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Synthesis, characterization, antibacterial evaluation and molecular docking study of 4-{[4-(5-phenyl-1,3,4-oxadiazol-2-yl)phenoxy]methyl}benzonitrile derivatives

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Novel series of 1,3,4-oxadiazole derivatives were synthesized by the reaction between respective acid hydrazides with various aldehydes using chloramine-T (CAT). The structural characterization of compounds **7a-e** were confirmed by LC-MS and ^{1}H NMR, ^{13}C NMR spectroscopy and they were subjected for biological evaluation and molecular docking studies. The synthesized compounds exhibited comparable activity with the standard.

Keywords: Oxadiazole, molecular docking, antibacterial, chloramine-T.

Introduction

Heterocycles have been gaining interest of researchers since few decades owing to their extensive applications. Among the various five membered heterocycles, 1,3,4-oxadiazoles have attracted much attention because of their applications as advanced functional materials, liquid crystals[1] and pharmacokinetic profiles because of their lipophilicity[2]. They have been found to exhibit wide range of biological activities like antitumor[3], anti-inflammatory[4], antibacterial[5], antifungal[6], anti-tubercular[7], anti-proliferative[8], anti-diabetic[3], analgesic[9], anti-convulsant[10], antiviral[11] and antioxidant[12].

A number of synthetic methods have been employed to synthesize this motif from monoacyl hydrazides using $POCl_3$[5], CS_2, KOH[13] in acidic medium, BrCN, $NaHCO_3$ in dioxane[14], CDI, TEA in THF[13], CAN, CH_2Cl_2(dry)[14], TsCl, pyridine in THF[15], green synthesis by using silica sulfuric acid[16], Pd-cat.[17], NH_4 in EtOH[18], PPh_3, $ClCCCl_3$, DIEA, CH_3CN[19], by microwave method[20], chloramine-T[21].

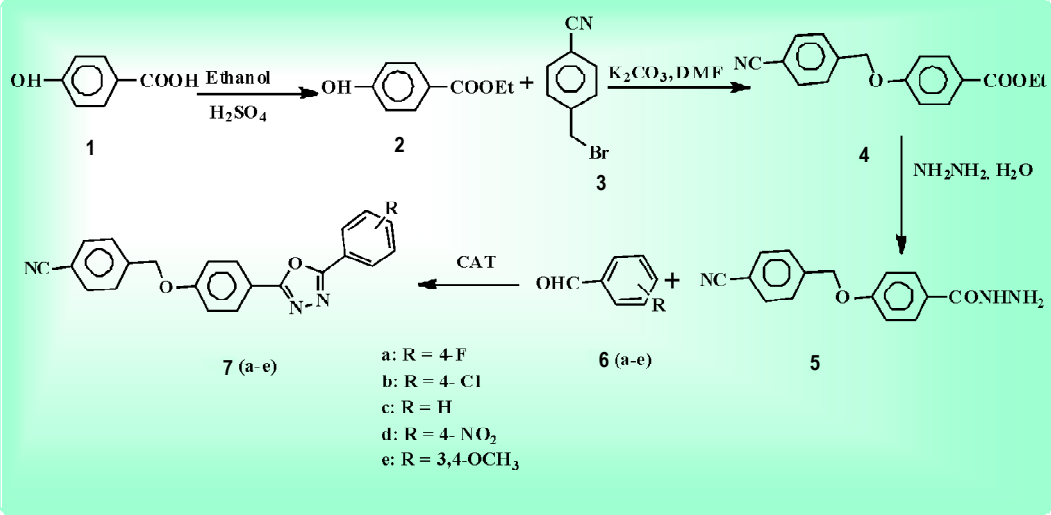
Previously, our research group has reported the synthesis of oxadiazoles and their biological activities[22], of late the liquid crystalline properties were also studied[23]. In this present work we report the synthesis of 4-{[4-(5-phenyl-1,3,4-oxadiazol-2-yl)phenoxy]methyl}benzonitrile derivatives and their antibacterial activity *in vitro* and molecular docking studies to understand the interaction with bacterial protein targets.

Experimental

Materials and methods:

The chemical ingredients, viz. anisaldehyde, 4-nitro benzaldehyde, 4-chloro benzaldehyde, 4-fluoro benzaldehyde, benzaldehyde, 3,4-dimethoxy benzaldehyde were procured from LOBA Chemie, India. Potassium carbonate, dimethyl formamide and diethyl ether was procured from RANKEM, India. The NMR of the synthesized compounds was obtained from AGILENT (400 MHz) NMR spectrometer.

Synthesized compounds were subjected to investigation of antibacterial property by agar well diffusion method. The Gram-negative bacteria such as *Salmonella typhi* (MTCC 733), *Escherichia coli* (MTCC 1698) and Gram-positive bacteria such as *Bacillus cereus* (MTCC 121), *Staphylococcus aureus* (MTCC 6908) were used in this study. 100 mL of Nutrient Agar and cotton swaps were prepared and steril-



Scheme 1. Scheme for synthesis of **7a-e**.

ized. The 20 mL agar was poured in each clean petri dish and was placed in laminar air flow chamber. After solidification, 0.1 mL of the human pathogenic bacteria was spread on nutrient agar. The agar plates were punctured using puncture apparatus for the introduction of synthesized compounds of 100 µg/mL concentration, and the sample volume being 75 µL. The plates were incubated for 24 h at 37°C. After incubation the zone of inhibition was measured. DMSO (solvent) was added into one well which is the negative control and in the other well, Gentamicin Sulfate (HIMEDIA) was used as positive control.

Scheme of synthesis:

General procedure for synthesis of ***4***[24,25]*:*

Mixture of *p*-hydroxy benzoate (**2**, 1.0 g, 6 mmol) and *p*-cyano benzyl bromide (**3**, 1.1 g, 6 mmol) and K_2CO_3 (2.49 g, 18.06 mmol) and dimethyl formamide (20 mL) were taken in a round bottom flask and were subjected to stirring at room temperature (28°C) for 8 h. The crude product was extracted into ether layer and dried over anhydrous Na_2SO_4.

General procedure for one pot synthesis of ***7a-e***[26]*:*

Mixture of **5** (1.0 g, 3.5 mmol) and **6c** (0.37 g, 3.5 mmol) were dissolved in ethanol and refluxed for 4 h, and to it chloramine-T (2.95 g, 10 mmol) was added and continued to reflux for 8 h. After the completion of reaction, ethanol was removed and the crude product was extracted in the ether layer and it was dried over anhydrous Na_2SO_4. The crude product was further subjected to purification by recrystallization using methanol.

Spectral data:

*4-{[4-(5-(4-Fluorophenyl)-1,3,4-oxadiazol-2-yl)phenoxy] methyl}benzonitrile (**7a**):* Prepared from **5** and **6a**. White solid; yield: 74%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.06 (d, 1H, ArH, *J* 8.8 Hz), 7.99 (d, 1H, ArH, *J* 8.8 Hz), 7.78 (d, 1H, ArH, *J* 8 Hz), 7.67 (d, 3H, ArH, *J* 8 Hz), 7.54 (d, 2H, ArH, *J* 8.4 Hz), 7.24 (d, 1H, ArH, *J* 8.4 Hz), 7.05 (d, 1H, ArH, *J* 8.8 Hz), 6.94 (d, 2H, ArH, *J* 8.8 Hz), 5.15 (s, 2H, OCH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.14, 161.67, 141.69, 131.65, 127.53, 123.75, 118.53, 116.62, 114.31, 111.96, 76.94, 68.87, 60.72, 14.32. Anal. Calcd. $C_{22}H_{14}FN_3O_2$ for: C, 71.15; H, 3.80; N, 11.32; Found: C, 71.46; H, 3.21; N, 11.24. LCMS [M+1]: Calcd. for $C_{22}H_{14}FN_3O_2$: 371.11, Found: 372.04.

*4-{[4-(5-(4-Chlorophenyl)-1,3,4-oxadiazol-2-yl)phenoxy] methyl}benzonitrile (**7b**):* Prepared from **5** and **6b**. White solid; yield: 75%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.07 (d, 1H, ArH, *J* 8.8 Hz), 7.98 (d, 1H, ArH, *J* 8.8 Hz), 7.77 (d, 1H, ArH, *J* 8 Hz), 7.65 (d, 3H, ArH, *J* 8 Hz), 7.55 (d, 2H, ArH, *J* 8.4 Hz), 7.23 (d, 1H, ArH, *J* 8.4 Hz), 7.04 (d, 1H, ArH, *J* 8.8 Hz), 6.95 (d, 2H, ArH, *J* 8.8 Hz), 5.15 (s, 2H, OCH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.13, 161.68, 141.70, 132.44, 131.64, 127.54, 123.77, 118.53, 114.32, 111.95, 76.69, 68.89, 60.73, 14.33. Anal. Calcd. $C_{22}H_{14}ClN_3O_2$ for: C, 68.13; H, 3.64; N, 10.83; Found: C, 68.22; H, 3.44; N, 10.28. LCMS [M+1]: Calcd. for $C_{22}H_{14}ClN_3O_2$: 387.08, Found: 388.01.

*4-{[4-(5-Phenyl-1,3,4-oxadiazol-2-yl)phenoxy]methyl} benzonitrile **(7c)**:* Prepared from **5** and **6c**. White solid; yield: 74%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.06 (d, 1H, ArH, *J* 8.8 Hz), 7.97 (d, 1H, ArH, *J* 8.8 Hz), 7.78 (d, 2H, ArH, *J* 8 Hz), 7.67 (d, 3H, ArH, *J* 8 Hz), 7.57 (d, 2H, ArH, *J* 8.4 Hz), 7.25 (d, 1H, ArH, *J* 8.4 Hz), 7.02 (d, 1H, ArH, *J* 8.8 Hz), 6.94 (d, 2H, ArH, *J* 8.8 Hz), 5.14 (s, 2H, OCH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.12, 161.66, 141.67, 131.64, 127.52, 123.73, 118.51, 116.61, 114.30, 111.94, 76.94, 68.85, 60.71, 14.31. Anal. Calcd. $C_{22}H_{15}N_3O_2$ for: C, 74.78; H, 4.28; N, 11.89; Found: C, 74.21; H, 4.24; N, 11.81. LCMS [M+1]: Calcd. for $C_{22}H_{15}N_3O_2$: 353.12, Found: 354.06.

*4-{[4-(5-(4-Nitrophenyl)-1,3,4-oxadiazol-2-yl)phenoxy] methyl}benzonitrile (**7d**):* Prepared from **5** and **6d**. White solid; yield: 72%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.08 (d, 1H, ArH, *J* 8.8 Hz), 7.96 (d, 1H, ArH, *J* 8.8 Hz), 7.78 (d, 1H, ArH, *J* 8 Hz), 7.67 (d, 3H, ArH, *J* 8 Hz), 7.57 (d, 2H, ArH, *J* 8.4 Hz), 7.27 (d, 1H, ArH, *J* 8.4 Hz), 7.05 (d, 1H, ArH, *J* 8.8 Hz), 6.98 (d, 2H, ArH, *J* 8.8 Hz), 5.18 (s, 2H, OCH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 166.10, 161.63, 141.65, 131.54, 127.42, 123.71, 118.50, 116.59, 114.29, 111.92, 76.92, 68.83, 60.69, 14.29. Anal. Calcd. $C_{22}H_{14}N_4O_4$ for: C, 66.33; H, 3.54; N, 14.06; Found: C, 66.23; H, 3.14; N, 14.01. LCMS [M+1]: Calcd. for $C_{22}H_{14}N_4O_4$: 398.10, Found: 399.03.

*4-{[4-(5-(3,4-Dimethoxyphenyl)-1,3,4-oxadiazol-2-yl) phenoxy]methyl}benzonitrile **(7e)**:* Prepared from **5** and **6d**. White solid; yield: 70%; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.07 (d, 1H, ArH, *J* 8.8 Hz), 7.99 (d, 1H, ArH, *J* 8.8 Hz), 7.79 (d, 1H, ArH, *J* 8 Hz), 7.69 (d, 3H, ArH, *J* 8 Hz), 7.55 (d, 2H, ArH, *J* 8.4 Hz), 7.29 (d, 1H, ArH, *J* 8.4 Hz), 7.07 (d, 1H, ArH, *J* 8.8 Hz), 6.95 (d, 1H, ArH, *J* 8.8 Hz), 5.16 (s, 2H, OCH_2), 3.95 (s, 6H, CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 163.93, 160.70, 149.41, 141.61, 132.47, 131.63, 129.67, 128.73, 127.56, 126.45, 120.27, 118.47, 116.62, 115.27, 114.32, 111.13, 109.52, 69.03, 68.91, 60.71, 56.16, 56.02, 14.31. Anal. Calcd. $C_{24}H_{19}N_3O_4$ for: C, 69.72; H, 4.63; N, 10.16; Found: C, 69.56; H, 4.27; N, 10.11. LCMS [M+1]: Calcd. for $C_{24}H_{19}N_3O_4$: 413.14, Found: 414.12.

Biological assay:

The synthesized compounds **7a-e** were investigated for their antibacterial property. The property was analyzed by checking the formation of inhibition zone, the obtained results are tabulated and represented graphically in Table 1 and Fig. 1 respectively.

Table 1. Zone of inhibition (mm)

	7a	**7b**	**7c**	**7d**	**7e**	Positive control (Gentamicin sulphate)
Escherichia coli	–	13	–	–	–	24
Staphylococcus aureus	12	14	–	–	–	24
Salmonela typhi	12	–	14	18	19	24
Bacillus cereus	22	18	15	18	19	24

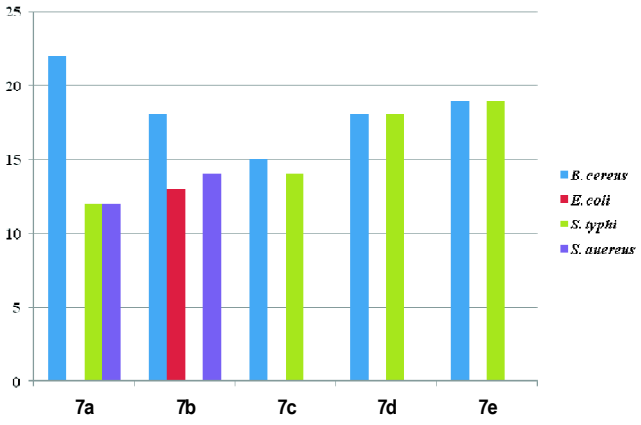


Fig. 1. Graphical representation of zone of inhibition exhibited by **7a-e**.

The appearance of inhibition zone shows the antibacterial activity of the synthesized compounds. However the exact mechanism of the inhibition caused by the compounds is not known well, probably it might be due to the interaction of the synthesized compound with DNA gyrase, which is involved in the DNA replication and transcription mechanism, which disrupts the ATPase activity of replication process by binding to 24 kDa C-terminal subdomain part of N-terminal B subunit of type II topoisomerase[27].

The results indicate good inhibitory activity against both Gram-negative bacterial strains by compound **7a**, this might be attributed to the presence of fluorine atom, as halogens are known to exhibit good inhibitory activity. Compound **7b** exhibited inhibitory activity against both Gram-positive bacterial strains and even one of the Gram-negative bacterial strains, probably due to the presence of chlorine atom. Both the compounds **7c** and **7d** exhibited inhibitory activity against both Gram-negative bacterial strains. It is evident from the results that, halogens do contribute to the inhibition activity.

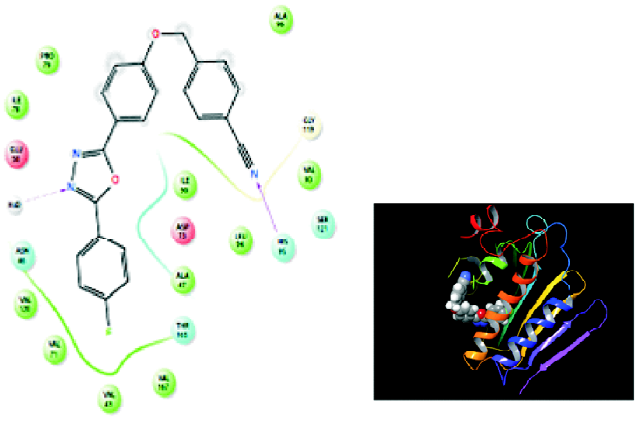
Molecular docking studies:

The molecular docking studies was carried out to corre-

late the antibacterial activity of the title compounds. Molecular docking was performed targeting the DNA gyrase (PDB ID: 1 KZN) with the synthesized ligands (**7a-e**) in order to determine the possible binding interactions of highly potent molecules. Most of the compounds showed good docking scores and potent interactions with different amino acid residues and the results are tabulated in Table 2. Among the series of compounds, those possessing electron withdrawing group, especially F (**7a**) gave the highest docking scores. The binding interactions of the synthesized derivatives are displayed in Fig. 2. Compound **7a** showed hydrogen bond interactions with HIS95. Compound **7b** showed π-π interactions with Arg136, Arg76.

Table 2. Molecular docking studies with 1 KZN

Entry	Docking score	H-bond interactions with amino acid residues	π-π stacking interactions
7a	–4.283	HIS95	NF
7b	–2.753	NF	Arg136, Arg76
7c	–3.726	NF	NF
7d	–3.417	Arg136	NF
7e	–3.648	Arg136	NF



A B

A and B are molecular interactions of **7a** with 1 KZN

Conclusion

We have reported the synthesis of new series of 1,3,4-oxadiazole derivatives by the reaction between respective acid hydrazides with various aldehydes using CAT. The structures of the synthesized compounds were confirmed by spectroscopy techniques. The synthesized compounds were subjected for antibacterial assay by well diffusion method. The synthesized compound **7a** exhibited comparable result with the standard drug gentamicin sulphate. Further molecular docking studies were carried out to study the interaction of our compound with bacterial protein and we found that, *in vitro* antibacterial assay is in agreement with the *in silico* studies.

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