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# Synthesis and characterization of bioactive isoxazole and 1,3,4-oxadiazole heterocycle containing scaffolds

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Herein we have disclosed the design, synthesis, and study of the biological property of isoxazole and 1,3,4-oxadiazole containing framework. Set of 2-(4-(4-nitrophenoxy)phenyl)-5-(4-(5-(4-(pentyloxy)phenyl)isoxazol-3-yl)phenyl)-1,3,4-oxadiazole derivatives have been constructed. Synthesized compounds have been screened for anti-fungal and anti-bacterial activities. Among them, Compound 8a have shown excellent anti-fungal activity against *C.albicans* fungi. Compound 8b and 8c have exhibited significant anti-bacterial activity with MIC 50 µg/mL against *S. pyogenus* and *S. aureus* pathogens, respectively.

**Keywords:** Isoxazole, 1,3,4-oxadiazole, Biheterocycles, Anti-fungal, Anti-bacterial

The isoxazole and 1,3,5-oxadiazole heterocycles are privileged frameworks embedded in various important pharmaceutical active compounds natural products and (Fig. 1). The wide range of pharmaceutical significance of these heterocycles attracted exploration protocols for designing and synthesizing a variety of heterocycle-containing isoxazole and 1,3,5-oxadiazole scaffolds and their applications. The isoxazole heterocycle containing organic framework is well explored for the pharmacological activity[1], and emerges as a crucial pharmacophore. They display a wide variety of applications in the therapeutic interest, like analgesic[2], anti-inflammatory[3], antioxidant[4], antimicrobial,[5] anticancer[6], CNS depressant[7], anti-tubercular[8] and other miscellaneous biological activities[9] like GABA (γ-aminobutyric acid) agonistic activity, inhibitory activity, and antihypertensive activity. Isoxazole derivatives as key intermediates provide the synthetic route for various complex natural products[10].

Besides this, the versatile biological activity of several azole heterocyclic motifs has fascinated researchers' significant attention for the design and exploration of their therapeutic importance. Among the class of azole, 1,3,4-oxadiazole emerge as an important isomer as it has a wide range of activity; such as platelet aggregation inhibitory[11], HIV integrase inhibitory[12], tyrosinase inhibitory[13], anti-inflammatory[14], anticancer[15], antimalarial[16], angiogenesis inhibitor[17], anti-bacterial[18], anticonvulsant[19], hypoglycaemic[20], analgesic[21], antitubercular[22], vasodilatory[23], cytotoxic[24], hypolipidemic[25], antiedema[26], anti-allergic agent[27], anti-fungal[28], and antiemetic, diuretic, and dopamine transporters[29]. Alongside these, it has other applications in material science and agriculture like electroluminescent materials[30], organic multilayer light-emitting diode[31], plant growth hormones[32], insecticidal[33], herbicidal[34].

Considering the vast biological importance of both 5 membered heterocyclic motifs, we have synthesized and characterized series of new organic frameworks by combining both isoxazole and 1,3,4-oxadiazole in one molecule. Further, all synthesized compounds were subjected to anti-bacterial and anti-fungal activities.

## Experimental Details

### Materials and methods

Intermediated B benzhydrezide derivative was prepared in the lab as per the literature report[34]. Required reagents, solvents, and catalysts were procured from various chemical vendors Spectrochem, Avra chemicals, and S.D. fine chem., otherwise stated. Anhydrous solvent, when required were prepared according to standard drying methods.

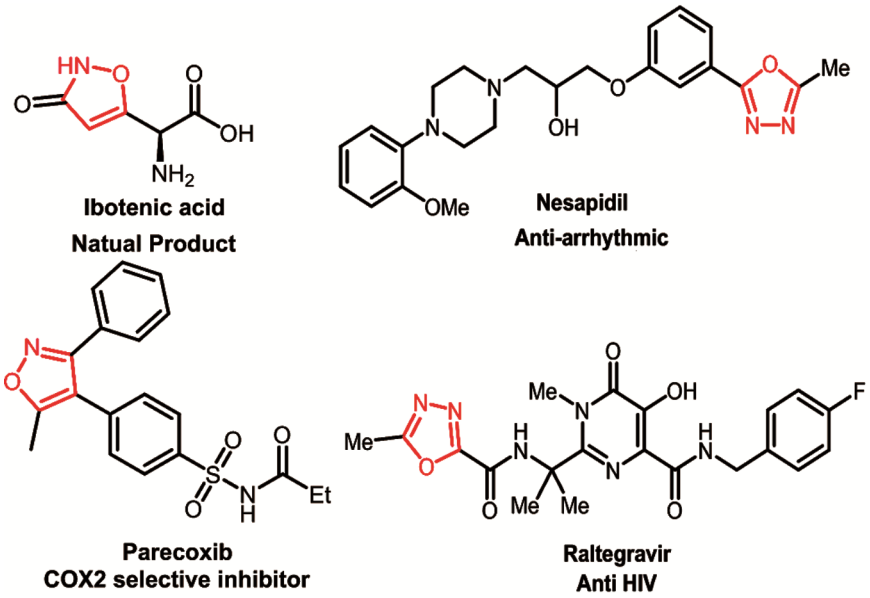



Fig. 1 — Marketed drugs and Natural products containing isoxazole and 1,3,5-oxadiazole heterocycles

The melting point was recorded in the open capillary in Büchi Melting point B-545. $^{1}$H and $^{13}$C NMR spectra were recorded at 400 MHz and 101 MHz, respectively, on bruker AV400 Avance using $CDCl_3$ purchased from Merck as internal standard ($CDCl_3$ at 7.27 ppm for $^{1}$H and 77.00 ppm for $^{13}$C) with reference to TMS. Chemical shifts (δ) are given in ppm. IR analysis was carried out on Perkin- Elmer and Shimadzu FTIR. Mass analysis was carried out in Shimadzu LCMS, showing the molecular ion peak. The activity was carried out at Microcare laboratory, Surat. The strains were procured from the Institute of Microbial Technology, Chandigarh.

### Synthesis procedure

#### *(E)-N'-(4-phenoxybenzylidene)-4-(5-(4-(pentyloxy)phenyl) isoxazol-3-yl)benzohydrazide derivatives (7a-7h)*

A mixture of 5 (1.28 gm, 0.0035 mol, 1 eq.) and aldehyde 6(a-h) (0.0039 mol, 1.1 eq.) in the presence of catalytic acetic acid(10%) was refluxed in 40 ml of ethanol for 7 hr. After completion of the reaction, the mixture was cooled, precipitates were collected and recrystallized by ethanol to give pure (E)-N'-(4-phenoxybenzylidene)-4-(5-(4-(pentyloxy)phenyl) isoxazol-3-yl)benzohydrazide derivatives (7a-7h) in high yield (90%).[36]

#### *2-(4-(4-nitrophenoxy)phenyl)-5-(4-(5-(4-(pentyloxy)phenyl) isoxazol-3-yl)phenyl)-1,3,4-oxadiazole (8a)*

To the stirred solution of 7a-h (1.7 gm, 0.0026 mol, 1 eq.) in DMSO (10 mL) cesium carbonate (1.71 g, 0.0052 mol, 2 eq.) and molecular iodine (1 gm, 0.0039 mol, 1.5 eq.) were added sequence and the mixture was stirred at 80° C for 60 min. The conversion was monitored by the TLC technique. After completion of the reaction, it was allowed to cool to room temperature, and then the content was poured into ice-cold water followed by treatment of 5% aqueous sodium thiosulphate (20 mL). The obtained white precipitate was filtered under a vacuum. The crude product was then treated with hot methanol (15 ml) for 15 to 20 min and cooled to room temperature. The resulting mixture was stirred for 30 min at room temperature and filtered under vacuum to afford the analytically pure product (1.5 gm) in good yield. All the characterization spectra are given in Supplementary Information.

### Analytical data

**2-(4-(4-nitrophenoxy)phenyl)-5-(4-(5-(4-(pentyloxy) phenyl)isoxazol-3-yl)phenyl)-1,3,4-oxadiazole (8a):** White solid; Overall yield: 46%;m.p. =201°C; IR (KBr) cm$^{-1}$: 3883, 3660, 3435, 3328, 3210, 3125, 3025, 2942, 1997, 1735, 1599, 1552, 1484, 1381, 1262, 1190, 1021, 836, 776, 684;$^{1}$H NMR (400 MHz, $CDCl_3$) δ:8.24 (d, $J$ = 8.5 Hz, 2H), 8.17 (d, $J$ = 8.9 Hz, 2H), 8.04 (d, $J$ = 8.5 Hz, 3H), 7.78 (d, $J$ = 8.8 Hz, 2H), 7.63 (ddd, $J$ = 8.3, 7.5, 1.7 Hz, 1H), 7.39 – 7.32 (m, 1H), 7.20 (dd, $J$ = 8.3, 1.1 Hz, 1H), 7.16 (d, $J$ = 8.9 Hz, 2H), 7.00 (d, $J$ = 8.9 Hz, 2H), 6.77 (s, 1H), 4.03 (t, $J$ = 6.6 Hz, 2H), 1.87 – 1.78 (m, 2H), 1.52 – 1.35 (m, 4H), 0.95 (t, $J$ = 7.1 Hz, 3H);$^{13}$C NMR (101 MHz, $CDCl_3$) δ: 186.15, 171.09, 164.20, 164.09,

161.95, 160.97, 159.48, 148.92, 142.10, 134.55, 132.49, 129.12, 127.48, 127.46, 127.42, 126.06, 124.88, 122.38, 119.55, 118.55, 114.99, 95.97, 68.23, 28.86, 28.17, 22.45, 14.02.; MS (m/z): 588.1.

**4-(2-bromo-4-(5-(4-(5-(4-(pentyloxy)phenyl)isoxazol-3-yl)phenyl)-1,3,4-oxadiazol-2-yl)pheno-xy) benzonitrile (8b):** White solid; Overall yield: 45%; m.p. =210°C; IR (KBr) $cm^{-1}$: 3643, 3549, 3380, 3211, 3126, 3025, 2939, 2533, 2354, 2256, 1696, 1601, 1530, 1487, 1422, 1352, 1244, 1179, 1012, 817; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.26 (d, *J* = 8.3 Hz, 2H), 8.05 (d, *J* = 8.4 Hz, 2H), 7.84 – 7.75 (m, 4H), 7.68 (d, *J* = 7.0 Hz, 2H), 7.13 (dd, *J* = 14.8, 8.9 Hz, 3H), 7.00 (d, *J* = 8.8 Hz, 2H), 6.77 (s, 1H), 4.03 (t, *J* = 6.5 Hz, 2H), 1.87 – 1.77 (m, 2H), 1.50 – 1.36 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 189.77, 171.15, 164.94, 162.93, 161.89, 160.99, 160.24, 154.64, 136.49, 134.48, 132.89, 127.64, 127.54, 127.49, 126.83, 124.62, 124.22, 122.85, 119.72, 118.64, 115.00, 95.95, 68.24, 28.86, 28.17, 22.45, 14.02; MS (m/z): 646.3 and 648.5.

**2-(4-(2-nitrophenoxy)phenyl)-5-(4-(5-(4-(pentyloxy) phenyl)isoxazol-3-yl)phenyl)-1,3,4-oxadiazole (8c):** White solid, Overall yield: 45%; m.p. =201°C; IR (KBr) $cm^{-1}$: 3813, 3655, 3376, 3308, 3221, 3124, 3083, 3023, 2948, 1729, 1693, 1603, 1525, 1494, 1426, 1347, 1249, 1183, 1126, 1028, 949, 832, 768, 685; $^1$H NMR (400 MHz, $CDCl_3$) δ:8.30 – 8.21 (m, 5H), 8.04 (d, *J* = 8.4 Hz, 2H), 7.78 (d, *J* = 8.5 Hz, 2H), 7.28 – 7.21 (m, 3H), 7.14 (d, *J* = 9.1 Hz, 2H), 7.00 (d, *J* = 8.9 Hz, 2H), 6.77 (s, 1H), 4.02 (t, *J* = 6.5 Hz, 2H), 1.86 – 1.77 (m, 2H), 1.50 – 1.35 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ:191.77, 178.46, 171.13, 164.23, 164.05, 161.93, 161.87, 160.99, 158.10, 143.55, 132.60, 129.32, 127.49, 127.45, 126.15, 124.91, 120.56, 120.46, 119.74, 118.31, 115.00, 95.95, 68.24, 28.86, 28.17, 22.46, 14.03; MS (m/z): 589.0.

**2-(4-(5-(4-(pentyloxy)phenyl)isoxazol-3-yl)phenyl)-5-(4-phenoxyphenyl)-1,3,4-oxadiazole (8d):** White solid; Overall yield: 43%; m.p. =198°C; IR (KBr) $cm^{-1}$: 3438, 3308, 3262, 3027, 2956, 1997, 1735, 1599, 1413, 1309, 1255, 1022, 837, 777, 685; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.00 (d, *J* = 8.5 Hz, 2H), 7.70 (d, *J* = 8.8 Hz, 2H), 7.61 (d, *J* = 8.3, 7.5, 1.7 Hz, 2H), 7.38 – 7.32 (m, 5H), 7.20 (dd, *J* = 8.3, 1.1 Hz, 2H), 7.12 (d, *J* = 8.9 Hz, 2H), 7.00 (d, *J* = 8.9 Hz, 2H), 6.75 (s, 1H), 4.03 (t, *J* = 6.6 Hz, 2H), 1.87 – 1.78 (m, 2H), 1.52 – 1.35 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H);$^{13}$C NMR (101 MHz, $CDCl_3$) δ: 171.19, 166.10, 164.19, 160.90, 159.28, 148.62, 142.10, 134.55, 132.49, 130.12, 129.12, 127.48, 127.42, 126.06, 124.88, 122.38, 119.55, 118.55, 114.99, 95.97, 68.23, 28.86, 28.17, 22.45, 14.02.; MS (m/z): 543.1.

**2-(3-methoxy-4-phenoxyphenyl)-5-(4-(5-(4-(pentyloxy)phenyl)isoxazol-3-yl)phenyl)-1,3,4-oxadiazole (8e):** White solid; Overall yield: 48%; m.p. =196°C; **IR (KBr) $cm^{-1}$:** 3645, 3355, 3333, 3208, 3134, 3025, 2942, 1997, 1751, 1599, 1552, 1486, 1381, 1262, 1190, 1170,1021, 836, 776, 684; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.20 (d, *J* = 8.3 Hz, 2H), 8.00 (d, *J* = 8.4 Hz, 2H), 7.84 – 7.75 (m, 6H), 7.68 (d, *J* = 7.0 Hz, 2H), 7.14 (dd, *J* = 14.8, 8.9 Hz, 2H), 7.00 (d, *J* = 8.8 Hz, 2H), 6.75 (s, 1H), 4.03 (t, *J* = 6.5 Hz, 2H), 3.73 (s, 3H), 1.87 – 1.77 (m, 2H), 1.50 – 1.36 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 182.27, 171.13, 165.94, 162.53, 161.99, 160.19, 158.34, 154.64, 136.49, 135.48, 132.32, 127.64, 127.54, 127.49, 126.83, 125.38, 125.01, 124.62, 124.22, 122.85, 119.72, 118.64, 115.00, 95.95, 68.24, 28.86, 28.17, 22.45, 14.02; MS (m/z): 573.2.

**2-(3-nitro-4-(2-nitrophenoxy)phenyl)-5-(4-(5-(4-(pentyloxy)phenyl)isoxazol-3-yl)phenyl)-1,3,4-oxadiazole (8f):** White solid; Overall yield: 35%; m.p. =209°C; IR (KBr) $cm^{-1}$: 3889, 3551, 3365, 3319, 3222, 3120, 3045, 2943, 1999, 1745, 1588, 1559, 1454, 1291, 1232, 1198, 1027, 838, 779, 687; 8.31 – 8.20 (m, 4H), 8.06 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 8.5 Hz, 2H), 7.32 – 7.23 (m, 3H), 7.16 (d, *J* = 9.1 Hz, 2H), 7.01 (d, *J* = 8.9 Hz, 2H), 6.76 (s, 1H), 4.02 (t, *J* = 6.5 Hz, 2H), 1.86 – 1.77 (m, 2H), 1.50 – 1.35 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 192.35, 189.72, 179.35, 173.43, 166.25, 165.15, 161.97, 161.89, 160.89, 158.12, 143.59, 132.67, 129.36, 126.59, 126.45, 126.15, 124.97, 120.58, 120.48, 119.64, 118.22, 115.12, 95.95, 68.24, 28.86, 28.17, 22.46, 14.03; MS (m/z): 633.2.

**2-(3-chloro-4-phenoxyphenyl)-5-(4-(5-(4-(pentyloxy)phenyl)isoxazol-3-yl)phenyl)-1,3,4-oxadiazole (8g):** White solid; Overall yield: 41%; m.p. =203°C; IR (KBr) $cm^{-1}$: 3664, 3422, 3301, 3199, 3113, 3012, 2944, 1977, 1730, 1589, 1582, 1474, 1401, 1264, 1199, 1022, 837, 774, 680; $^1$H NMR (400 MHz, $CDCl_3$) δ: 8.09 (d, *J* = 8.4 Hz, 2H), 7.74 – 7.65 (m, 5H), 7.62 (d, *J* = 7.0 Hz, 2H), 7.36 (d, *J* = 8.3 Hz, 2H), 7.10 (dd, *J* = 14.8, 8.9 Hz, 3H), 6.99 (d, *J* = 8.8 Hz, 2H), 6.76 (s, 1H), 4.03 (t, *J* = 6.5 Hz, 2H), 1.87 – 1.77 (m, 2H), 1.50 – 1.36 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 172.15, 166.65,

163.39, 162.48, 161.99, 160.75, 155.32, 137.83, 134.52, 132.92, 127.69, 127.62, 127.55, 127.49, 126.93, 124.72, 124.22, 122.85, 119.72, 118.64, 115.00, 95.95, 68.24, 28.86, 28.17, 22.45, 14.02; MS (m/z): 577.2 and 579.2.

**2-(4-(2-chlorophenoxy)phenyl)-5-(4-(5-(4-(pentyloxy)phenyl)isoxazol-3-yl)phenyl)-1,3,4-oxadiazole (8h):** White solid; Overall yield: 40%; m.p. =201°C; IR (KBr) $cm^{-1}$: 3664, 3422, 3301, 3199, 3113, 3012, 2944, 1977, 1730, 1589, 1582, 1474, 1401, 1264, 1199, 1022, 837, 774, 680; $^1$H NMR (400 MHz, $CDCl_3$) δ:8.03 (d, *J* = 8.4 Hz, 2H), 7.74 (d, *J* = 8.5 Hz, 2H), 7.50 – 7.41 (m, 5H),7.24 – 7.17 (m, 3H), 7.11 (d, *J* = 9.1 Hz, 2H), 7.00 (d, *J* = 8.9 Hz, 2H), 6.77 (s, 1H), 4.02 (t, *J* = 6.5 Hz, 2H), 1.86 – 1.77 (m, 2H), 1.50 – 1.35 (m, 4H), 0.95 (t, *J* = 7.1 Hz, 3H); $^{13}$C NMR (101 MHz, $CDCl_3$) δ: 178.46, 171.13, 164.23, 164.05, 161.93, 161.87, 160.99, 158.10, 143.55, 132.60, 129.32, 127.98, 127.59, 127.48, 126.25, 125.11, 120.36, 120.16, 119.54, 118.22, 115.23, 95.32, 69.04, 28.86, 28.17, 22.46, 14.03; MS (m/z): 577.2 and 579.2.

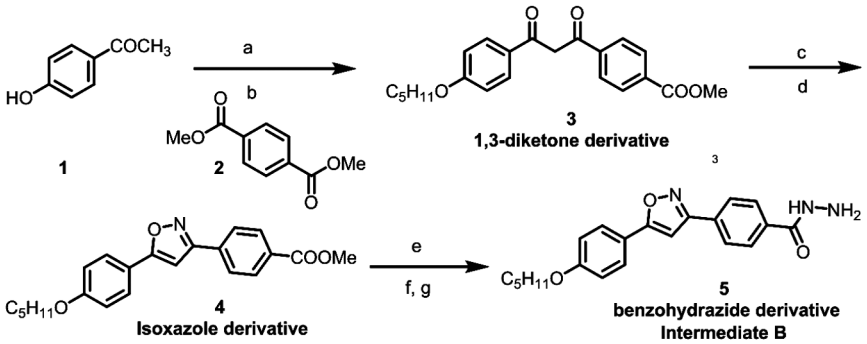
**Biological activity**

Synthesized compounds 8a-h were evaluated for *in vitro* anti-fungal and anti-bacterial studies against three pathogenic fungi (e.g., *C.Albicans, A. Niger, and A. Clavatus*) and four pathogenic bacteria (e.g., *E.Coli, P.Aeruginosa, S.Aureus, and S.Pyogenus*) using Sabouraud Dextrose Agar and Mueller Hinton Broth as nutritional medium, respectively, using Broth Dilution Method. To sterilize the growth media, it was autoclaved at 120°C for half an hour and allowed to cool at room temperature. The medium was mixed with the dimethyl sulphoxide (DMSO) solution of compound 8a-h. The stain was incubated for 72 h at 37°C temperature. Inoculum size for test strain was adjusted to 108 CFU (Colony Forming Unit) per milliliter by comparing turbidity and screened for the activity. Pure DMSO solvent was mixed with media and could serve as blank control. A commercially available drugs Nystatin and Greseofulvin were used as positive control/reference standards for anti-fungal activity. For the anti-bacterial activity, drugs Gentamycin, Ampicillin, ChloramphenicoL, and Ciprofloxacin were used as the positive control. For the activity, two-stage screening was done, primary and secondary screening. In primary Screening, 1000 µg/mL, 500 µg/mL, and 250 µg/mL concentrations of the synthesized compounds were taken. From these screening, the compounds found active were further tested in the second set of dilution against all microorganisms. The secondary screening was carried out with only the compounds which found active in primary screening. The stock solutions were similarly diluted to obtain 200 µg/mL, 100 µg/mL, 50 µg/mL, 25 µg/mL, 12.5 µg/mL, and 6.25 µg/mL concentrations. The highest dilution, which displayed at least 99% inhibition zone, was considered as a MIC for both activities.

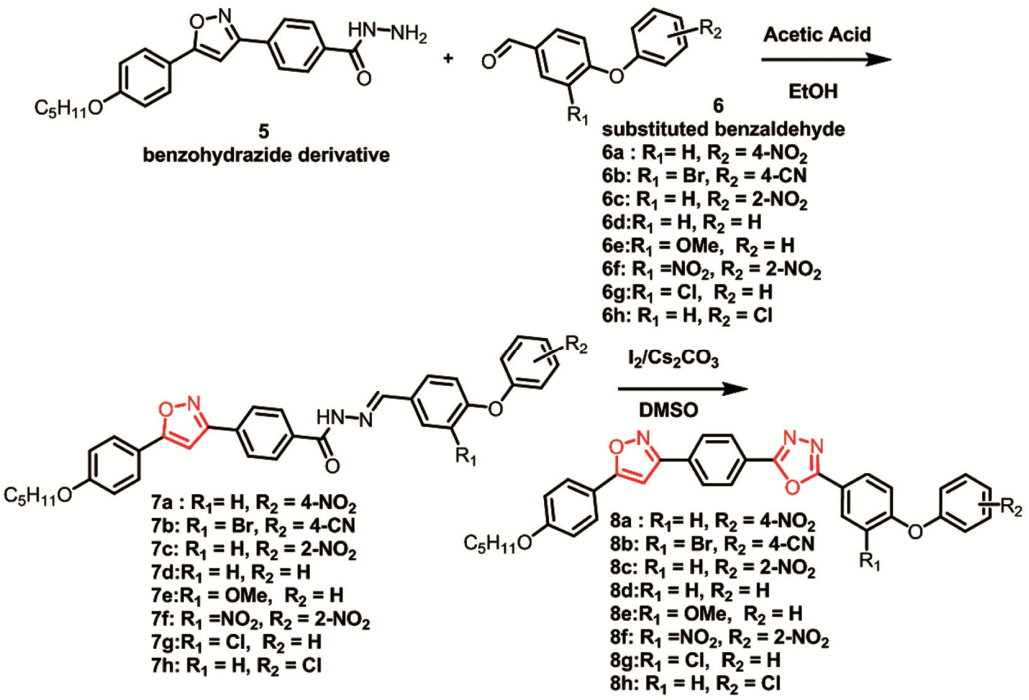
## Results and Discussion

Synthesis of the targeted framework of the sequential chemical transformations has archived us with readily available compound 4-hydroxy acetophenone (Scheme 1 and 2). The target compound was prepared via synthesis of intermediate B containing isoxazole core heterocycle by reported method followed the construction of 1,3,4-oxadiazole core.

Synthesis of target intermediate molecule B (Scheme 1) was achieved by a reported method[35], First, the 4-hydroxy acetophenone (1) was protected, and further, it was converted to the 1,3-diketone derivative (3) using crossed Claisen condensation reaction with the dimethyl terephthalate (2) in excellent yield. Next, the 1,3-diketone (3) reacted




Scheme 1 — Synthesis of intermediate B




Scheme 2 — Synthesis of target compound 8a-h

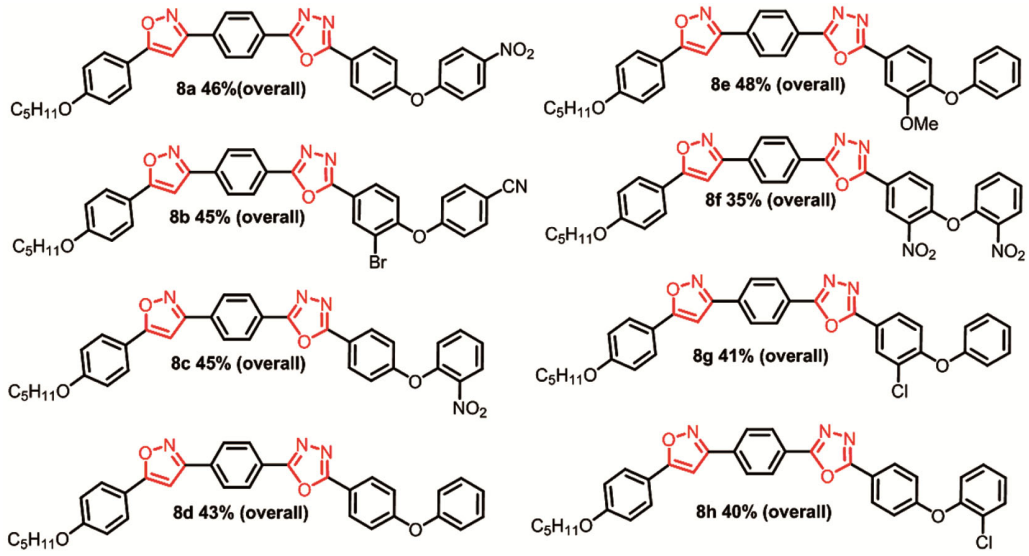



Fig. 2 — Structure of synthesized target compound 8a-h

with ammonium formate and followed by the reaction with ammonium hydroxide, the Isoxazole heterocycle (4) was obtained in good yield. Further, the ester group present on the side chain phenyl ring was hydrolyzed to carboxylic acid and followed by an acid-amine coupling reaction using HOBt/EDCI with hydrazine hydrate. The reaction was smooth, and compound (4) was converted to its benzohydrazide derivative (5) in good yielding, i.e., intermediate B. After having intermediate B in hand, we have further proceeded with constructing the other heterocyclic ring. The benzohydrazide derivative (intermediate B) was reacted with various bromo, and nitro substituted benzaldehyde (6a-h)in the presence of acetic acid in ethanol gave the benzylidenebenzohydrazide (7a-h) derivative in excellent yield(>90%).

The compounds 7 (a-h) were treated with the $I_2/CsCO_3$/DMSO, which gives the desired compound 8a-h (Fig. 2) in excellent yields (overall ~46% yield over 9 liner steps). After having synthesized

compound 8a-h in hand, we have screened those for anti-fungal and anti-bacterial activity. As described in Table 1, compounds 8a-h were screened for the *in vitro* anti-fungal activity against three pathogenic fungi *C. albicans*, *A. niger*, *A. clavatus*. Readily available known anti-fungal drugs Nystatin and Greseofulvin as reference slandered. Among screened compounds 8a-h, 8a exhibits excellent anti-fungal activity against *C. albicans* with 250 μg/mL MIC. Compound 8a and 8b have shown comparable activity with Greseofulvin against the *C. albicans* fungi, i.e., MIC value 500 μg/mL, with other fungi, *A. niger*, *A. clavatus* the synthesized compounds required high concentration (> =1000 μg/mL) for the minimum inhibition as compared to both the reference slandered. Other compounds required very high concentrations for the minimal inhibition of bacterial culture compared to the reference drug.

Besides this, the synthesized molecules (8a-h) were also tested for anti-bacterial activity. The study was performed against four bacteria, *E. coli*, *P. aeruginosa*, *S. aureus*, and *S. pyogenus* with Gentamycin, Ampicillin, Chloramphenicol, and Ciprofloxacin drugs as a reference standard, and obtained results are tabulated in Table 2. Delightfully compound 8a required half concentration (50 μg/mL) as compared to standard Ampicillin (100 μg/mL) and comparable with Chloramphenicol (50 μg/mL) for the minimum inhibition against *E. coli*. Against *P. aeruginosa* compound 8a and 8b showed excellent activity as both depicted 25 μg/mL MIC as compared to standard Ampicillin (100 μg/mL), Chloramphenicol (50 μg/mL), and Ciprofloxacin (25 μg/mL). In comparison, compound 8c required a higher concentration (125 μg/mL) for minimal inhibition. Compound 8c showed comparable activity with standard drugs Chloramphenicol and Ciprofloxacin (MIC = 50 μg/mL) and more significant activity as compared to Ampicillin (MIC = 250 μg/mL) against *S. aureus* culture. Compound 8b exhibited comparable anti-bacterial activity with Chloramphenicol and Ciprofloxacin (MIC = 50 μg/mL) against *S. pyogenus* culture. Other compounds required higher concentrations for the minimal inhibition of bacteria as compared to the reference drug.

Table 1 — Antifungal activity

| Sr. No. | Sample Code | Minimal fungicidal concentration (μg/mL) | | |
|---|---|---|---|---|
| | | *C. albicans* MTCC 227 | *A. niger* MTCC 282 | *A.clavatus* MTCC 1383 |
| 1 | 8a | 250 | 500 | 1000 |
| 2 | 8b | 500 | 1000 | 1000 |
| 3 | 8c | 500 | 1000 | >1000 |
| 4 | 8d | 1000 | 1000 | >1000 |
| 5 | 8e | >1000 | 1000 | >1000 |
| 6 | 8f | >1000 | >1000 | 1000 |
| 7 | 8g | 1000 | >1000 | >1000 |
| 8 | 8h | 750 | >1000 | >1000 |
| 9 | Nystatin | 100 | 100 | 100 |
| 10 | Greseofulvin | 500 | 100 | 100 |

Table 2 — Antibacterial activity

| Sr. No. | Sample Code | Minimal Inhibition Concentration (μg/mL) | | | |
|---|---|---|---|---|---|
| | | *E. coli* MTCC 443 | *P. aeruginosa* MTCC 1688 | *S. aureus* MTCC 96 | *S. pyogenus* MTCC 442 |
| 1 | 8a | 50 | 25 | 62.5 | 100 |
| 2 | 8b | 100 | 25 | 62.5 | 50 |
| 3 | 8c | 100 | 125 | 50 | 250 |
| 4 | 8d | 75 | 100 | 100 | 250 |
| 5 | 8e | 100 | 100 | 100 | 250 |
| 6 | 8f | 100 | >100 | 250 | >250 |
| 7 | 8g | 75 | 100 | 62.5 | 100 |
| 8 | 8h | 100 | 125 | 100 | 250 |
| 9 | Gentamycin | 0.05 | 1 | 0.25 | 0.5 |
| 10 | Ampicillin | 100 | 100 | 250 | 100 |
| 11 | Chloramphenicol | 50 | 50 | 50 | 50 |
| 12 | Ciprofloxacin | 25 | 25 | 50 | 50 |

## Conclusion

In conclusion, we have synthesized series of the organic framework containing two biologically important heterocycles, i.e., isoxazole and 1,3,5-oxadiazole. The synthesised compounds were screened for the anti-fungal and anti-bacterial activity Compound 8a exhibited significant anti-fungal activity with MIC 250 μg/mL against *C. albicans* fungi. Compounds 8b and 8c showed significant anti-bacterial activity with MIC 50 μg/mL against *S. pyogenus* and *S. aureus* pathogens, respectively. This work could inspire further investigation in the design and synthesis of a biheterocylic framework for anti-fungal, anti-bacterial, and other biologically active agents.

## Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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