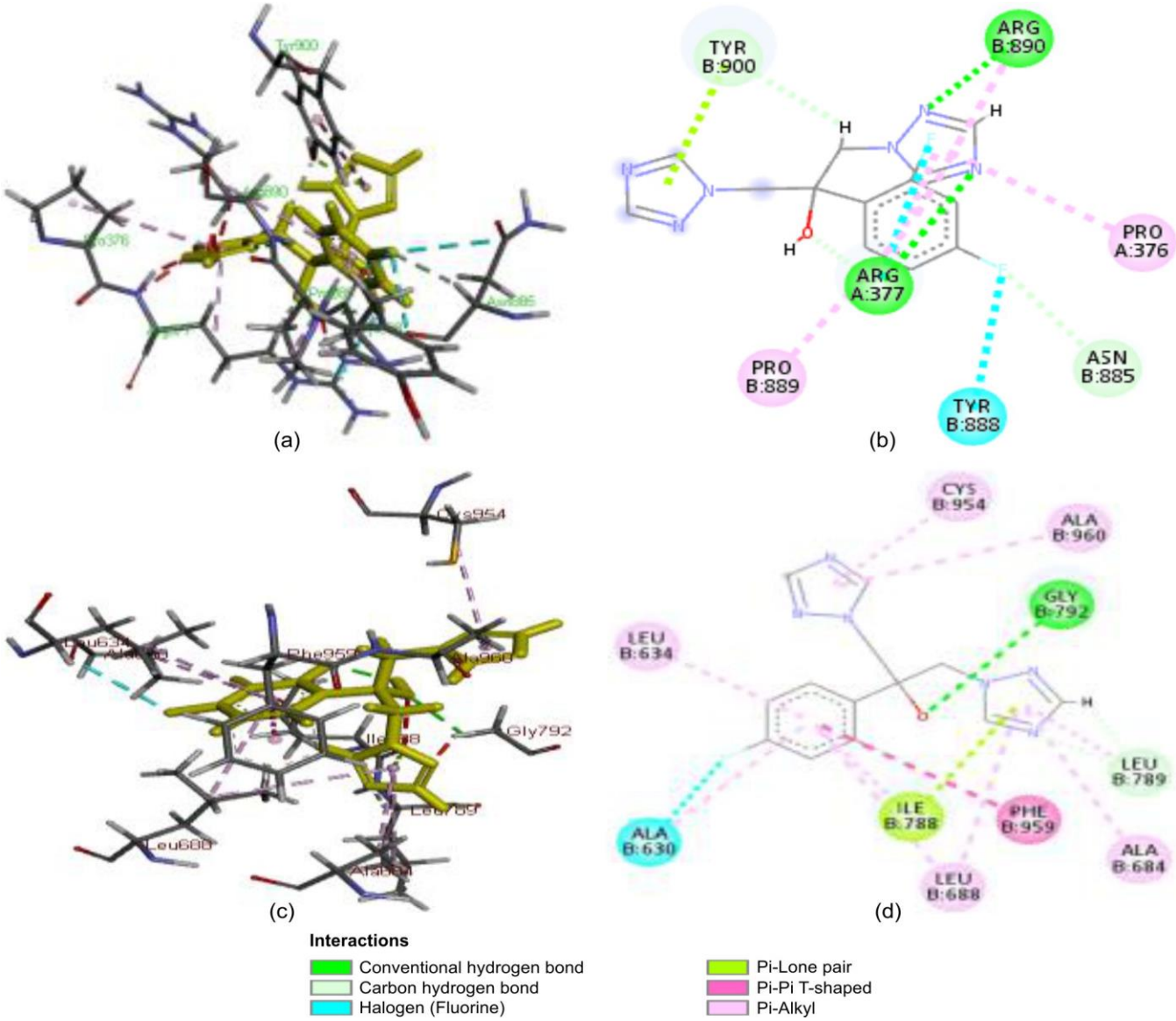


Fig. 2. Ligand–receptor interactions of fluconazole with *A. niger* EstA in 3D (a) and 2D (b) modes and with *C. albicans* sterol 14α-demethylase (CYP51) in 3D (c) and 2D (d) modes

ligand within the predicted binding pocket. Fluconazole exhibited a different interaction pattern with ARG890 (conventional hydrogen bond; 2.46 Å), TYR900 (carbon–hydrogen bond; 2.05 Å), TYR888 (halogen interaction; 2.99 Å) and PRO889 (π-alkyl interaction; 5.09 Å). The distinct interaction profiles suggest differences in the binding orientations of the two ligands within EstA.

Docking interaction with *C. albicans* CYP51 (PDB: 5TZ1): Compound **2AC09** formed a conventional hydrogen bond with LYS90 (1.74 Å) and a carbon–hydrogen bond with HIS377 (2.49 Å). Hydrophobic π-alkyl contacts were observed with ALA117 (3.59 Å), ALA61 (5.18 Å) and LEU87 (4.24 Å). Fluconazole showed interactions with GLY792 (hydrogen bond; 2.46 Å), ALA630 (halogen interaction; 3.65 Å), LEU634 (π-alkyl interaction; 4.65 Å), CYS954 (4.75 Å) and ALA960 (5.02 Å). The predicted poses suggest that both compounds interact with hydrophobic regions of the CYP51 binding pocket, although their individual interaction patterns differ.

Conclusion

The synthesized 1,3,4-oxadiazole derivatives **2AC07-2AC13** were successfully synthesized and characterized. The biological evaluation revealed distinct antimicrobial profiles, with compound **2AC11** exhibiting concentration-dependent antibacterial activity against *B. subtilis* and *P. aeruginosa*, while compounds **2AC07-2AC09** showed antifungal activity against the tested fungal strains. Among these derivatives, **2AC09** displayed the broadest antifungal spectrum and the largest inhibition zones, although fluconazole remained more active in the agar-well diffusion assay. Molecular docking of compound **2AC09** against *A. niger* EstA and *C. albicans* CYP51 revealed favourable predicted binding energies and specific ligand–protein interactions, providing preliminary insight into its possible molecular recognition at these targets. Phytotoxicity assessment using tomato seeds revealed inhibitory effects of all seven derivatives on at least one parameter of seed germination or seedling growth, with root elongation generally being the most sensitive endpoint. Compounds **2AC09** and **2AC07** exhibited the strongest phytotoxic effects, whereas **2AC11** showed the lowest response. The observed differences in antimicrobial and phytotoxic activities suggest that structural modifications influence these biological responses in distinct ways. Compound **2AC11** merits further investigation as an antibacterial lead, while compound **2AC09** requires further antifungal optimization. Compounds **2AC07** and **2AC09** may be considered preliminary candidates for agrochemical growth-inhibitory applications.

CONFLICT OF INTEREST

The authors declare that there is no conflicts of interest 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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