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# Synthesis of Novel Substituted 3 -Phenyl-5-(3-phenylisoxazole-5-yl)-1, 2, 4-Oxadiazoles Catalyzed by Cu-HAP and Antimicrobial Evaluation of Biological Activity

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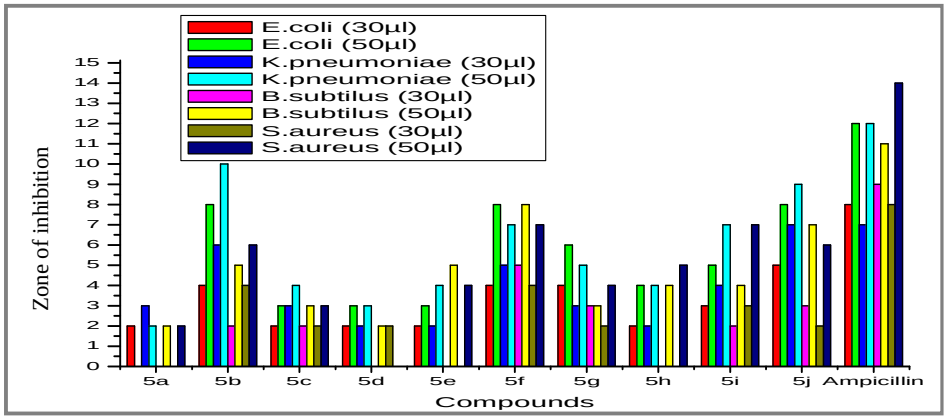
## ABSTRACT

*A novel approach for the synthesis of substituted 3-phenyl-5-(3-phenylisoxazole-5-yl)-1,2,4-oxadiazole* ***(6a-j)*** *were synthesized by the reaction of ethyl-3-phenyl isoxazole-5-carbboxylates* ***(4a-c)*** *and amide oximes* ***(5a-g)*** *using a catalytic amount of Cu-HAP as catalyst at heating conditions in the presence of ethanol and catalytic amount of DMF media. The catalyst was quantitatively recovered from reaction mixture by simple filtration and reused for three cycles with consistence activity. All these compounds have been characterized by modern spectral techniques such as IR, $^{1}$H NMR, Mass etc. Evaluation of synthesized compounds for antimicrobial activity against specific bacterial strains like 1) Escherichia coli 2) Klebsiella pneumoniae 3) Bacillus subtilis 4) Staphylococcus aureus, along with antifungal activity against 1) Aspergillus niger, 2) Aspergillus foetidus 3) Candida albicans and 4) Candida Rogosa.*

## Graphical Abstract




Antibacterial activity of compounds **6a-j** against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia*

**Keywords:** Cu-HAP, Green chemistry, 1, 2, 4-Oxadiazole, 3-Phenyl isoxazole-5-carbboxylates, Amide oximes, Antimicrobial activity.

## INTRODUCTION

In the past few decades, the synthesis of new heterocyclic compounds has been a topic of great attention due to their extensive applicability. 1, 2, 4-oxadaizoles are a class of important of heterocycles which have been well documented throughout the literature due to their biological significance. The 1, 2, 4-Oxadaizoles have often been used as bioisosters of esters, they are present in various biologically active compounds, such as benzodiazepine receptor ligands, muscarine receptor agonists and 5-HT3 receptor antagonists [**1**]. 1, 2, 4-oxadiazole derivatives possess human tryptase inhibitory activity [**2**], anti-inflammatory [**3**], antitumor [**4**] and antifungal activities [**5**]. Recent studies have proved anti-inflammatory properties, anti-tumor activities [**6**] and strong neuroprotective agents as well [**7**].

The chemical and pharmaceutical industries are always under pressure to progress more environmentally friendly organic reaction methodologies. In this regard, the increasing demand for cleaner procedures supported by stringent environmental laws necessitates use of eco-friendly and discriminating catalysts. Hydroxyapatites (HAP) possess $Ca^{2+}$ sites surrounded by $PO_4^{3-}$ tetrahedral parallel to the hexagonal axis, which have attracted considerable interest in view of their potential usefulness as biomaterials, adsorbents, and ion exchangers. Apatites are metal basic phosphates, various kinds of cations and anions can be readily introduced into their frame work due to their large ion-exchange ability and such exchanged apatites are already in use in several organic transformations.

The introduction of transition metal cations, such as Ru, Pd, Cu and Zn into the apatite framework could generate stable monomeric phosphate complexes, which exhibit prominent catalytic performances for various reactions like 2, 4-dioxo esters, Isoxazole preparations and 1, 2, 4-oxadiazole using Cu-HAP as a catalyst [**8**]. Copper(II)-exchanged hydroxyapatite, prepared by ion-exchanging of Ca(II) in calcium hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] with $Cu(NO_3)_2$ at 70$^o$C in water, functions as a reusable heterogeneous catalyst with neither reducing agents nor bases for 1, 2, 4-oxadiazole at 80$^o$C in DMF as a solvent. We would use Cu-HAP, easily prepared from [$Ca_{10}(PO_4)_6(OH)_2$] and $Cu(NO_3)_2$ in water similarly to Pd-HAP. Thus we would establish a reusable process of Cu-HAP in DMF under air with the aim of advancing ion-exchanged Cu-HAP for environmentally benign catalysis [**9**].

## MATERIALS AND METHODS

**Chemistry:** All reactions were carried out under nitrogen atmosphere in oven-dried glassware with magnetic stirring**.** All the chemicals and solvents were purchased from SD Fine Chemicals, Bombay, India. Solvents were purified and dried according to the standard procedures. Silica gel (60–120 mesh) for column chromatography was purchased from M/s Acme Synthetic Chemicals (Mumbai, India) and pre-coated TLC plates (Silica gel 60F254) were purchased from Merck (Darmstadt, Germany). The $^1$H-NMR and $^{13}$C-NMR spectra were recorded on Bruker 400 and 100 MHz, respectively, and TMS was used as an internal standard. Chemical shifts relative to TMS as internal standards were given as δ values in ppm. Mass spectra were recorded using electron spray ionization on Waters e2695 Separators module (Waters, Milford, MA, USA) mass spectrometer. IR spectra were recorded on a Fourier transform (FT-IR), USA (Perkin-Elmer model 337) instrument. The melting points were determined on a Barnstead Electro Thermal 9200 Instrument.

**1) General procedure for the synthesis of ethyl-2,4-dioxo-4-phenylbutanoates (3a-c):** To a stirred solution of Acetophenone **(1a-c)** and diethyl oxalate **(2)** in DMF was added Cu-HAP (10 mol%) under nitrogen atmosphere. Then the reaction mixture was stirred for 5-6h at 110$^o$C, progress of the reaction was monitored by TLC, on completion of the starting material, the reaction mixture was cooled to room temperature and the catalyst was filtered. The filtrate was diluted with 50 mL of cold water and extracted with EtOAc (2x30 mL). The combined organic layer was dried with anhydrous $Na_2SO_4$, and

the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent to provide the desired product gave ethyl 3-oxo-3-phenylpropanoate **(3a-c)** [10-12].

**2) Synthesis of ethyl-3-phenyl isoxazole-5-carbboxylates (4a-c):** To a stirred solution of ethyl 2, 4-dioxo-4-phenylbutanoate **(3a-c)** in ethanol was added hydroxylamine hydrochloride under nitrogen atmosphere. Then the reaction mixture was refluxed for 3h, progress of the reaction was monitored by TLC, on completion of starting material most of the solvent was removed and the crude was dissolved in EtOAc, washed with water followed by saturated sodium chloride. The combined organic layers were dried over anhydrous sodium sulphate and then concentrated on reducer pressure to give ethyl 3-phenylisoxazole-5-carboxylate as a white solid **(4a-c)** [10-12], as the product is obtained in pure form and proceeded to the next step without any further purification.

**3) Synthesis of amide oximes (5a-g):** To a mixture of phenylacetonitrile (6.3g, 53.8 mmol) in EtOH (60 mL), $NH_2OH.HCl$ (7.5g, 107.6 mmol) and Et3N (10.8 g, 107.6 mmol) were added and refluxed for 3h. After completion of the reaction, EtOH was removed completely. To the residue water (100 mL) was added and extracted into ethyl acetate (100 mL), dried over $Na_2SO_4$ and concentrated to yield the amide oxime **(5a-g).**

**4) Synthesis of 3-phenyl-5-(3-phenylisoxazole-5-yl)-1, 2, 4-oxadiazole (6a-j):** To a stirred solution of ethyl 3-phenylisoxazole-5-carboxylate **(4a-c)** (1.2 mmol) in DMF was added Cu-HAP (10 mol %) and N'-hydroxybenzimidamides **(5a-g)** (1.8 mmol) were added and refluxed for 5 hours. After completion of the reaction by TLC monitoring, on completion of the starting material, the reaction mixture was cooled to room temperature and the catalyst was filtered. The filtrate was diluted with 50 mL of cold water and extracted with EtOAc (2x25 mL). The combined organic layer was dried with anhydrous $Na_2SO_4$, and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel using petroleum ether/ethyl acetate as eluent to provide the desired product gave the **1, 2, 4-oxadiazoles (6a-l)**.

**i) 3-Phenyl-5-(3-phenylisoxazole-5-yl)-1, 2, 4-oxadiazole (6a):** White solid, m.p. $80^0$C. Yield 60%. IR (KBr) $cm^{-1}$: 1608, 1448, 1346, 1028, 943, 765, 742, 680 and 675. $^1$H-NMR ($CDCl_3$, 400MHz): δ 8.20 – 8.18 (m, H-2''', 6''', 2H), 7.89 – 7.86 (m, H-2, 6, 2H), 7.54 – 7.51 (m, H-3, 4, 5, 3''',4''',5''', 6H), 7.17 (s, H-4', 1H). $^{13}$C-NMR ($CDCl_3$, 100MHz): δ 172.1, 168.8, 168.1, 151.5, 132.4, 131.7, 129.8, 127.6, 126.4, 126.1, 125.9, 100.9. . MASS (ESIMS): 290 [M+H].

**ii) 3-(2-Chlorophenyl)-5-(3-phenylisoxazol-5-yl)-1, 2, 4-oxadiazole (6b):** White solid, m.p. $88^0$C. Yield 64%. IR (KBr) $cm^{-1}$: 1618, 1442, 1327, 1082, 947, 758, and 678. $^1$H-NMR ($CDCl_3$, 400MHz): δ 8.07 (ddd, J = 9.7, 7.7, 4.0Hz, 3H), 7.94 (s, 1H), 7.76 (d, J = 8.0Hz, 1H), 7.70 (ddd, J = 9.0, 7.2, 1.5Hz, 1H), 7.63-7.60 (m, 4H). $^{13}$C-NMR ($CDCl_3$, 100MHz): δ 172.1, 168.3, 168.1, 166.7, 151.5, 137.2, 136.3, 131.8, 130.0, 129.8, 129.7, 129.4, 129.2, 126.4, 100.9. MASS (ESIMS): 324[M+H], 326 [M+H+2].

**iii) 3-(4-Chlorophenyl)-5-(3-phenylisoxazol-5-yl)-1, 2, 4-oxadiazole (6c):** White solid, m.p. $73^0$C. Yield 58 %. IR (KBr) $cm^{-1}$: 1643, 1606, 1381, 1332, 1093, 941. $^1$H-NMR ($CDCl_3$, 400MHz): δ 8.16-8.10 (m, H-2''',6''', 2H), 8.05-8.01 (m, H-2,6, 2H), 7.91 (s, H-4', 1H), 7.73-7.59 (m, H-3,4,5,3''',5''', 5H). MASS (ESIMS): 324[M+H], 326 [M+H+2].

**iv) 3-(3-Chlorophenyl)-5-(3-phenylisoxazol-5-yl)-1, 2, 4-oxadiazole (6d):** White solid, m.p. $86^0$C. Yield 71%. IR (KBr) $cm^{-1}$: 1612, 1577, 1342, 1307, 1022, 943. 1H NMR (400MHz, dmso) δ 8.07 (s, H-2''',1H), 8.06 – 8.01 (m, H-4''',5''',6''', 3H), 7.93 (s, H-4', 1H), 7.74 (d, J = 8.1 Hz, H-2, 1H), 7.65 (dd, J = 14.8, 6.6 Hz, H-6, 1H), 7.59 (d, J = 3.9 Hz, H-3,4,5, 3H). $^{13}$C NMR (101 MHz, dmso) δ 172.1, 168.5, 167.7, 151.6, 134.5, 132.3, 132.0, 131.8, 129.9, 127.8, 127.2, 126.4, 126.3, 126.1, 101.2. MASS (ESIMS): 324[M+H], 326 [M+H+2].

**v) 5-(3-Phenylisoxazol-5-yl)-3-(o-tolyl)-1,2,4-oxadiazole (6e):** White solid, m.p. $77^0$C. Yield 62%.IR (KBr) $cm^{-1}$: 1620, 1604, 1564, 1448, 1338, 1244, 945. 1H NMR (400MHz, dmso) δ 8.08 – 7.97 (m, H-2,6,6''', 3H), 7.89 (s, 1H), 7.59 (dd, J = 5.0, 1.3 Hz, H-3,4,5, 3H), 7.52 (t, J = 7.2 Hz, H-5''', 1H), 7.44 (dd, J = 13.8, 7.0 Hz, H-3''',4''', 2H), 2.61 (s, $CH_3$, 3H). $^{13}$C-NMR (101 MHz, dmso) δ 172.1, 169.4, 167.1, 151.6, 138.2, 132.0, 131.8, 131.7, 130.3, 129.8, 126.8, 126.4, 126.1, 125.2, 100.9, 22.1. MASS (ESIMS): 304 [M+H].

**vi) 3-(4-Bromophenyl)-5-(3-phenylisoxazol-5-yl)-1, 2, 4-oxadiazole (6f):** White solid, m.p. $73^0$C. Yield 73%. IR (KBr) $cm^{-1}$: 1597, 1564, 1444, 1404, 1382, 1332, 1238, 937. $^1$H-NMR (400 MHz, dmso) δ 8.02 (d, J = 8.0 Hz, H-2''',3''',5''',6''', 4H), 7.91 (s, H-4', 1H), 7.83 (d, J = 8.3 Hz, H-2,6, 2H), 7.59 (d, J = 3.7 Hz, H-3,4,5, 3H). $^{13}$C-NMR (101 MHz, dmso) δ 172.1, 168.3, 168.2, 151.5, 133.0, 131.8, 129.9, 129.6, 126.4, 126.1, 126.1, 125.1, 100.9. MASS (ESIMS): 368[M+H], 370[M+H+2].

**vii) 3-(2-Fluorophenyl)-5-(3-phenylisoxazol-5-yl)-1, 2, 4-oxadiazole (6g):** White solid, m.p. $82^0$C. Yield 78%. IR (KBr) $cm^{-1}$: 1612, 1570, 1485, 1450, 143, 1390, 1334, 1230, 758.1H-NMR (400 MHz, dmso) δ 8.10 (t, J = 7.1 Hz, H-6''', 1H), 8.05 – 8.00 (m, H-2,6, 2H), 7.88 (s, H-4', 1H), 7.69 (dd, J = 12.8, 6.5 Hz, H-3''', 1H), 7.57 (d, J = 3.8 Hz, H-3,4,5, 3H), 7.47 (dd, J = 17.9, 9.9 Hz, H-4''',5''', 2H). $^{13}$C-NMR (101 MHz, dmso) δ 172.1, 167.7, 165.7, 165.6, 161.6, 159.0, 151.4, 134.5, 134.4, 131.8, 131.0, 129.8, 126.4, 126.1, 125.7, 125.7, 117.5, 117.3, 114.1, 114.0, 100.9. MASS (ESIMS): 308[M+H].

**viii) 3-(3-Chlorophenyl)-5-(3-(4-methoxyphenyl)isoxazol-5-yl)-1, 2, 4-oxadiazole (6h):** Off White solid, m.p. $89^0$C. Yield 63%IR (KBr) $cm^{-1}$ 1604, 1500, 1436, 1255, 1164, 1009, 933. $^1$H-NMR (400 MHz, dmso) δ 8.07 (d, J = 7.7 Hz, H-2''',6''', 2H), 7.98 (d, J = 8.6 Hz, H-2,6, 2H), 7.77 (s, H-4', 1H), 7.74 (d, J = 8.0 Hz, H-4''', 1H), 7.66 (t, J = 7.7 Hz, H-5''', 1H), 7.14 (d, J = 8.6 Hz, H-3,5, 2H), 3.84 (s, $OCH_3$, 3H). $^{13}$C-NMR (101 MHz, dmso) δ 172.2, 168.6, 167.8, 161.9, 151.3, 134.5, 132.3, 132.0, 128.3, 127.9, 127.2, 126.3, 118.7, 115.3, 99.4, 55.9. . MASS (ESIMS): 354[M+H], 356[M+H+2].

**ix) 5-(3-(4-Methoxyphenyl) isoxazol-5-yl)-3-(o-tolyl)-1, 2, 4-oxadiazole (6i):** White solid, m.p. $89^0$C. Yield 72% IR (KBr) $cm^{-}$1: 1614, 1608, 1514, 1448, 1344, 1263, 1184, 1155, 1018, 945. 1H-NMR (400 MHz, dmso) δ 7.98 (dd, J = 11.9, 8.5 Hz, H-4''',5''',6''', 3H), 7.71 (s, H-4', 1H), 7.54 – 7.47 (m, H-3''', 1H), 7.43 (dd, J = 13.5, 6.9 Hz, H-2,6, 2H), 7.12 (d, J = 8.6 Hz, H-3,5, 2H), 3.83 (s, $OCH_3$, 3H), 2.60 (s, $CH_3$, 3H). $^{13}$C-NMR (101 MHz, dmso) δ 172.1, 169.3, 167.2, 161.9, 151.4, 138.2, 132.0, 131.7, 130.2, 128.2, 126.8, 125.2, 118.8, 115.2, 99.3, 56.1, 22.0. . MASS (ESIMS): 334[M+H].

**x) 3-(4-Bromophenyl)-5-(3-(4-methoxyphenyl)isoxazol-5-yl)-1, 2, 4-oxadiazole (6j):** White solid, m.p. $89^0$C. Yield 67%. IR (KBr) $cm^{-1}$: 1614, 1600, 1506, 1473, 1452, 1415, 1263, 1182, 1134, 1016, 829, 756. 1H-NMR (400 MHz, dmso) δ 7.98 (dd, J = 11.9, 8.5 Hz, H-2''',3''',5''',6''',4H), 7.71 (s, H-4', 1H), 7.43 (dd, J = 13.5, 6.9 Hz, H-2, 6, 2H), 7.12 (d, J = 8.6 Hz, H-3,5, 2H), 3.83 (s, $OCH_3$, 3H). $^{13}$C NMR (100 MHz, dmso) δ 172.2, 168.4, 168.1, 161.9, 151.4, 133.0, 129.6, 128.2, 126.1, 125.1, 118.7, 115.3, 99.3, 55.9. MASS (ESIMS): 398[M+H], 400[M+H+2].

**1. Antibacterial activity by disc diffusion method:** The antibacterial activity of synthesized compounds was conducted against two gram positive bacteria viz., *Bacillus subtilis and Staphylococcus aureus* Gram negative bacterial strains of *Escherichia coli and Klebsiella pneumonia"* by using disc diffusion method. Ampicillin sodium was employed as standard to compare the results.

**[A] Preparation of Mueller-Hinton agar:** (1) Beef extract: 300 g, (2) Acid hydrolysate of casein: 17.5 g, (3) Starch: 1.5 g, (4) Agar: 17 g, (5) Distilled water: 1 Lit.

The above constituents were weighed and dissolved in water. The mixture was warmed on water bath till agar was dissolved. This was then sterilized in an autoclave at 15 lbs pressure and $121^o$C for

fifteen minutes. The sterilized medium (20 mL) was poured in sterilized Petri dishes under aseptic condition, allowing them to solidify on a plane table.

**[B] Preparation of Anti-bacterial Solution:** All the compounds were dissolved in DMSO and proper drug controls were used. Compound was taken at concentration of 1mg/ml for testing anti-bacterial activity. The compound diffused into the medium produced a concentration gradient. After the incubation period, the zones of inhibition were measured in mm. The tabulated results represent the actual readings against the control.

**[C] Test cultures:** Following common standard strains were used for screening the antibacterial activities:

| | |
|---|---|
| *Escherichia coli* | [Gram negative] MTCC – 443 |
| *Klebsiella pneumonia* | [Gram negative] MTCC – 424 |
| *Bacillus subtilis* | [Gram positive] MTCC – 96 |
| *Staphylococcus aureus* | [Gram positive] MTCC – 442 |

**[D] Inoculums' preparation:** The inoculum was standardized at 1× 106 CFU $mL^{-1}$ comparing with turbidity standard (0.5 MacFarland tube)

**2. Antifungal activity by paper disc method:** All those compounds screened for antibacterial activity were also tested for their antifungal activity. The fungi employed for screening were *Aspergillus niger*, *Aspergillus foetidus*, *Candida albicans* and *Candida rogosa*. Antifungal activity was tested at the following concentrations 50µg $\mu L^{-1}$ and fluconazole was employed as standard to compare the results.

**Medium Composition:** Potato infusion 200 gm, Dextrose 20 gm, Agar 20 gm, Distilled water 1L.

The medium was sterilized in the autoclave at 121°C (15 lbs) pressure for 15 min. The medium was cooled to 45-50°C and poured in 20 mL volume in each petridish and allowed to solidify. The antifungal activity screening is done by the paper disc method.

**Testing equipments:** Tubes of uniform size, paper discs and petridishes were employed.

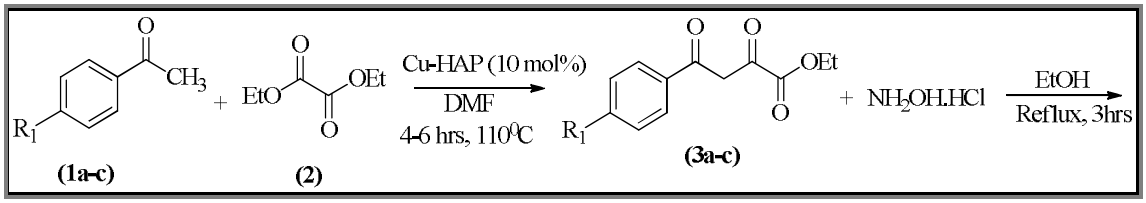
**Maintenance of sterility:** All required apparatus were sterilized before use and necessary precautions were taken to avoid contamination.

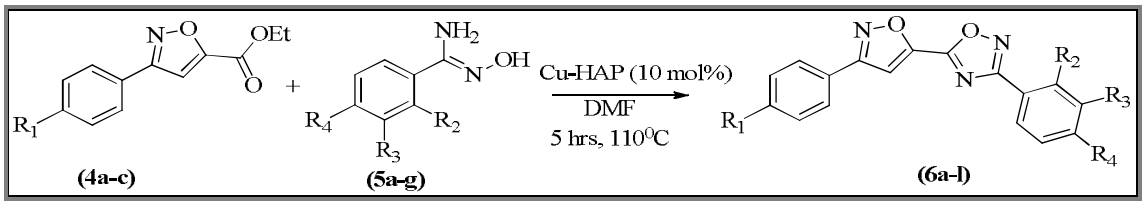
**Preparation of sample solutions:** The testing compounds 1mg was dissolved in 1 mL of DMSO. This gives the concentration of the sample compounds as 1µg $1\mu L^{-1}$. Two Different dilutions such as 30µg 30 $\mu L^{-1}$ and 50µg $50\mu L^{-1}$ were prepared from the sample solution.

**Anti-fungal testing:** Anti-fungal activity the synthesized compounds were screened for their anti-fungal activity against four fungi. They are *Aspergillus niger*, *Aspergillus foetidus*, *Candida albicans* and *Candida rogosa*. Potato Dextrose Agar (PDA) medium was prepared and about 15 mL of PDA was poured into each petriplate and allowed to solidify. 5 mm disc of seven day old culture of the test fungi was placed at the center of the petriplate and incubated at 26°C for 7 days. After incubation the percentage inhibition was measured and three replicates were maintained for each treatment. Fluconozole was used as the standard. All the synthesized compounds were tested (at the dosage of 50 µL of the novel compounds/petriplate, where concentration was 1 mg $mL^{-1}$) by poisoned food technique.

## RESULTS AND DISCUSSION

**Synthesis of 3-phenyl-5-(3-phenylisoxazole-5-yl)-1, 2, 4-oxadiazole (6a-j):** The reaction of acetophenones **(1a-c)** with diethyl oxalate **(2)** in the presence of Cu-HAP and DMF as a solvent results ethyl-2, 4-dioxo-4-phenylbutanoates **(3a-c)**. These are ethyl-2, 4-dioxo-4-phenyl but anoates on reaction with hydroxylamine hydrochloride in ethanol as a solvent yields ethyl-3-phenyl isoxazole-5-carboxylates **(4a-c)**. Further carboxylates explored **(4a-c)** on reaction with amide oximes **(5a-g)** which are prepared from corresponding amines, in toluene and potassium carbonate as base affords title **(6a-j)** compounds in almost good to excellent yields.



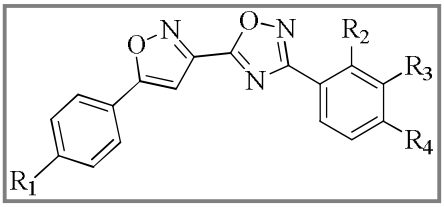




In the IR spectrum **6a**, peaks were observed at 2245cm$^{-1}$(C=N), 1608, 1448cm$^{-1}$ (C=C), 765, 680 cm$^{-1}$ (mono substituted benzene) 742, 675 cm$^{-1}$ (mono substituted benzene). In the $^{1}$H-NMR of spectrum **6a,** the newly formed 3-phenyl-1, 2, 4-oxadiazole protons of H-2''', 6''' appeared as a multiplet at δ 8.20-8.18, H-3, 4, 5, 3''', 4''', 5''' appeared as multiplet at δ 7.54-7.51, H-2, 6 observed as multiplet in the range δ 7.89-7.86 and proton H-4' appeared as a singlet. In the $^{13}$C-NMR spectrum of **6a,** the carbon signal assignments are as follows 172.1, 168.8, 168.1, 151.5, 132.4, 131.7, 129.8, 127.6, 126.4, 126.1, 125.9, and 100.9. In the mass spectrum of **6a**, molecular ion peak was observed at m/z 290 [M+H].

**Antibacterial Activity:** "All the synthesized substituted 3-phenyl-5-(3-phenylisoxazole-5-yl)-1, 2, 4-oxadiazoles (Table 1, Figure 1) **6a-j** were screened for their antibacterial activity against different types of bacterial strains" they are "Gram positive bacterial strains of *Bacillus subtilis and Staphylococcus aureus* Gram negative bacterial strains of *Escherichia coli and Klebsiella pneumoniae"* at a concentration of 30 µg mL$^{-1}$ and 50 µg mL$^{-1}$ [**13-15**]. Some of the synthesized compounds showed high activity and some showed moderate activity compared to standard drug *Ampicillin* at a concentration of 30µg mL$^{-1}$ and 50 µg mL$^{-1}$.

The antibacterial activity of compound **6h,** (R = -$OCH_3$) **6i** (R = -$OCH_3$, R2 = -$CH_3$), **6j** (R = -$OCH_3$, R3 = -Br), **6b** (R1 = -Cl) and **6f** (R3 = -Cl) showed good zone of inhibition against *Escherichia coli*, *Klebsiella pneumoniae* , *Bacillus subtilis and Staphylococcus aureus* compared to the standard drug at a concentration of 30 µg mL$^{-1}$ and 50 µg mL$^{-1}$. Whereas, the compounds **6a, 6b, 6c, 6e** and **6g** were showing, moderate activity against all the bacterial strains when compared to standard drug. It leads us to conclude that from table 1 and figure 1 methoxy, chloro, and bromide substituted compounds showed higher zone of inhibition could be attributed the presence of an electron donating groups when compared with other compounds. Furthermore, substitutions like -$CH_3$, and H, did not provide any significant change in the levels of activity against bacterial strains.

**Table 1.** Evaluation of anti-bacterial activity of synthesized Isoxazole based Oxadiazoles.




| Compound | R | R1 | R2 | R3 | Gram Negative | | | | Gram Positive | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *E. coli* | | *K. pneumonia* | | *B. subtilis* | | *S. aureus* | |
| | | | | | 30µL | 50µL | 30µL | 50µL | 30µL | 50µL | 30µL | 50µL |
| 6a | H | H | H | H | 2 | - | 3 | 2 | - | 2 | - | 2 |
| 6b | H | Cl | H | H | 4 | 8 | 6 | 10 | 2 | 5 | 4 | 6 |
| 6c | H | H | Cl | H | 2 | 3 | 3 | 4 | 2 | 3 | 2 | 3 |
| 6d | H | H | H | Cl | 2 | 3 | 2 | 3 | - | 2 | 2 | - |
| 6e | H | CH3 | H | H | 2 | 3 | 2 | 4 | | 5 | - | 4 |
| 6f | H | H | H | Br | 4 | 8 | 5 | 7 | 5 | 8 | 4 | 7 |
| 6g | H | F | H | H | 4 | 6 | 3 | 5 | 3 | 3 | 2 | 4 |
| 6h | OCH3 | H | Cl | H | 2 | 4 | 2 | 4 | | 4 | - | 5 |
| 6i | OCH3 | CH3 | H | H | 3 | 5 | 4 | 7 | 2 | 4 | 3 | 7 |
| 6j | OCH3 | H | H | Br | 5 | 8 | 7 | 9 | 3 | 7 | 2 | 6 |
| | Ampicillin | | | | 8 | 12 | 7 | 12 | 9 | 11 | 8 | 14 |

In vitro Antibacterial activity of Compounds (Concentration used 30µg 30µL$^{-1}$ and 50µg 50µL$^{-1}$)

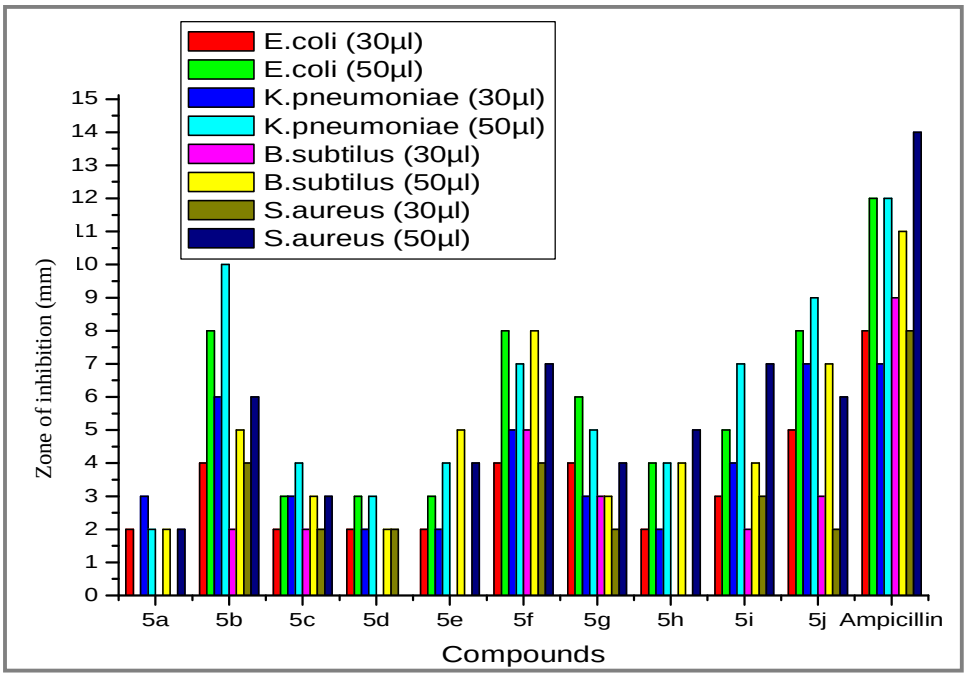
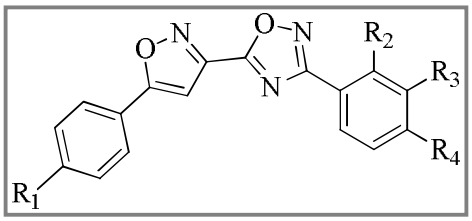



**Figure 1**. Antibacterial activity of compounds **6a-j** against *Bacillus subtilis, Staphylococcus aureus, Escherichia coli and Klebsiella pneumonia.*

**Antifungal Activity:** The antifungal activity of substituted 3 -phenyl-5-(3-phenylisoxazole-5-yl)-1, 2, 4-oxadiazoles derivatives (Table 2, Figure 2) have been evaluated against *A. niger, A. foetidus, C. albicans and C. Rogosa* by employing fluconazole as the standard drug concentration of 30 µg mL$^{-1}$ and 50 µg mL$^{-1}$. The antifungal activity of compounds **6g** (R = -F), **6h,** (R = -$OCH_3$) **6e** (R1 = -$CH_3$) and **6i** (R = -$OCH_3$, R2 = -$CH_3$), showed good zone of inhibition against *A. niger*, *A. foetidus,*

*C. albicans and C. Rogosa* compared to the standard drug. Due to fluorine electro negativity nature compound **6g** showed more activity than other Halo substituted compounds. The electron donating groups such as methoxy and methyl substituted groups showed better antifungal activity and which could seen in the case of **6h, 6e** and **6i**. Whereas the compounds **6a, 6b, 6c, 6d, 6f** and **6j** were showing moderate activity against all the fungal strains when compared to standard drug fluconazole at a concentration of 30 µg $mL^{-1}$ and 50 µg $mL^{-1}$.

**Table 2.** Evaluation of anti-fungal activity of synthesized Isoxazole based Oxadiazoles.




| Compound | R | R1 | R2 | R3 | *A. Niger* | | *A. foetidus* | | *C. albicans* | | *C. Rogosa* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | 30µL | 50µL | 30µL | 50µL | 30µL | 50µL | 30µL | 50µL |
| 6a | H | H | H | H | 05 | 08 | 05 | 07 | 06 | 09 | 06 | 08 |
| 6b | H | Cl | H | H | 03 | 05 | - | 03 | 02 | 03 | - | 06 |
| 6c | H | H | Cl | H | 07 | 10 | 08 | 12 | 08 | 11 | 07 | 10 |
| 6d | H | H | H | Cl | 04 | 04 | - | 05 | - | 04 | 03 | - |
| 6e | H | CH3 | H | H | 09 | 11 | 09 | 12 | 08 | 10 | 09 | 12 |
| 6f | H | H | H | Br | 03 | 05 | 07 | 09 | 05 | 08 | 05 | 07 |
| 6g | H | F | H | H | 07 | 09 | 07 | 08 | 05 | 08 | 07 | 09 |
| 6h | OCH3 | H | Cl | H | 07 | 09 | 07 | 08 | 09 | 11 | 06 | 10 |
| 6i | OCH3 | CH3 | H | H | 04 | 06 | 03 | 07 | 05 | 10 | 05 | 07 |
| 6j | OCH3 | H | H | Br | 03 | 05 | 05 | 06 | 04 | 05 | 04 | 07 |
| | Fluconazole | | | | 05 | 11 | 04 | 10 | 07 | 12 | 09 | 13 |

In vitro Antifungal activity of Compounds (Concentration used 30µg 30µ$L^{-1}$ and used 50µg 50µ$L^{-1}$)

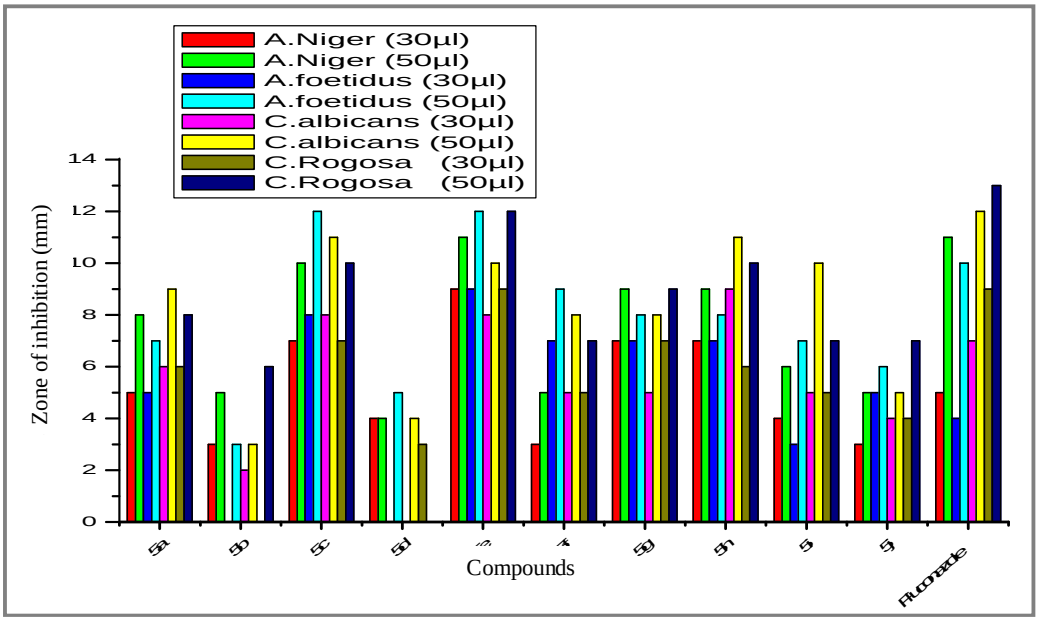



**Figure 2.** Anti fungal activity of compounds **6a-j** against *A. niger, A. foetidus, C. albicans and C. Rogosa*

## CONCLUSIONS

Developed a new and efficient method for the synthesis of substituted 3-phenyl-5-(3-phenylisoxazole-5-yl)-1, 2, 4-oxadiazoles in excellent yields using Cu-HAP as catalyst at heating conditions in DMF media. Compared to other methods, this new method has the lead of good yields, inexpensive reagents, easily available, easy workup, mild reaction conditions, environmentally friendly reaction conditions, reusable catalyst makes this method simple, clean, practical, and economically viable. The *in vitro* antibacterial, antifungal evaluation showed that most of the synthesized substituted 3-phenyl-5-(3-phenylisoxazole-5-yl)-1, 2, 4-oxadiazoles derivatives exhibited moderate to good zone of inhibition. From the results of antibacterial and antifungal activity of compounds it is interesting to note that substituent's like methoxy, methyl and fluoro substituent's shows better antibacterial and antifungal activity compared to other substituted compounds. Noticeably, compound **6h**, **6i**, **6j and 6g** were most potent compound *in vitro* activity against bacterial and fungal strains.

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