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Synthesis and antimicrobial studies of a new series of 2-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}-5-substituted-1,3,4-oxadiazoles

S.L. Gaonkar *, K.M.L. Rai *, B. Prabhuswamy

Department of Studies in Chemistry, University of Mysore, Manasagangotri, Mysore 570 006, India

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Abstract

A series of novel 2-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}-5-substituted-1,3,4-oxadiazoles were synthesized by the oxidative cyclisation of hydrazones derived from 4-[2-(5-ethylpyridin-2-yl)ethoxy]benzaldehyde and aroylhydrazines using chloramine-T as oxidant. IR, NMR and elemental analysis characterized the newly synthesized compounds. The synthesized compounds were evaluated for their antimicrobial activity and were compared with standard drugs. The compounds demonstrated potent to weak antimicrobial activity. Out of the compounds studied, compounds **8c** and **8d** showed significant inhibition. Compounds **8b**, **8f**, **8k** and **8e** showed moderate activity. The minimum inhibitory concentration of the compounds was in the range of 8–26 μg ml^{-1} against bacteria and 8–24 μg ml^{-1} against fungi. The title compounds represent a novel class of potent antimicrobial agents.

Keywords: Synthesis; 1,3,4-oxadiazoles; Antimicrobial activity; Chloramine-T

1. Introduction

1,3,4-Oxadiazoles are an important class of heterocyclic compounds with broad spectrum of biological activities. Substituted 1,3,4-oxadiazoles have revealed antibacterial [1–3], antimycobacterial [4], antifungal [5,6], anti-inflammatory [7,8], analgesic [9], anticonvulsant [10,11], antihypoglycemic [12], and insecticidal properties [13]. Compounds possessing oxadiazole moiety show anticancer [14] and tyrosinase inhibitory activity [15]. Oxadiazoles find use as fluorescent whiteners [16] and also act as muscle relaxants [17].

Pioglitazone is a well known pharmaceutically active compound used as insulin sensitizing agent in the treatment of diabetes [18]. 4-[2-(5-ethylpyridin-2-yl)ethoxy]benzaldehyde is a main active metabolite, which is one of the key intermediate of pioglitazone. The biological activity of pyridine derivatives is well established [19–21]. This broad spectrum of biological activity of 1,3,4-oxadiazoles, and pyridine derivatives prompted us to synthesize and evaluate antimicrobial activity of novel 2-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}-5-substituted-1,3,4-oxadiazoles. In our laboratory Rai et al. [22] reported the use of chloramine-T as an efficient reagent for the synthesis of 2-amino-5-substituted-1,3,4-oxadiazoles with good yield and purity by the oxidative cyclization of semicarbazones derived from aromatic aldehydes. We have synthesized 2,5-substituted-1,3,4-oxadiazoles using chloramine-T by the oxidative cyclization of hydrazones derived from aromatic aldehyde and aroylhydrazines.

In this paper, we describe the synthesis and antimicrobial activity of a series of novel 2-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}-5-substituted-1,3,4-oxadiazole derivatives bearing 6-membered pyridine and 5-membered oxadiazole moiety.

2. Chemistry

Aromatic acid **1** was esterified with ethanol using sulfuric acid as catalyst and the resulting ester **2** was refluxed with hy-

* Corresponding author. Tel.: +91 821 251 5110; fax: +91 821 242 1263.
E-mail addresses: gaonkarslg@rediffmail.com (S.L. Gaonkar), kmlrai@yahoo.com (K.M.L. Rai).

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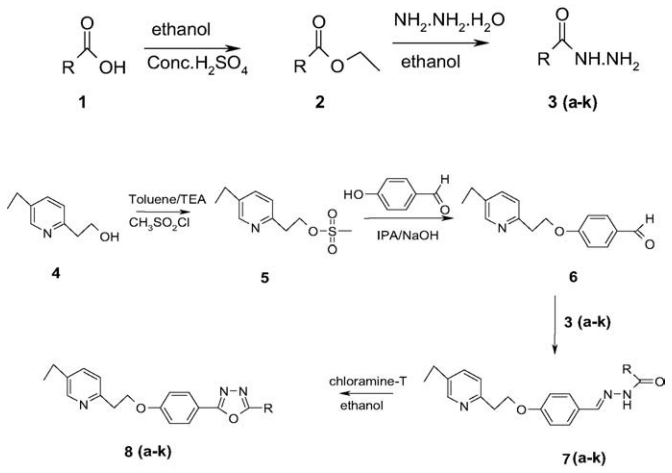

Scheme 1. Where R = (a) phenyl, (b) 4-chlorophenyl, (c) 2,3-dichlorophenyl, (d) 2,4-dichlorophenyl, (e) 4-methoxyphenyl, (f) 4-nitrophenyl, (g) 2-nitrophenyl, (h) 4-tolyl, (i) 2-tolyl, (j) 3-pyridinyl, (k) 4-pyridinyl.

drazine hydrate in ethanol to give aroylhydrazine **3(a–k).** Aroylhydrazine **3** was condensed with 4-[2-(5-ethylpyridyl) ethoxy]benzaldehyde **6** to gave aroyl hydrazone **7(a–k)** which was oxidatively cyclized in presence of oxidant chloramine-T to gave 2-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}-5-substituted-1,3,4-oxadiazoles derivatives **8(a–k)** in good quality and yield. The aldehyde **6** was synthesized by treating 2-(5-ethylpyridin-2-yl)ethanol **4** with triethylamine and methanesulfonyl chloride to get an ester **5** which was refluxed with 4-hydroxybenzaldehyde and NaOH flakes to get 4-[2-(5-ethyl-pyridin-2yl)ethoxy]benzaldehyde **6** as a thick oil. The synthesis and X-ray crystallographic studies of **4** have been reported [23] (Scheme 1).

3. Results and discussion

3.1. Chemistry

Chloramine-T was used as an efficient reagent for the oxidative cyclization of aroyl hydrazone **7(a–k)** to gave 1,3,4-oxadiazoles **8(a–k)** in good purity and yield. IR, 1H NMR and elemental analyses characterized all the synthesized 1,3,4-oxadiazoles. The IR spectrum of oxadiazoles **8(a–k)** showed the absence of amide carbonyl frequency in the region 1760–1650 cm^{-1} and the NH frequency in the region 3400–3200 cm^{-1} and showed a new peak at 1630–1640 cm^{-1} due to C=N frequency. ^1H NMR showed peaks due to aromatic protons and other substituents in the expected region.

3.2. Antimicrobial activity

All synthesized oxadiazole derivatives were evaluated for antimicrobial activity by disc diffusion and microdilution method against the various strains. Streptomycin and tetracycline were used as standard drugs against bacteria and nystatin was used against fungi. In all the determinations tests were performed in triplicate and the results were taken as a mean of at least three determinations. Among the series of oxadiazoles synthesized, compounds **8c**, **8d** shown significant inhibition. Compounds **8b**, **8f**, **8k** and **8e** shown moderate activity. The significant inhibition shown by **8c** and **8d** may be due to the presence of two chloro groups present on benzene ring at tached to C_5 of oxadiazole moiety. The moderate activity of **8b, 8f** and **8e** might be due to chloro group, nitro group and methoxy group respectively at para position of benzene ring. The moderate activity of **8k** may be due to 4-pyridinyl ring attached C_5 of oxadiazole moiety. Remaining compounds were not active against any of the strains tested. (Tables 1–4).

4. Conclusion

In conclusion 2-{4-[2-(5-ethylpyridin-2-yl)ethoxy]phenyl}-5-substituted-1,3,4-oxadiazoles derivatives were synthesized and their antimicrobial activities have been evaluated. Compounds **8c** and **8d** demonstrated potent inhibition against all the strains tested. Further research in this area is under progress in our laboratory.

Table 1
Minimal inhibitory concentration (MIC) in μg ml^{-1} of synthesized compounds against tested bacterial strains by microdilution method

Compound	Minimal inhibitory concentration (MIC) in μg ml^{-1}				
	Bacillus substilis	*Escherichia coli*	*Pseudomonas fluorescens*	*Xanthomonas campestris pvs.*	*Xanthomonas oryzae*
8a	26	28	25	28	23
8b	18	14	12	09	12
8c	14	11	10	08	11
8d	15	12	11	09	11
8e	20	15	14	11	15
8f	19	14	13	10	13
8g	20	18	16	15	18
8h	26	24	20	23	28
8i	25	24	21	22	27
8j	24	19	19	16	21
8k	20	14	14	10	15
Streptomycin	19	13	12	–	–
Tetracycline	–	–	–	09	13

Table 2
Minimal inhibitory concentration (MIC) in μg ml^{-1} of synthesized compounds against tested fungal strains by microdilution method

Compound	Minimal inhibitory concentration (MIC) in μg ml^{-1}					
	Aspergillus niger	*Aspergillus flavus*	*Fusarium oxysporum*	*Trichoderma* species	*Fusarium monaliforme*	*Penicillum* species
8a	21	23	20	21	19	22
8b	16	15	11	12	10	11
8c	12	12	08	11	08	08
8d	13	12	10	11	08	09
8e	17	16	12	14	10	12
8f	16	14	12	12	10	11
8g	16	17	13	12	10	12
8h	22	23	19	21	22	24
8i	23	22	21	24	19	22
8j	18	19	16	16	13	15
8k	16	17	11	13	10	11
Nystatin	15	15	11	12	9	10

Table 3
Inhibitory zone (diameter) mm of synthesized compounds against tested bacterial strains by disk diffusion method

Compound	*Bacillus substilis*	*Escherichia coli*	*Pseudomonas fluorescens*	*Xanthomonas campestris pvs.*	*Xanthomonas oryzae*
8a	06 mm	06 mm	07 mm	05 mm	05 mm
8b	14 mm	13 mm	16 mm	13 mm	11 mm
8c	17 mm	18 mm	21 mm	16 mm	15 mm
8d	16 mm	17 mm	19 mm	15 mm	14 mm
8e	10 mm	12 mm	14 mm	10 mm	09 mm
8f	12 mm	13 mm	15 mm	10 mm	11 mm
8g	07 mm	08 mm	07 mm	06 mm	07 mm
8h	05 mm	06 mm	07 mm	05 mm	04 mm
8i	04 mm	04 mm	06 mm	05 mm	06 mm
8j	09 mm	09 mm	11 mm	07 mm	06 mm
8k	12 mm	12 mm	16 mm	11 mm	10 mm
Streptomycin	13 mm	14 mm	17 mm	–	–
Tetracycline	–	–	–	12 mm	12 mm

Streptomycin sulfate (25 μg per disc); Tetracycline (25 μg per disc) were used as positive reference standard antibiotic discs, Synthesized compounds (25 μg per disc).

Table 4
Inhibitory zone (diameter) mm of synthesized compounds against tested fungal strains by disk diffusion method

Compound	*Aspergillus niger*	*Aspergillus flavus*	*Fusarium oxysporum*	*Trichoderma species*	*Fusarium monaliforme*	*Penicillum species*
8a	04 mm	05 mm	05 mm	04 mm	03 mm	02 mm
8b	07 mm	09 mm	12 mm	15 mm	10 mm	09 mm
8c	12 mm	13 mm	17 mm	21 mm	14 mm	13 mm
8d	11 mm	12mm	15 mm	19 mm	12 mm	11 mm
8e	07 mm	08 mm	12 mm	13 mm	10 mm	08 mm
8f	07 mm	09 mm	13 mm	14 mm	10 mm	08 mm
8g	05 mm	06 mm	10 mm	10 mm	08 mm	06mm
8h	04 mm	06 mm	09 mm	10 mm	08 mm	06 mm
8i	03 mm	05 mm	08 mm	09 mm	07mm	05 mm
8j	05 mm	07 mm	10 mm	10 mm	08 mm	06 mm
8k	07 mm	09 mm	12 mm	14 mm	10 mm	08 mm
Nystatin	08	10	14	16	12	10

Nystatin (25 μg per disc) was used as positive reference standard antifungal disc, Synthesized compounds (25 μg per disc).

5. Experimental section

5.1. Chemistry

Melting points were determined on Thomas Hoover melting point apparatus and are uncorrected. ^{1}H NMR (300 MHz) spectra were measured on a Bruker AM FT spectrometer using $CDCl_3$ using tetramethylsilane (TMS) as internal standard. The chemical shifts are expressed in δ and following abbreviations were used. s = singlet, d = doublet, t = triplet and m = multiplet. Infrared (IR) spectra were measured on Shimadzu 8300 spectrometer. Elemental analyses were determined on Vario-EL instrument. Thin layer chromatography (TLC) was done on precoated silica gel G plates using chloroform-acetone as eluent.

5.1.1. General procedure for the synthesis of aroyl hydrazines *3(a–k)*

A solution of respective aromatic acid **1** (1 g), ethanol (10 ml), and catalytic amount of conc. H_2SO_4 were refluxed for 3 h. The reaction mixture was cooled and the solid formed was filtered to get ester **2,** which was refluxed with 98% hydrazine hydrate (2 ml) in ethanol (10 ml) for 2 h. After com-

pletion of the reaction by TLC (toluene/ethyl acetate/DEA, 7.5:2.5:1) the reaction mixture was cooled and the solid formed was filtered and washed with chilled ethanol (1 ml) to get corresponding aroyl hydrazines **3(a–k)**.

*5.1.2. Procedure for the synthesis of 4-[2-(5-ethylpyridyl)-ethoxy]benzaldehyde **6***

A solution of 2-(5-ethyl-pyridin-2-yl)ethanol **4** (15.1 g, 0.1 mol) in toluene (100 ml) and triethylamine (20.2 g, 0.2 mol) were treated with methanesulfonyl chloride (11.4 g, 0.1 mol) at 0–5 °C. The reaction mixture was stirred at r.t. for 1 h. After completion of the reaction the precipitate was filtered off and the filtrate was washed first with water (50 ml) then 5% sodium bicarbonate (50 ml) and finally with water (50 ml) and dried (anhy. Na_2SO_4). It was then concentrated under reduced pressure to give **5** as oil, which was refluxed with 4-hydroxybenzaldehyde (12.2g, 0.1 mol) and NaOH flakes (5.0 g, 0.12 mol) in isopropyl alcohol (300 ml) for 6 h. After completion of the reaction the solvent was removed under vacuum. The residue was dissolved in ethyl acetate (300 ml), washed with 5% aq. NaOH (100 ml), water (100 ml) and dried (anhy. Na_2SO_4). The solvent was removed under reduced pressure to gave oily residue which was purified by column chromatography using ethyl acetate-*n*-hexane (3:7) as an eluent to gave 4-[2-(5-ethylpyridyl)-ethoxy]benzaldehyde **6** as yellow oil (18 g, 71%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.29 (t, J = 7.6 Hz, 3H), 2.68 (q, J = 7.6 Hz, 2H), 3.29 (t, J = 6.3 Hz, 2H), 4.40 (t, J = 7.0 Hz, 2H), 7.0 (d, J = 8.8 Hz, 2H), 7.21 (d, J = 8.2 Hz, 1H), 7.50–7.75 (m, 3H), 8.39 (s, 1H), 9.85 (s, 1H). IR (KBr pellets cm^{-1}) ν 3060, 2950, 2835, 1732, 1635, 1430, 1204. Anal. CHN: calcd C 75.27, H 6.71, N 5.49, found C 75.19, H 6.79, N 5.46.

*5.1.3. General procedure for the synthesis of aroyl hydrazones **7(I–XI)***

An equimolar mixture of aroyl hydrazine **3(a–k)** and 4-[2-(5-ethylpyridyl)ethoxy]benzaldehyde **6** were refluxed in ethanol (10 vol.) for 3 h. The progress of the reaction was monitored by TLC (toluene/ethyl acetate/DEA, 7.5:2.5:1). After completion of the reaction, the mass was cooled and the solid formed was filtered to give aroyl hydrazones **7(a–k)**, which were used directly for next reaction.

*5.1.3.1. Synthesis of 5-ethyl-2-{2-[4-(5-phenyl [1,3,4]oxadiazol-2-yl)phenoxy]ethyl} pyridine **8a***. **Typical procedure**. A mixture of aroyl hydrazone **7a** (1.0 g, 2.68 mmol) and chloramine-T.3H_2O (0.91 g, 3.2 mmol) in ethanol (10 ml) were refluxed under stirring for 3 h. The reaction mass was then concentrated under reduced pressure and the residue was extracted into diethyl ether (2 × 10 ml), washed with 10% NaOH solution (10 ml), water (10 ml), finally with brine solutions and dried (anhy. Na_2SO_4). Ether was evaporated and the resulting residue was stirred in *n*-hexane. The solid formed was filtered and recrystalised from ethanol to gave **8a** as a white crystalline solid (0.82 g, 81%), m.p. 139–141 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.27 (t, J = 7.6 Hz, 3H), 2.65 (q, J = 7.6 Hz, 2H), 3.28 (t, J = 6.3 Hz, 2H), 4.36 (t, J = 7.0 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 7.20–7.60 (m, 9H), 8.41 (s, 1H). IR (KBr pellets cm^{-1}) ν 3070, 2955, 2835, 1635, 1460, 1204. Anal. CHN: calcd C 74.37, H 5.70, N 11.31, found C 74.46, H 5.76, N 11.27.

The same procedure was used in all cases.

*5.1.3.2. Synthesis of 2-(2-{4-[5-(4-chlorophenyl) [1,3,4]oxadiazol-2-yl]phenoxy}ethyl)-5-ethylpyridine **8b***. Obtained from **7b** (1.0 g, 2.46 mmol) and chloramine-T.3H_2O (0.83 g, 2.95 mmol) as a white crystalline solid (0.78 g, 79%), m.p. 165–167 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.27 (t, J = 7.6 Hz, 3H), 2.65 (q, J = 7.6 Hz, 2H), 3.28 (t, J = 6.3 Hz, 2H), 4.36 (t, J = 7.0 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 7.20–7.65 (m, 8H), 8.44 (s, 1H). IR (KBr pellets cm^{-1}) ν 3070, 2952, 2835, 1632, 1584, 1478, 1446, 1210. Anal. CHN: calcd C 68.06, H 4.97, N 10.35, found C 68.01, H 5.02, N 10.31.

*5.1.3.3. Synthesis of 2-(2-{4-[5-(2,3-dichlorophenyl) [1,3,4]oxadiazol-2-yl]-phenoxy} ethyl)-5-ethylpyridine **8c***. Obtained from **7c** (1.0 g, 2.27 mmol) and chloramine-T.3H_2O (0.76 g, 2.72 mmol) as a white crystalline solid (0.77 g, 78%), m.p. 172–175 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.27 (t, J = 7.6 Hz, 3H), 2.65 (q, J = 7.6 Hz, 2H), 3.28 (t, J = 6.3 Hz, 2H), 4.36 (t, J = 7.0 Hz, 2H), 6.93 (d, J = 8.8 Hz, 2H), 7.15–7.60 (m, 7H), 8.41 (s, 1H). IR (KBr pellets cm^{-1}) ν 3072, 2954, 2835, 1630, 1590, 1478, 1446, 1215. Anal. CHN: calcd C 62.74, H 4.35, N 9.54, found C 62.79, H 4.40, N 9.49.

*5.1.3.4. Synthesis of 2-(2-{4-[5-(2,4-dichlorophenyl) [1,3,4]oxadiazol-2-yl]phenoxy} ethyl)-5-ethylpyridine **8d***. Obtained from **7d** (1.0 g, 2.27 mmol) and chloramine-T.3H_2O (0.76 g, 2.72 mmol) as a white crystalline solid (0.78 g, 80%), m.p. 178–180 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.27 (t, J = 7.6 Hz, 3H), 2.65 (q, J = 7.6 Hz, 2H), 3.28 (t, J = 6.3 Hz, 2H), 4.36 (t, J = 7.0 Hz, 2H), 6.95 (d, J = 8.8 Hz, 2H), 7.20–7.60 (m, 7H), 8.44 (s, 1H). IR (KBr pellets cm^{-1}) ν 3070, 2956, 2835, 1632, 1582, 1476, 1444, 1215. Anal. CHN: calcd C 62.74, H 4.35, N 9.54, found C 62.71, H 4.42, N 9.56.

*5.1.3.5. Synthesis of 5-ethyl-2-(2-{4-[5-(4-methoxyphenyl) [1,3,4]oxadiazol-2-yl]phenoxy} ethyl)pyridine **8e***. Obtained from **7e** (1.0 g, 2.48 mmol) and chloramine-T.3H_2O (0.84 g, 2.97 mmol) as a yellow oil (0.82 g, 83%). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.29 (t, J = 7.6 Hz, 3H), 2.68 (q, J = 7.6 Hz, 2H), 3.31 (t, J = 6.3 Hz, 2H), 3.79 (s, 3H), 4.29 (t, J = 7.0 Hz, 2H), 6.90–6.95 (m, 4H), 7.20–7.60 (m, 6H), 8.46 (s, 1H). IR (KBr pellets cm^{-1}) ν 3064, 2952, 2833, 1636, 1442, 1247. Anal. CHN: calcd C 71.80, H 5.77, N 10.47, found C 71.74, H 5.79, N 10.43.

*5.1.3.6. Synthesis of 5-ethyl-2-(2-{4-[5-(4-nitrophenyl) [1,3,4] oxadiazol-2-yl]phenoxy} ethyl)pyridine **8f***. Obtained from **7f** (1.0 g, 2.38 mmol) and chloramine-T.3H_2O (0.81 g, 2.87 mmol) as a white crystalline solid (0.80 g, 81%), m.p. 176–178 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.25 (t, J = 7.6 Hz, 3H), 2.68 (q, J = 7.6 Hz, 2H), 3.29 (t, J = 6.3 Hz,

2H), 4.32 (t, $J = 7.0$ Hz, 2H), 6.91 (d, $J = 8.8$ Hz, 2H), 7.20–7.70 (m, 6H), 8.22 (d, $J = 8.8$ Hz, 2H), 8.45 (s, 1H). IR (KBr pellets cm^{-1}) ν 3090, 2952, 2836, 1636, 1528, 1482, 1342, 1247. Anal. CHN: calcd C 66.34, H 4.84, N 13.45, found C 66.42, H 4.89, N 13.40.

5.1.3.7. Synthesis of 5-ethyl-2-(2-{4-[5-(2-nitrophenyl) [1,3,4] oxadiazol-2-yl]phenoxy} ethyl)pyridine ***8g***. Obtained from **7g** (1.0 g, 2.38 mmol) and chloramine-T.$3H_2O$ (0.81 g, 2.87 mmol) as a white crystalline solid (0.79 g, 80%), m.p. 181–184 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.24 (t, $J = 7.6$ Hz, 3H), 2.62 (q, $J = 7.6$ Hz, 2H), 3.31 (t, $J = 6.3$ Hz, 2H), 4.30 (t, $J = 7.0$ Hz, 2H), 6.93 (d, $J = 8.8$ Hz, 2H), 7.22 (d, $J = 8.2$ Hz, 1H), 7.35 (d, $J = 8.8$ Hz, 2H), 7.50–7.75 (m, 4H), 8.22 (d, $J = 8.6$ Hz, 1H), 8.48 (s, 1H). IR (KBr pellets cm^{-1}) ν 3092, 2954, 2834, 1636, 1528, 1482, 1345, 1242. Anal. CHN: calcd C 66.34, H 4.84, N 13.45, found C 66.40, H 4.90, N 13.41.

5.1.3.8. Synthesis of 5-ethyl-2-{2-[4-(5-p-tolyl [1,3,4]oxadiazol-2-yl)phenoxy]ethyl} pyridine ***8h***. Obtained from **7h** (1.0 g, 2.57 mmol) and chloramine-T.$3H_2O$ (0.87 g, 3.10 mmol) as a white crystalline solid (0.77 g, 78%), m.p. 144–147 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.28 (t, $J = 7.6$ Hz, 3H), 2.29 (s, 3H), 2.62 (q, $J = 7.6$ Hz, 2H), 3.32 (t, $J = 6.3$ Hz, 2H), 4.29 (t, $J = 7.0$ Hz, 2H), 6.94 (d, $J = 8.8$ Hz, 2H), 7.15 (d, $J = 8.8$ Hz, 2H), 7.21 (d, $J = 8.2$ Hz, 1H), 7.4–7.65 (m, 5H), 8.48 (s, 1H). IR (KBr pellets cm^{-1}) ν 3072, 2930, 2834, 1636, 1605, 1486, 1242. Anal. CHN: calcd C 74.78, H 6.01, N 10.90, found C 74.82, H 6.09, N 10.81.

5.1.3.9. Synthesis of 5-ethyl-2-{2-[4-(5-o-tolyl [1,3,4]oxadiazol-2-yl)phenoxy]ethyl} pyridine 8i. Obtained from **7i** (1.0 g, 2.57 mmol) and chloramine-T.$3H_2O$ (0.87 g, 3.10 mmol) as a white crystalline solid (0.76 g, 77%), m.p. 138–140 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ 1.26 (t, $J = 7.6$ Hz, 3H), 2.31 (s, 3H), 2.61 (q, $J = 7.6$ Hz, 2H), 3.32 (t, $J = 6.3$ Hz, 2H), 4.29 (t, $J = 7.0$ Hz, 2H), 6.91 (d, $J = 8.8$ Hz, 2H), 7.1–7.4 (m, 7H), 7.6 (d, $J = 7.6$ Hz, 1H), 8.46 (s, 1H). IR (KBr pellets cm^{-1}) ν 3078, 2935, 2832, 1634, 1605, 1484, 1245. Anal. CHN: calcd C 74.78, H 6.01, N 10.90, found C 74.84, H 6.05, N 10.85.

5.1.3.10. Synthesis of 5-ethyl-2-{2-[4-(5-pyridin-3-yl [1,3,4]oxadiazol-2-yl)phenoxy] ethyl}pyridine ***8j***. Obtained from **7j** (1.0 g, 2.67 mmol) and chloramine-T.$3H_2O$ (0.90 g, 3.20 mmol) as a yellow oil (0.77 g, 79%). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.25 (t, $J = 7.6$ Hz, 3H), 2.61 (q, $J = 7.6$ Hz, 2H), 3.29 (t, $J = 6.3$ Hz, 2H), 4.26 (t, $J = 7.0$ Hz, 2H), 6.95 (d, $J = 8.8$ Hz, 2H), 7.22 (d, $J = 8.2$ Hz, 1H), 7.4–7.65 (m, 4H), 7.95 (d, $J = 7.6$ Hz, 1H), 8.62–8.88 (m, 3H). IR (KBr pellets cm^{-1}) ν 3082, 3030, 2945, 2835, 1636, 1482, 1212. Anal. CHN: calcd C 70.95, H 5.41, N 15.04, found C 70.89, H 5.49, N 15.11.

5.1.3.11. Synthesis of 5-ethyl-2-{2-[4-(5-pyridin-4-yl [1,3,4]oxadiazol-2-yl)phenoxy] ethyl}pyridine ***8k***. Obtained from **7k** (1.0 g, 2.67 mmol) and chloramine-T.$3H_2O$ (0.90 g, 3.20 mmol) as a yellow oil (0.78 g, 80%). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.28 (t, $J = 7.6$ Hz, 3H), 2.63 (q, $J = 7.6$ Hz, 2H), 3.28 (t, $J = 6.3$ Hz, 2H), 4.28 (t, $J = 7.0$ Hz, 2H), 6.94 (d, $J = 8.8$ Hz, 2H), 7.21 (d, $J = 8.2$ Hz, 1H), 7.41 (d, $J = 8.8$ Hz, 2H), 7.62–7.68 (m, 3H), 8.52–8.58 (m, 3H). IR (KBr pellets cm^{-1}) ν 3084, 3030, 2948, 2835, 1635, 1480, 1210.Anal. CHN: calcd C 70.95, H 5.41, N 15.04, found C 71.02, H 5.46, N 14.99.

5.2. Biology

5.2.1. Materials and methods

Five bacteria and six fungal species were used as the antimicrobial test strains namely: *Bacillus substilis, Escherichia coli, Pseudomonas fluorescens, Xanthomonas campestris pvs., Xanthomonas oryzae, Aspergillus niger, Aspergillus flavus, Fusarium oxysporum, Trichoderma* species, *Fusarium monaliforme* and *Penicillum* species. The bacterial strains were maintained on the LB agar medium and the filamentous fungi were maintained on Potato dextrose agar (PDA) medium at 28 °C. The agar disk diffusion method [24] was used to test antimicrobial activity using potato dextrose agar medium.

The bacteria inoculum was prepared by suspending in 9 ml of sterile water for colonies from 24 hours culture on LB agar medium. For the filamentous fungi, the inoculum was prepared with the spores derived from 5 to 15 days culture on PDA medium. The mycelia were covered with 10 ml of distilled water and the conidia were scraped using sterile pipette. The spores were recovered after filtration on sterile absorbent cotton and were resuspended in sterile distilled water.

The cell density of each inoculum was adjusted with hemocytometer in order to obtain a final concentration of approximately 10^4 and 10^6 CFU ml^{-1} for the bacteria and filamentous fungi respectively.

Nystatin (Himedia) was used as positive control for fungi and streptomycin and tetracycline for bacteria. Each disk contained 25 μg of standard drugs and 25 μg synthesized compounds. Plates were first kept at 4 °C for at least 2 hours to allow the diffusion of chemicals, and then incubated at 28 °C. Inhibition zones were measured after 24 hours of incubation for bacteria and after 48 hours of incubation for fungi.

The microdilution method [25] was used to evaluate the minimum inhibitory concentration (MIC) of all the synthesized compounds. The Nutrient liquid medium and Potato dextrose liquid medium were used as test media. Tests were performed in 96-well round bottom sterile culture plates. The suspensions of yeast and filamentous fungi were adjusted in sterile water to match the density of a 0.5 McFarland Standard. The wells of a microdilution plate were inoculated with 180 ml of the culture medium containing a final inoculum of 0.5–2.5 × 10^3 CFU ml^{-1}. All the compounds previously solubilised in DMSO were serially diluted two folds in the liquid medium to give a range of concentration from 640 to 0.1 μg ml^{-1}. Twenty microliter of each concentration were added to wells containing culture suspension except the growth control well. The final concentration

ranged from 64 to 0.01 μg ml^{-1}. Plates were incubated at 35 °C for 48 hours. Fungal growth was assessed at 494 nm by measuring the optical density in each well using an enzyme immunoassay multiwell reader (Sigma diagnostic).

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