RESEARCH ARTICLE

Synthesis, Characterization and Molecular Docking Studies of Benzyl Scaffold Bearing 1, 3, 4-oxadiazole as Promising Antimicrobial Agents

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ABSTRACT

Background: The derivatives of heterocyclic ring 1,3,4-Oxadiazole are used as an antimicrobial agent for the treatment of microbial infections.

Objective: In this study, a new series of benzyl moiety possessing 1,3,4-oxadiazole derivatives have been synthesized by the reaction of 4-(benzylamino) benzohydrazide with substituted aromatic acids in the presence of cyclizing agent phosphorus oxychloride.

Method: The structures of synthesized compounds were analyzed by chromatographic data and characterized by spectral analysis (FT-IR, ^{1}H-NMR, C^{13} NMR and mass spectrum). All synthetic derivatives were evaluated for their *in-vitro* antimicrobial activity against selected microbial strains; few listed in "priority pathogens" by World Health Organization (WHO). Antibacterial activity of synthetic compounds were assayed against two gram-positive strains *Bacillus subtilis*, *Escherichia coli,* and two gram-negative strains *Staphylococcus aureus*, *Pseudomonas aeruginosa*. Antifungal activity of synthetic compounds was screened against *Candida albicans* and *Aspergillus spp*. The potent derivatives were subjected to in-silico molecular docking studies with enzyme peptide deformylase.

Results and discussion*: in-vitro* antimicrobial assay indicated that compounds 1f, 1j, 1i, 1h and 1g exhibited good antimicrobial activity as compared to commercially available antibiotics Cefixime and Econazole drugs.

Conclusion: In this novel series of benzyl moiety containing 1,3,4-oxadiazole derivatives, five compounds exhibited potent antimicrobial activity against selected microbial strains.

Keywords: Benzyl, priority pathogens, *in-vitro* antimicrobial activity, in-silico molecular docking study, peptide deformylase, WHO.

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Conflict of interest: None

INTRODUCTION

Molecular modification[1,2] of a previously known lead structure is the most suitable approach for the discovery of a new structure with an improved pharmacokinetic profile and therapeutic effect.[3] Antimicrobial agents are widely used for the treatment of microbial infections in humans and animals.[4] Due to excessive use of antimicrobial drugs, antimicrobial resistance is a serious problem in both human and animal medicine, adapted by various mechanism.[5,6] Hence, considering the future aspects of antimicrobials, screening of novel structures is a substantial need for new and potent antimicrobial agents with minimum toxic effects.[7,8] Heterocyclic derivatives have attracted attention of researchers due to their many medicinal values.[9] Oxadiazoles are five-membered heterocyclic compounds bearing two nitrogen and one oxygen atom.[10] Among them, 1,3,4-oxadiazole have shown a broad range of pharmacological properties including antiviral[11], antineoplastic,[12] anti-mycobacterium,[13] anti-inflammatory,[14] and antimicrobial activities. During the last few decades, many researchers reported new antimicrobial agents having 1,3,4-oxadiazole ring.[15-17]

As per a previous literature survey gram-positive bacterial pathogens are not readily resistant to antimicrobial agents as compared to gram-negative bacterial pathogens.[18] These differences in resistance mechanism may be due to their distinctive cell wall composition.[19] Gram-negative bacteria have a multilayered cell wall and a specific periplasmic space in the outer cell membrane, which is not present in gram-positive

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bacterial pathogens.[20] Hence, the gram-negative bacterial cell wall is more lipophilic in contrast to gram-positive bacteria.[21] WHO has published a list of antibiotic-resistant pathogens hazardous for human in 2017. In WHO list, the maximum number of priority pathogens belongs to gram negative bacteria category. [22]

Computational techniques or virtual screening (VS) are important ways for drug designing and pharmacophore identification.[23,24] In-silico molecular docking is one of the basic approaches for structural based drug design, which has been used since the 1980s.[25,26] Computer programs based on various algorithms have been developed to perform molecular modeling as well as molecular docking studies.[27,28] So, computational studies and other bioinformatics tools are highly effective steps in drug design and discovery.[29,30] Molecular docking studies requires two basic components viz. ligand and active site of selected / target protein.[31]

Peptide deformylase (PDF) is an active and specific mononuclear iron-containing enzyme, belongs to the subclass of the metalloprotease category.[32,33] This enzyme is required for protein maturation by removal of N-formyl group from N-terminal methionine polypeptide chain through iron-mediated catalysis.[34,35] Peptide deformylase is vital and extremely conserved enzyme in various bacterial pathogens but it is not essential for protein synthesis in eukaryotic cells[36] and hence it is an interesting target for creating new antibacterial agents.[37,38]

This research paper reports the synthesis of a novel series of benzyl moiety contains 2,5-disubstituted 1,3,4-oxadiazole derivatives and evaluation of their antimicrobial activity supported with molecular docking studies using peptide deformylase as target enzyme.

MATERIALS AND METHODS

Chemistry

Synthetic approach for a series of 2,5-disubstituted 1,3,4-oxadiazole analogs holding benzyl moiety is depicted in Scheme-1. The *para* aminobenzoic acid was used as a starting material and masking the carboxylic group by esterification process (Fischer method) before benzylation.[39,40] Benzylated product was converted into respective hydrazide by using hydrazine hydrate through simple reactions. Further, respective hydrazide was made to react with various substituted aromatic acids in the presence of cyclo-dehydrating agent $POCl_3$ under specified conditions and the desired products were obtained. The completion of reactions and purity of synthesized compounds were examined by thin-layer chromatography (TLC) method. The synthetic strategy for obtaining benzyl moiety bearing 2,5-disubstituted 1,3,4-oxadiazole derivatives is depicted in Scheme-1.

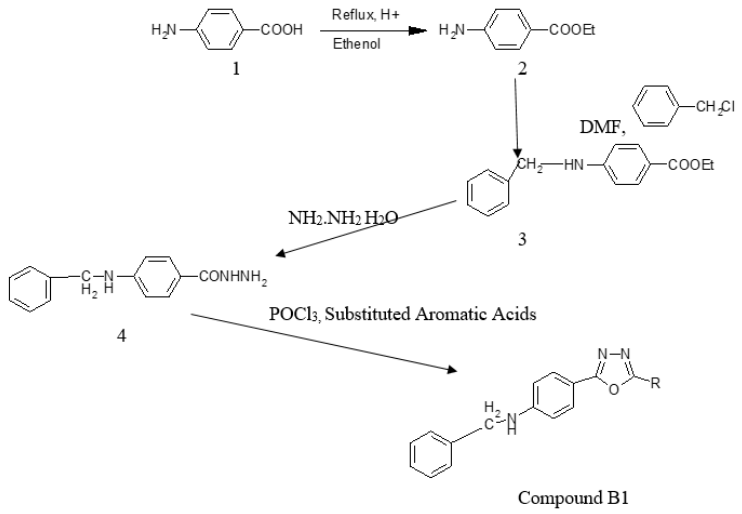
Experimental Methods

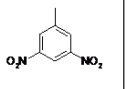
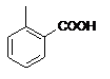
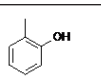
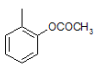
Chemical reagents and solvent were procured from local commercial suppliers. Silica gel G coated TLC plate was used for reaction monitoring and for identification of retardation factor (R_f) values with hexane, ethyl acetate and ethanol as a mobile phase. Spot visualization of TLC plate was done by iodine chamber. Melting points of synthesized compounds were determined by open capillary method using digital melting point apparatus and found uncorrected. The λ_{max} of synthesized compounds was obtained by using double beam UV-visible spectrophotometer (Shimadzu Pharma Spec 1700). The physical descriptions and UV data of synthesized compounds are given in Table 1. The Perkin Elmer IR spectrometer was used for obtaining IR spectra using the KBr pellet method. Mass spectra were obtained through a mass spectrometer by the ESI method. ^{1}H NMR and ^{13}C NMR spectra were recorded by operating 400 MHz JNM-ECS spectrometer using DMSO-D6, showing chemical shift valves on δ ppm scale. Auto Dock Vina version software was applied for molecular docking studies.

General procedure for synthesis of N-benzyl-4-[5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl]aniline (B1 a-j)

- *Synthesis of 4-amino benzoic Acid Ethyl Ester*

To ethanolic solution of PABA (0.1mol), concentrated H_2SO_4 (1 ml) was added drop-wise. The reaction mixture was gently



Compound no.	R	Compound no.	R
B1a		B1f	
B1b		B1g	
B1c		B1h	
B1d		B1j	
B1e		B1i	

Scheme 1: for synthesis of 1,3,4-oxadiazole derivatives.

Table 1: Analytical description of synthesized 2,5 disubstituted Oxadiazole derivatives

S.No	*Comp.*	*Molecular Formula*	*Solubility*	*R_f Value*	*λ_{max} (nm)*
1	B1 a	$C_{20}H_{16}ON_4$	DMSO,Ethanol,$CHCl_3$	0.52	271
2	B1 b	$C_{22}H_{17}O_3N_3$	DMSO,Ethanol,$CHCl_3$	0.69	277
3	B1 c	$C_{21}H_{17}O_2N_3$	DMSO,Ethanol,$CHCl_3$	0.64	276
4	B1 d	$C_{23}H_{19}O_3N_3$	DMSO,Ethanol,$CHCl_3$	0.34	271
5	B1 e	$C_{21}H_{17}ON_3$	DMSO,Ethanol,$CHCl_3$	0.39	263
6	B1 f	$C_{21}H_{15}O_5N_5$	DMSO,Ethanol,$CHCl_3$	0.52	320
7	B1 g	$C_{21}H_{16}O_3N_4$	DMSO,Ethanol,$CHCl_3$	0.48	291
8	B1 h	$C_{21}H_{18}ON_4$	DMSO,Ethanol,$CHCl_3$	0.46	320
9	B1 i	$C_{22}H_{19}O_2N_3$	DMSO,Ethanol,$CHCl_3$	0.68	277
10	B1 j	$C_{22}H_{19}ON_3$	DMSO,Ethanol,$CHCl_3$	0.51	263

Mobile Phase for TLC: Hexane: Ethyl acetate: Ethanol (5:3:2); λ_{max} in Ethanol

refluxed for 60–75 minutes, then cooled, poured in 30 mL ice-cold water and then slowly added 10% Na_2CO_3 solution to make the mixture neutral to slightly basic (approx. pH 8). The resulting solid was filtered, dried, and recrystallized from ethanol to obtain ester compound 1 (Lit. Reported MP 90-92°C)

General Procedure for the Synthesis of 4-(benzyl amino) benzoic Acid Ethyl Ester

Taken solution of compound 1 (1g) and KOH (0.6g) in DMF (20 mL) and added benzyl chloride (1.5mL) drop-wise at low temperature. The reaction mixture was refluxed for appropriate time. The completion of reaction was checked by TLC. After the completion of the reaction, the reaction mixture was poured in to a separating funnel containing 20mL ethyl acetate. Mixture was shaken vigorously and allowed to stand for some time forming two separate layers. Upper layer was collected and neutralized by using sodium carbonate solution. Finally DMF was distilled off and desired compound 2 was obtained.[41] Yield: 65%, B.P.: 148-150°C; IR (cm^{-1}): 3343.99 (NH),3000.0 (CH aromatic), 2983.3 (CH methylene), 2878.20(CH methyl), 1680.88 (C=O),1598.09 (C-O),1310.92(CN),1172.21(C-C); ^{1}H-NMR ($CDCl_3$, ppm): 7.84 (d, 2H, J=3.9),7.52(d, 2H,J=3.3),7.04 (s,3H), 6.62-6.60(d, 2H, J=3.9),4.32-4.02(m, 4H, Methylene), 1.35-1.32(t,3H, methyl); MS: $[M+H]^+$326.1

General Procedure for the Synthesis of 4-(Benzyl amino) Benzoic acid hydrazide

Taken solution of Compound 2 (0.01 mol) in ethanol (20 mL), added hydrazine hydrate (0.02 mol) and refluxed the mixture for about 5 hrs on steam bath. After cooling, the resulting solid was filtered, dried and recrystallized from ethanol to obtain compound 3. Yield: 62%, M.P.: 152-154°C; IR (cm^{-1}): 3339.05(NH_2) 3005.0 (CH aromatic), 2908.3 (CH methylene), 1679.80 (C=O), 1309.92(CN), 1170.89 (C-C); MS: $[M+H]^+$328.3

General Procedure for Synthesis of N-benzyl-4-[5-(pyridine-4-yl)-1,3,4-oxadiazol-2-yl]aniline (B1 a-j)

A mixture of respective aryl hydrazide (3) (0.01 mol) and nicotinic acid (0.01 mol) was dissolved in about 5ml of phosphorus oxychloride. The reaction mixture was refluxed on a water bath for 7-8 hrs. After completion of the reaction, the mixture was cooled at room temperature. Excess of phosphorous oxychloride was distilled off, and the mixture was gradually poured into crushed ice by continuous stirring for 10 minutes and sodium bicarbonate (20%) was added for neutralization. The resulting mixture was filtered, and the residue was washed with cold water and $NaHCO_3$ solution. The separated yellow solid was dried and recrystallized from ethanol to obtain the final compound B1 a. A similar procedure was adopted for synthesis of compound B1b-j.[42] The corresponding R for B1a-j is shown below.

Characterization of Synthesized Compounds B1a-j

2-(pyridine-4-yl)-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (a)

Yield: 70.6%, M.P: 174-178°C, light yellow solid; IR (cm^{-1}): 3416.01(NH),3093.07,3059.6(CH aromatic),2889.27(Aliphatic CH) 1676.50(CN), 1605.98,1575.15,1457.30 (C – C =C), 1346.86,1289.19(CN), 1053.16 (C-O-C), 899.73,784.24,757.61(oop bending); ^{1}H NMR (DMSO-D6,400 MHz,δ ppm): 7.91 (d, 2H, pyridyl, (J = 3.9), 7.6-7.5 (t,2H, aromatic,) 7.47-7.44 (m, 9H, Aromatic, (J = 3.7), 5.81(s, H, NH), 4.50 (d, 2H, methylene, (J = 1.9),; ^{13} C NMR (DMSO-d_6,400 MHz, **δ** ppm)164.98, 164.81, 149.77, 144.30, 133.30,131.70, 131.27, 129.72, 127.98, 123.22, 120.00, 117.37, 113.04, 44.04; MS: m/z calculated for $C_{20}H_{16}N_4O$ $[M+H]^+$329.3 , found : 328.3

2-(o-carboxyphenyl)-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (b)

Yield: 55.8%, M.P/B.P: 192-194°C, light yellow liquid; IR (cm^{-1}): 3390.07(OH,NH),3001.09(CH aromatic),2941.99(Aliphatic CH), 1791.62 (C=O) 1602.90(CN),1534.66,1466.38 (C – C =C),1413.18 (OH), 1282.22(CN),1233.66(C-O-C), 1071.75 (C-O-C), 845.73,794.24,708.08 (oop bending); ^{1}H NMR (DMSO-D6,400 MHz,δ ppm): 10.32 (s,1H, benzoic acid), 8.73 (d, 2H, carboxyphenyl, (J = 1.9), 8.23 (d,2H, aromatic, (J = 2.0), 7.57-7.47 (m, 9H, Aromatic),), 5.82(s,b, H, NH), 4.43 (d, 2H, methylene, (J = 2.2); ^{13} C NMR (DMSO-d_6,400 MHz, δ ppm) 172.43,167.68, 161.63, 142.04, 136.18, 131.72,130.79, 129.71, 119.81, 117.60, 113.08, 42.03; MS: m/z calculated for $C_{22}H_{17}N_3O_3$ $[M+H]^+$372.2 , found : 371.12

2-(o-hydroxyphenyl)-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (c)

Yield: 40.2%, M.P/B.P: 180-182°C, yellowish brown solid; IR (cm^{-1}): 3359.00(OH,NH),3009.07,3059.6(CH aromatic),2954.56, 2821.24(Aliphatic CH) 1633.44(CN), 1605.54,1573.27,1540.34 (C – C =C), 1454.93 (OH), 1346.86,1292.66(CO)), 1078.59,1044.56 (C-O-C), 875.84,547.48(oop bending); ^{1}H NMR (DMSO-d6,400 MHz,δ ppm): 7.76-7.73(dd, 4H, aromatic),7.57-7.44 (m,5H, aromatic), 6.91-6.86 (dd, 4H, Aromatic),6.48(s, 1H, OH) 5.68(s,b, H, NH), 4.50 (d, 2H, methylene, (J = 2.0); ^{13} C NMR (DMSO-d_6,400 MHz, δ ppm) 172.43,166.63, 161.63, 151.36, 143.08, 136.08, 131.72,130.79, 119.61, 117.70, 113.28, 45.81.04; MS: m/z calculated for $C_{21}H_{17}N_3O_2$ $[M+H]^+$344.13 , found : 343.13

2-{5-[4-(benzylamino) phenyl]-1,3,4-oxadiazol-2yl)}phenyl acetate B 1 (d)

Yield: 69.5%, M.P/B.P: 198-200°C, light brown solid; IR (cm^{-1}): 3405.02(NH),3095.72, (CH aromatic),2873.02, 2761.42(Aliphatic CH), 1708.96(C=O), 1693.67, 1579(CN), 1602.35, 1473.46 (C – C =C), 1258.53,1220.15(CO)), 1075.16, (C-O-C), 892.71, 823.32,755.14.48(oop bending); ^{1}H NMR (DMSO-D6,400 MHz,δ ppm): 7.89-7.73(m, 4H, aromatic),7.65-7.55 (m,4H, aromatic), 7.48-7.7.31 (m, 5H, Aromatic), 4.08(s,b, H, NH), 4.90 (d, 2H, methylene, (J = 2.5),2.1(s,3H, methyl); ^{13} C NMR (DMSO-d_6,400 MHz, δ ppm)168.02, 165.65, 144.00, 134.46,131.73, 130.06, 128.23, 124.28, 117.39, 113.06, 53.07, 29.03; MS: m/z calculated for $C_{23}H_{19}N_3O_3$ $[M+H]^+$386.3 , found : 385.14

2-phenyl-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (e)

Yield: 71.4%, M.P/B.P: 166-168°C, Off white solid; IR (cm^{-1}): 3423.29(NH),3082.72(CH aromatic),2883.02(Aliphatic CH) 1676.50(CN), 1643.67,1579.04 (C – C =C), 1303.17(CN),1258.53(C-O str), 1075.16 (C-O-C), 892.17,823.32, 755.14(oop bending); ^{1}H NMR (DMSO-D6,400 MHz,δ ppm): 7.91 (d, 2H, aromatic),7.60-7.44 (m,12H, aromatic), 5.01(s, H, NH), 4.10(d, 2H, methylene, J=2.06);^{13} C NMR (DMSO-d_6,400 MHz, δ ppm)167.98,167.81, 164.31, 154.01, 143.02, 133.30,131.70, 131.70131.27, 129.72,129.01, 128.00, 117.37, 113.04, 46.30; MS: m/z calculated for $C_{21}H_{17}N_3O$ $[M+H]^+$328.3, found : 327.13

2-(3,5-dinitrophenyl)-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (f)

Yield: 47.2%, M.P / B.P: 212-214°C, light yellowish brown solid; IR (cm^{-1}): 3405.29(NH),3095.02, (CH aromatic),2873.02(Aliphatic CH) 1693.67(CN), 1602.54,1540.38.15, (C – C =C),1523.45,1393.17(N-O asymm, symm), 1247.66,1200.90(C-O str), 1075.59 (C-O-C), 836.73,745.24,706.61(oop bending); ^{1}H NMR (DMSO-d6,400 MHz,δ ppm): 9.02 (s, 3H, nitrophenyl), 8.23 (d,2H, aromatic, (J = 4.1), 7.57-7.47 (m, 7H, Aromatic), 6.50 (d, 1H, NH, (J = 3.2), 4.43(d, 2H, methylene, J=2.2); ^{13} C NMR (DMSO-d_6,400 MHz, δ ppm) 169.70,166.13,161.66, 150.69, 144.40,136.16,134.30, 131.90,131.73,130.89, 130.79, 126.58, 124.57, 124.29, 119.68, 117.60, 113.07, 46.45; MS: m/z calculated for $C_{21}H_{15}N_5O_5$ $[M+H]^+$418.3, found : 417.10

2-(p-nitophenyl)-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (g)

Yield: 58.9%, M.P / B.P: 202-204°C, light yellow solid; IR (cm^{-1}): 3323.29(NH), 3007.94(CH aromatic),2855.05(Aliphatic CH) 1650.48(CN), 1453.64 (C – C =C), 1377.59 (N-O asymm.),1241.21(CO), 1097.57 (C-O-C), 858.49,724.49,757.61(oop bending); ^{1}H NMR(DMSO-d6,400 MHz,δ ppm): 8.50 (d, 2H, nitrophenyl, J=4.6), 7.91 (d,2H, nitrophenyl, (J=3.9), 7.40-7.24 (m, 9H, Aromatic), 6.50 (d, 1H, NH, (J = 3.0), 4.41(s, 2H, methylene, J=2.2)); ^{13} C NMR (DMSO-d_6,400 MHz, δ ppm)166.10, 164.66, 154.09,150.69, 144.36, 131.90, 131.73,130.89,130.79, 126.58, 124.57, 124.29, 119.68, 117.60, 113.07, 43.64; MS: m/z calculated for $C_{21}H_{18}N_4O_3$ $[M+H]^+$373.4 , found : 372.12

2-(p-aminophenyl)-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (h)

Yield: 66.3%, M.P/B.P: 170-172°C, yellowish brown solid; IR (cm^{-1}): 3430.68(NH),3064.96 (CH aromatic),2930.89(Aliphatic CH), 1696.47, 1595.66(CN),1635.46 (NH bending, m) 1602.98, (C – C =C), 1282.32, 1233.42 (CO), 1070.97 (C-O-C), 878.76,707.53,706.61(oop bending); ^{1}H NMR (DMSO-d6,400 MHz,δ ppm): 7.86 (d,4H, aromatic, (J = 3.9), 7.56 (4, 2H, Aromatic J=3.78), 7.33 (d, 5H, Aromatic, (J = 3.6),6.50(d, 2H. NH, J=3.3), 6.2(s, 2H, NH2) 4.18(d, 2H, methylene, J=2.0) ^{13} C NMR (DMSO-d_6,400 MHz, δ ppm)168.0, 164.72, 148.86, 143.84, 133.11,131.72, 129.44, 128.72,127.94, 113.11, 44.43; MS: m/z calculated for $C_{21}H_{18}N_4O$ $[M+H]^+$343.1 , found : 342.14

2-(p-methoxyphenyl)-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (i)

Yield: 45.1%, M.P /B.P: 196-198°C, dark brown solid; IR (cm^{-1}): 3359.00(NH),3009.93(CH aromatic),2821.24(Aliphatic CH), 1633.44(CN), 1605.95,1535.00,1498.38 (C – C =C), 1281.97,1233.25(CO), 1011.24 (C-O-C), 876.23,764.06,708.18(oop bending); ^{1}H NMR(DMSO-d6,400 MHz,δ ppm): 7.89-7.13 (m, 13H, Aromatic), 6.30 (d, 1H, NH, (J = 3.2), 4.33(d, 2H, methylene, J=2.1),3.82 (s, 3H, Methoxy); ^{13} C NMR (DMSO-d_6,400 MHz, δ ppm)166.81, 159.97,153.62, 143.72, 133.30,131.70, 131.27, 129.72,129.00 128.90,117.37, 113.04, 54.62, 45.25; MS: m/z calculated for $C_{22}H_{16}N_3O_2$ $[M+H]^+$358.2 , found : 357.14

2-(benzyl)-5-[4-(benzylamino)-phenyl]-1,3,4-oxadiazole B 1 (j)

Yield: 71.9%, M.P / B.P: 158-160°C, light yellow liquid; IR (cm^{-1}): 3323.29(NH) ,3055.24(CH aromatic),2954.74,2870.26(Aliphatic CH), 1644.31, 1543.84(CN),1543.84,1455.24 (C – C =C), 1310.66,1282.08(CO), 1078.45 (C-O-C), 875.66,845.07 (oop bending); ^{1}H NMR (DMSO-d6,400 MHz,δ ppm): 7.76-7.73 (q, 4H, Aromatic), 7.5-7.4(m, 10H, Aromatic), 6.50 (d, 1H, NH, (J = 3.2), 4.18(d, 4H, methylene, J=4.3); ^{13} C NMR (DMSO-d_6,400 MHz, δ ppm)166.0, 148.86, 143.04, 133.39,131.70, 131.72, 129.72, 127.44, 128.81,113.11, 44.23; MS: m/z calculated for $C_{22}H_{19}N_3O$ $[M+H]^+$342.1 , found : 341.15

In-vitro Antimicrobial Evaluations

Antibacterial Studies

The novel synthesized compounds were evaluated for their antibacterial activity against *Bacillus substilis, Staphylococcus aureus, Escherichia coli,* and *Pseudomonas aeruginosa*. The antibacterial evaluation was evaluated by disc diffusion method.[43, 44] Sterilized nutrient agar media (20 mL) was poured into each labeled petri dish and solidified. Freshly prepared bacterial inoculum was spread on the surface of agar media for the growth of the bacterial lawn. Then discs were immersed in different concentrations of test compounds and were placed on top of agar surface. Cefixime was taken as a positive control, and DMSO was used as blank. All Petri dishes were incubated at 37°C for 24–48 hrs. The results were recorded by measuring diameter of zone of inhibition produced by various concentrations of the test compounds. The bacterial zone of inhibition data for test compounds are presented in Table 2

MIC Determination

Minimum Inhibitory Concentration of synthesized compounds was determined by using broth dilution method.[45, 46] Aqueous solution of Mueller Hinton broth was prepared, sterilized and poured into labeled tubes. The tubes were inoculated by fresh bacterial log culture, containing 5×10^5 CFU/mL concentration. DMSO was used as a solvent for preparation of stock solution of test compounds and reference drug. The reference drug and test compound solutions were added in to inoculated tubes and incubated at 35 ± 2°C for 20-24 hrs. One set was prepared for each test compound against test organism with 6.5, 12.5, 25, 50, 100, 200 and 400 mg/L concentration. MIC values of synthesized compounds were noted at the end of incubation time by observing the lowest concentration of tubes with visible growth. The MIC data of synthesized compounds are shown in Table 4.

Antifungal Activity

Newly synthesized compounds were also analyzed for their antifungal activity through disc diffusion method.[47,48] Two fungal strains *Aspergillus flavus* and *Candida albicans* were taken in DMSO for this experiment. Sterile sabourands agar media was poured into pre-sterilized Petri plates and allowed for solidification. PDA plates were inoculated with 100 µL of fresh fungal strain of log culture for lawning. Further paper

Table 2: Antibacterial activity of the synthesized 2,5-disubstiuted 1,3,4-oxadiazole derivatives

		Zone of inhibition in mm			
Compound	*Concentration (µg/ml)*	*S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*
B1 (a)	100	-	-	10.0 ± 0.3	-
	200	-	-	11.0 ± 0.2	-
	400	-	9.0 ± 0.4	12.0 ± 0.2	-
B1 (b)	100	-	-	-	-
	200	-	-	-	-
	400	-	-	-	-
B1 (c)	100	07.2 ± 0.2	-	10.1 ± 0.4	-
	200	10.0 ± 0.4	-	10.0 ± 0.9	05.0 ± 0.2
	400	12.4 ± 0.1	-	12.2 ± 0.3	09.7 ± 0.1
B1 (d)	100	-	-	-	-
	200	-	-	-	1.0 ± 0.4
	400	-	5.0 ± 0.3	-	1.2 ± 0.3
B1 (e)	100	-	-	09.0 ± 0.3	-
	200	-	10.0 ± 0.6	15.2 ± 0.6	-
	400	-	12.3 ± 0.4	16.4 ± 0.2	-
B1 (f)	100	10.4 ± 0.3	07.2 ± 0.4	11.2 ± 0.5	8.1 ± 0.6
	200	15.0 ± 0.2	10.8 ± 0.1	15.7 ± 0.2	10.8 ± 0.4
	400	20.1 ± 0.4	18.1 ± 0.2	20.8 ± 0.3	11.1 ± 0.2
B1 (g)	100	06.1 ± 0.4	04.0 ± 0.4	10.4 ± 0.3	-
	200	12.5 ± 0.2	06.1 ± 0.4	13.3 ± 0.3	08.0 ± 0.2
	400	16.3 ± 0.4	13.6 ± 0.5	17.8 ± 0.1	12.2 ± 0.4
B1 (h)	100	08.2 ± 0.4	05.0 ± 0.3	10.2 ± 0.1	-
	200	11.0 ± 0.2	10.2 ± 0.2	14.6 ± 0.6	-
	400	17.2 ± 0.5	14.1 ± 0.7	18.1 ± 0.3	11.2 ± 0.4
B1 (i)	100	05.2 ± 0.5	09.0 ± 0.4	10.0 ± 0.2	09.0 ± 0.4
	200	11.0 ± 0.2	12.2 ± 0.1	16.0 ± 0.6	12.2 ± 0.2
	400	16.4 ± 0.1	18.8 ± 0.6	18.2 ± 0.3	12.5 ± 0.3
B1 (j)	100	09.2 ± 0.2	10.0 ± 0.2	10.0 ± 0.6	-
	200	10.0 ± 0.1	12.0 ± 0.6	16.6 ± 0.2	-
	400	16.3 ± 0.4	19.0 ± 0.4	20.4 ± 0.2	9.2 ± 0.7
Cefixime	100	08.6 ± 0.3	07.2 ± 0.2	10.1 ± 0.3	-
	200	11.7 ± 0.2	12.7 ± 0.4	15.4 ± 0.1	-
	400	17.2 ± 0.4	18.9 ± 0.1	20.8 ± 0.3	10.8 ± 0.1

discs immersed in different dilutions of test compounds were placed on its surface. These plates were incubated at 37°C for 24-48 hours. The fungal activity of synthesized compounds was compared with econazole as the standard drug and DMSO as blank. A fungal inhibition halo zone was created around the tested compounds. The diameter of inhibition zone of all the synthetic compounds was measured in millimeters and data are given in Table 3.

In-silico Molecular Docking Studies

Molecular Docking studies were performed for synthesized 2, 5-disubstituted 1, 3, and 4-oxadiazole derivatives by using Auto Dock/Vina software.[49,50] In correlation to *in- vitro* antimicrobial activity, it was worthwhile to carry out *in- silico* studies to predict the binding affinity and orientation at the active site of the protein.[51,52] The structure of synthesized compounds was drawn in Chem Sketch Draw tool (Chem-Sketch freeware) allocated with proper 2-D orientation, and also checked for structural drawing error. A 2-D structure was converted into 3D representation with minimum energy by using Chembio3D. The energy minimized ligand molecules were used as input for Auto Dock Vina suitable for molecular docking simulation.[53] The protein Peptide deformylase (PDF) was selected as target macromolecule.[54,55,56] The PDB coordinate file named 'PDF- 1G2A.pdb'was used as a receptor molecule. Water molecules and other interfered groups were removed from receptor molecule and rebuild the missing side chain in receptors automatically.

The interesting region of macromolecule was covered by grid box in *x, y* and *z* direction. The graphic user interface program "MGL Tools" was utilized for a grid box set to carry out the docking simulation. The grid box volume was set to 60, 60, and 60 Å for *x, y*, and *z* dimensions which included all the interesting amino acids residues in the considered active pocket of selected protein/macromolecule. Auto Grid 4.0 program was utilized for obtaining grid maps, supported with Auto Dock 4.0.[57] The docking algorithm software provided with Auto Dock Vina was applied for identification of best-docked conformation between the ligand and protein. About ten conformers were considered for each ligand molecule during the docking process. PyMol was used to conclude docking

Table 3: Antifungal activity of synthesized 2,5-disubstiuted 1,3,4-oxadiazole derivatives in diameter of inhibition zone (mm)

Comp. code	*Candida albicans*					*Aspergillus flavus*				
	50	*100*	*250*	*500*	*1000*	*50*	*100*	*250*	*500*	*1000*
4a	3.2 ± 0.1	3.9 ± 0.2	8.8 ± 0.2	10.0 ± 0.4	14.2 ± 0.1	0.0 ± 0	10.6 ± 0.2	14.5 ± 0.8	15.4 ± 0.3	18.8 ± 0.7
4b	00 ± 0.0	00 ± 0.0	00 ± 0.0	00 ± 0.0	00 ± 0.0	00	00	00	00	00
4c	00 ± 0.0	00 ± 0.0	00 ± 0.0	00 ± 0.0	00 ± 0.0	00	00	00	00	00
4d	00 ± 0.0	2.4 ± 0.6	2.0 ± 0.03	6.9 ± 0.7	10.3 ± 0.4	00 ± 0	00 ± 0	4.0 ± 0.8	7.2 ± 0.2	12.6 ± 0.3
4e	00 ± 0.0	00 ± 0.0	00 ± 0.0	00 ± 0.0	00 ± 0.0	00	00	00	00	
4f	10.6 ± 0.7	15.8 ± 0.3	17.6 ± 0.1	19.7 ± 0.2	25.9 ± 0.1	11.0 ± 0.0	14.4 ± 0.6	20.2 ± 0.3	23.0 ± 0.6	26.8 ± 0.2
4g	3.0 ± 0.07	4.2 ± 0.05	5.0 ± 0.03	08.0 ± 0.2	12.5 ± 0.02	2.3 ± 0.05	10.4 ± 0.3	14.2 ± 0.6	15.4 ± 0.7	17.0 ± 0.2
4h	4.0 ± 0.2	9.0 ± 0.0	13.0 ± 0.3	15.2 ± 0.8	19.8 ± 0.5	8.3 ± 0.02	14.1 ± 0.2	18.4 ± 0.5	20.9 ± 0.4	22.6 ± 0.2
4i	5.3 ± 0.0	6.9 ± 0.0	10.1 ± 0.0	12.4 ± 0.07	16.1 ± 0.03	6.3 ± 0.05	12.0 ± 0.03	16.0 ± 0.02	16.0 ± 0.02	20.4 ± 0.03
4j	07.2 ± 0.4	13.6 ± 0.06	16.2 ± 0.08	19.2 ± 0.02	24.9 ± 0.02	09.2 ± 0.04	15.6 ± 0.06	21.0 ± 0.02	22.8 ± 0.06	26.2 ± 0.02
standard	15.0 ± 0.2	17.9 ± 0.7	19.0 ± 0.4	21.4 ± 0.3	26.4 ± 0.3	14.0 ± 0.6	17.4 ± 0.4	20.5 ± 0.1	26.4 ± 0.6	27.8 ± 0.4
DMSO	-	-	-	-	-	-	-	-	-	-

Table 4: Minimum Inhibitory concentration data of synthesized compounds B1a-B1j

	Gram-positive bacteria		*Gram-negative bacteria*	
Compound code	*S.aurus*	*Bacillus subtilis*	*E.coli*	*P.aureginosa*
B1a	NT	NT	NT	NT
B1b	NT.	NT	NT	NT
B1c	100	100	50	400
B1d	NT	NT	NT	NT
B1e	NT	NT	NT	NT
B1f	25	50	25	200
B1g	50	100	25	200
B1h	50	100	12.5	100
B1i	100	200	50	400
B1j	50	100	25	200
Standard Cefixime	25	50	12.5	100

MIC (µg/mL) value means lowest concentration of the compound to inhibit the visible growth of bacteria.
NT: not tested

simulation's final pictorial presentation between ligand and target macromolecule/ protein.[58]

RESULT AND DISCUSSION

Chemistry

Benzyl moiety has good antimicrobial properties and also enhances the lipophilicity of compound.[59,60] Therefore, benzyl ring containing 2,5-disubstituted1,3,4-oxadiazole were synthesized as per scheme-1. The structural confirmation of newly synthesized compounds B 1 (a-j) was studied by IR, ^{1}H NMR, ^{13}C NMR and mass spectral analysis. IR spectrum of compound (a) shows absorption band at 3416.01cm-1 due to NH group and aromaticity of compound was confirmed by absorption band aroused at 3093.07 and 3059.6 cm^{-1}. Absorption bands at 1578.50cm^{-1} and 1053.16 cm^{-1} are assigned for CN and C-O-C ring stretching of Oxadiazole respectively. Medium absorption band at 3000-2800 cm^{-1} range are shown due to aliphatic CH stretching in each IR spectrum. In ^{1}H NMR spectra, all proton signals appeared at an appropriate region with the expected chemical shift and integral values. Diagnostic peaks at δ 7.91 (d, 2-H), 7.6- 7.4 (m, 10-H) were observed in aromatic region for compound B1(a). A broad peak at δ5.8 (s, 2-H) was observed due to amine (NH) protons. The signal of methylene protons was seen at δ 4.5 (d, 2-H). Fundamental peaks at δ 165.1, 164.3, 149.7, 144.3, 133-120, 44,0 were seen in ^{13}C NMR spectrum which completely correlates with the proposed structure of compound B1(a). The structures of remaining derivative compounds were also characterized in a similar way. The delta (δ) values of aromatic and aliphatic protons were changed due to substitution by electron-donating groups or electron withdrawing groups at the different positions of phenyl ring. The mass spectrum of synthesized compounds was also expected as per the proposed molecular formula and molecular ion peak was coherent with calculated molecular weight. Analytical and spectral data of synthesized compounds were completely supportive with their proposed structures and detail spectral values are given in the experimental section.

Antimicrobial Studies

Antibacterial Activity

All the synthesized compounds were studied for their antibacterial activity against selected strains *Staphylcoccus aureus*, *Bacillus subtilis*, *E.coli* and *P. aeruginosa* by using disc diffusion method. Cefixime was taken as positive control and DMSO used as negative control. The antibacterial activity of test compounds was assayed on the basis of radius of inhibition zones. The average diameter of zones of inhibition (mm) were recorded and compared with standard drug Cefixime. Most of the compounds exhibited mild to moderate antibacterial activity against selected strains as per screening results (table-2). Promising inhibitory results of Compound B1f, B1j, B1h, B1i B1g, and B1c were obtained against selected bacterial strains. The compounds B1f, B1j and B1h exhibited activity nearly similar to that of cefixime ranging from 11.1 ± 0.2 to 20.1 ± 0.8 for B1f, 9.2 ± 0.7 to 20.4 ± 0.2 for B1j, 11.2 ± 0.8 to 18.1.1 ± 0.3 for B1h at the concentration 400µg/ml. The potent derivatives revealed that structural variations of compounds impacts biological activity. Compounds B1f, B1g, B1h, B1j having nitro, benzyl, methoxy and amino group showed good antibacterial activity against all strains except *P. aeruginosa*. Remaining compounds did not showed remarkable antibacterial activity. MIC values of synthesized compounds also indicated good antibacterial activity given in Table 4.

Antifungal activity

Antifungal activity of synthesized compounds was performed by disc diffusion method. Two fungal strains *Aspergillus flavus* and *Candida albicans* were taken for assessing antifungal activity. Antifungal screening results revealed that four compounds B1f, B1j, B1h, and B1i showed antifungal activity (table-3). Among these compounds, B1f and B1j exhibited promising results comparable with standard drug Econazole. The descending order of antifungal activity of synthesized compounds were B1f>B1j>B1h>B1i≥B1a≥B1g against selected fungal strains.

Molecular Docking Studies

In silico analysis of potentially active newly synthesized 1,3,4-oxadiazole derivatives were carried out against peptide deformylase protein and the docking results were also synchronized with *in-vitro* antimicrobial screening results. The docking analysis of tested compounds was performed through auto dock process to predict the binding affinity of ligand and best orientation conformation of each ligand. The binding affinities of docked ligand were assessed as binding energy or docking scores. The hydrophobic and hydrogen bond interactions between protein and each ligand molecule

Table 5: *In silico* molecular docking results of selected 2,5-disubstituted1,3,4-oxadiazole compounds

S.No.	*Ligand*	*Affinity (binding energy) in cal/mol*	*H –bonds*	*Hydrophobic interactions amino acids*
1.	B1 (f)	12.52	6	GLU42,GLU41,ARG97,LEU91,CYS90,GLN50,HIS136,GLU133,HIS132, GLN131,ARG97,GLY124
2.	B1 (h)	8.33	2	ARG97,GLU88,CYS90,ILE128,HIS132,CYS129,GLU133,GLY45,GLY43 ,LEU91,SER92
3.	B1 (i)	8.13	3	ILE128,HIS132,CYS129,GLU133,ILE44,GLY43,GLU41,LEU91,CYS90, GLU88,ARG97
4.	B1 (j)	9.19	2	GLU95,PRO94,ARG97,GLY89,CYS90,LEU91,ILE44,GLU133,CYS129,H IS132,ILE128,HIS132,ALA127
Standard	Cefixime	7.3	6	ILE44, GLU41,GLU42, GLY89, GLU95,ILE128, ILE86, HIS132,GLU133,GLU88

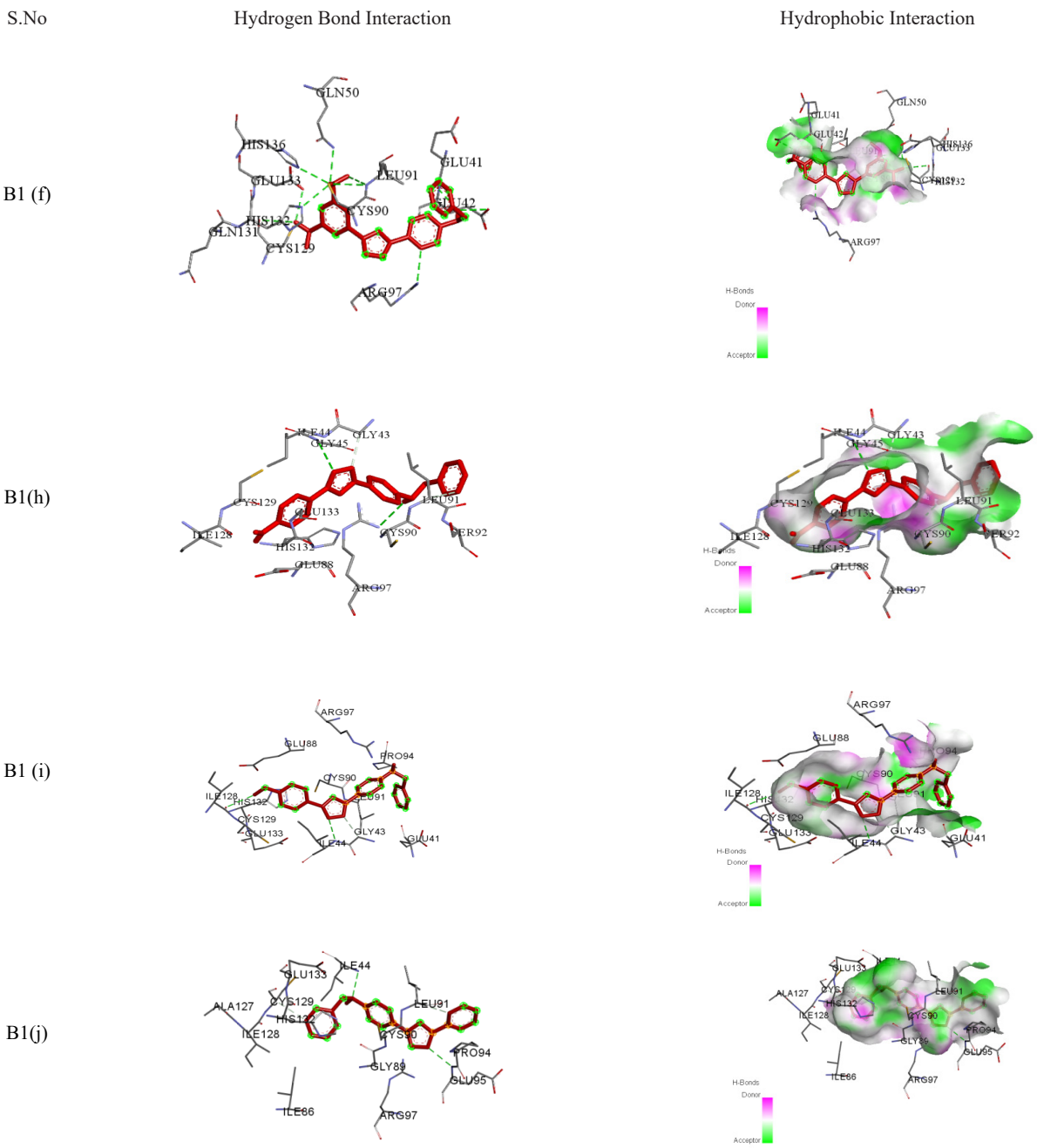


Figure 1: 3D pictorial representation of selected ligand molecule with peptide deformylase

B1f, B1h, B1i and blj were assessed within the binding pocket of protein-peptide deformylase 1G2A. All docked molecules exhibited excellent binding posed with amino acids of active pocket of selected protein as shown in Figure 1. The docked ligand molecules B1f, B1h, B1i and Blj were found with excellent docking confirmation with minimal binding affinity (-12.52, -8.33, -8.13 and -9.19 kJ mol^{-1}) than standard drug Cefixime (-7.3) and Econazole (-6.9). Molecular docking scores of compound B1f, B1h, B1i, and Blj are shown in Table 5.

CONCLUSION

A series of novel 2,5-disubstituted Oxadiazole derivatives fused with benzyl ring were synthesized and evaluated for their antimicrobial activity against microorganisms as per priority pathogens declared by WHO. Among synthesized compounds, B1f and B1j showed remarkable antimicrobial potential against tested microorganisms. *In-vitro* antimicrobial activity of tested compounds was also correlated with in silico molecular docking studies. Molecular docking results also conclude that the phenyl ring substituted with electron-withdrawing groups and benzyl ring attached to Oxadiazole enhanced the biological activity due to alteration in lipophilicity. Hence, based on the above results it can be concluded that the current study of Oxadiazole is suitable for further investigation in the field of antimicrobial drug designing.

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Supportive/supplementary materials:

Supplementary data will be provided on demand.

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