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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Recyclable CuO nanoparticles-catalyzed synthesis of novel-2,5-disubstituted 1,3,4-oxadiazoles as antiproliferative, antibacterial, and antifungal agents

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Abstract A series of new 2,5-disubstituted 1,3,4-oxadiazoles have been conveniently synthesized through an oxidative C–O coupling by direct C–H bond activation of *N*-aroyl-*N*-arylidinehydrazines using a catalytic quantity of CuO nanoparticles. Twenty compounds have been synthesized in good to excellent yields (75–90 %). All the synthesized compounds were evaluated for their in vitro antiproliferative, antibacterial, and antifungal activity. Compounds **8d** and **10d** are more promising antiproliferative agents with IC_{50} value of 3.66 and 3.89 μM in MCF-7 cell line, and compounds **8a** and **10a** were showed more potent antifungal activity than standard drug.

Keywords 1,3,4-Oxadiazoles · CuO nanoparticles · Antiproliferative · Antibacterial · Antifungal activity

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Introduction

Among five-membered aromatic heterocycles, 1,3,4-oxadiazoles are of particular interest in different areas of medicinal and pesticide chemistry (Dogan *et al*., 2002; Shi *et al*., 2001). Within drug discovery and development, a number of compounds containing an oxadiazole moiety are in late stage clinical trials, including Zibotentan as an anticancer agent (James and Growcott, 2009), Raltegravir as an antiretroviral drug for the treatment of HIV infection (Summa *et al*., 2008), and ABT-751 as tubulin polymerization inhibitor (Ouyang *et al*., 2006). It is clear that oxadiazoles are having a large impact on multiple drug discovery programs across a variety of disease areas, including anticancer, antibacterial, antifungal, antiviral, analgesic, anti-inflammatory, antihypertensive, anticonvulsant, and anti-diabetic properties (Boström *et al*., 2012; Oliveira *et al*., 2012).

Natural products represent a significant source of inspiration for the design of structural analogs with improved pharmacological profiles. As we know, salicylic acid was identified in willow bark extracts as an active anti-inflammatory compound. To our knowledge, the natural products salicylihalamides A and B are potent inhibitors of the vacuolar ATPase (V-ATPase) and are anticancer agents (Sugimoto *et al*., 2009). In particular, oxadiazoles derived from salicylic acid were established as hypnotic drug Fenadiazole (1), anticancer (2) (Aboraia *et al*., 2006), antiproliferative, EGFR inhibitory (3) (Liu *et al*., 2012), PI3K inhibitory, potential immunosuppressive (4) (Zhang *et al*., 2012), anticonvulsant activities (5) (Zarghi *et al*., 2005a), and oxadiazoles derived from thiosalicylic acid act as benzodiazepine receptor agonists (6) (Zarghi *et al*., 2005b) (Fig. 1).

Due to the biological significance of oxadiazoles that are derived from salicylic acid and thiosalicylic acid, we have

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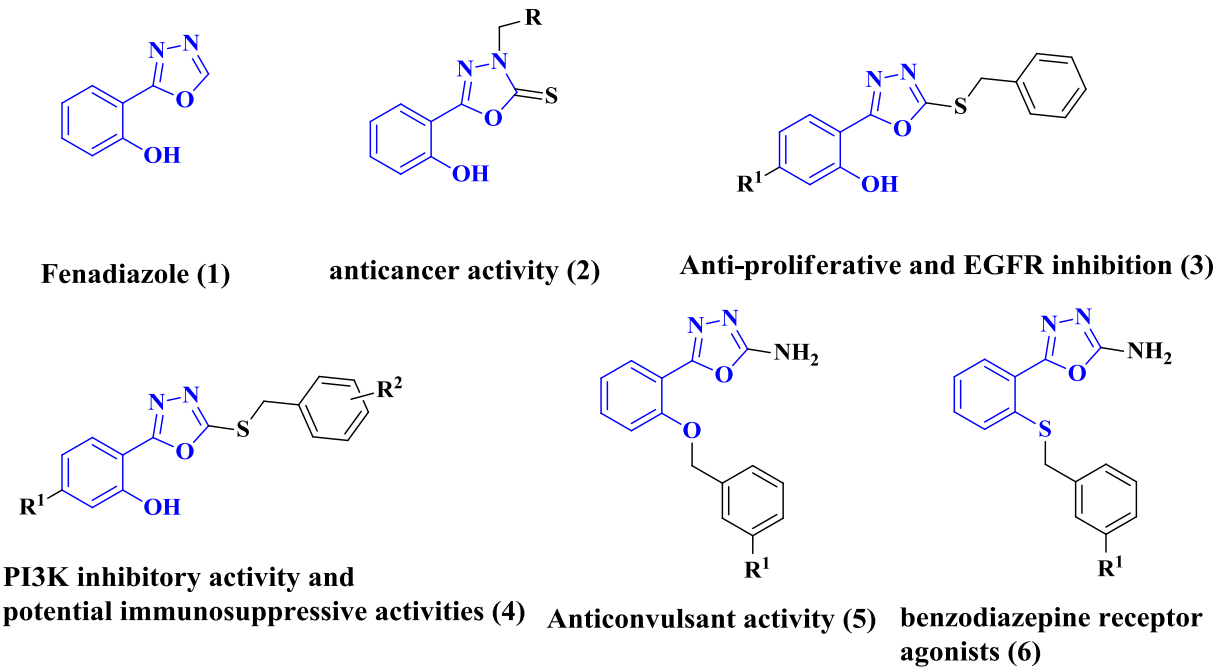


Fig. 1 Representative structure of biological active oxadiazoles derived from salicylic and thiosalicylic acid

developed a new protocol for the synthesis of 2,5-disubstituted 1,3,4-oxadiazoles from salicylic and thiosalicylic acid with an expectation to find more potent antiproliferative, antibacterial, and antifungal agents.

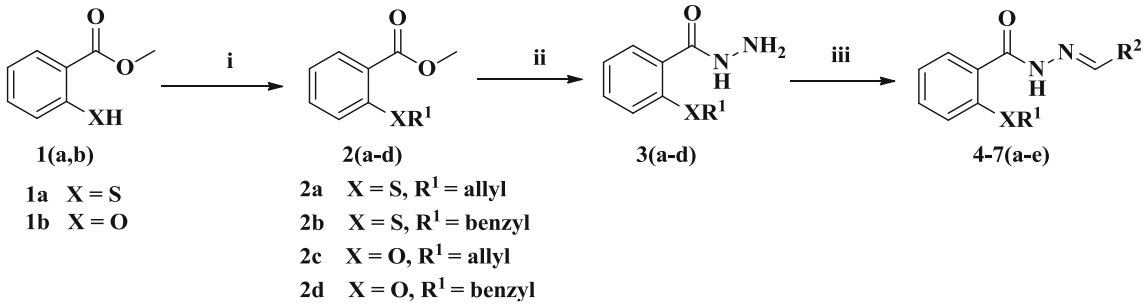
Various reagents have been reported for the synthesis of 2,5-disubstituted 1,3,4-oxadiazoles such as sodium chlorate platinum (Singh *et al*., 2013), hypervalent iodine's (Prakash *et al*., 2010), ceric ammonium nitrate (Dabiri *et al*., 2006), chloramine-T (Jedlovska and Lesko, 1994), lead dioxide in acetic acid (Milcent and Barbier, 1983), bromine/sodium acetate (Werber *et al*., 1977), potassium permanganate (Rostamizadeh and Housaini, 2004), mercuric oxide/iodine (Flidallah *et al*., 2002), trichlorophosphate (Lee *et al*., 2001), phosphorus oxychloride (Al-Talib *et al*., 1990), sulphuric acid (Short and Long, 1969), $Cu(OTf)_2$ (Guin *et al*., 2011), and CuI (Kawano *et al*., 2009).

Yet, the disadvantages associated with these methods are usage of expensive reagents, hazardous materials, multi step processes, long reaction times, and formation of undesired side products. In case of $Cu(OTf)_2$ catalyzed imine C–H functionalization, and C–H activation/arylation of 2-substituted 1,3,4-oxadiazole with CuI seems some advantages, but these catalytic systems i.e., $Cu(OTf)_2$ and CuI are not recyclable.

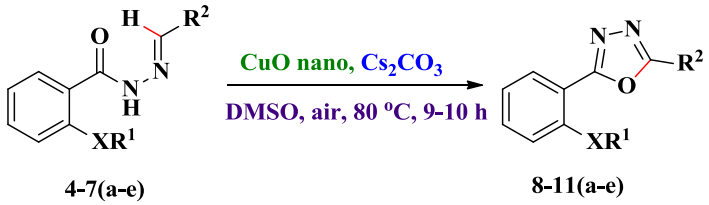
The C–H functionalization strategy has received substantial attention because of its economic, sustainable, and environmentally benign alternative for C–C and C–heteroatom bond formation. In the case of intra-molecular C–H hetero functionalization, heteroatoms act as directing groups as well as intra-molecular nucleophiles, are ideal, and atom-economical processes can be used for the construction of heterocyclic architectures (Stokes and Driver, 2011). A few reports have appeared in the literature on Cu catalyzed, $C(sp^3)$–H, and $C(sp^2)$–H bond activation and its further application to C–O bond formation (Kumar *et al*., 2011; Saberi and Heydari, 2013; Zhao *et al*., 2012; Zhang, 2010). Therefore, it is of utmost importance to develop economically and environmentally more viable procedure for the synthesis of 2,5-disubstituted 1,3,4-oxadiazoles from *N*-aroyl-*N*-arylidinehydrazines utilizing recyclable CuO nanoparticles as catalyst.

Nano CuO is a heterogeneous catalyst and has become an attractive both from economic and industrial points. The CuO nano-catalyzed reactions provide the advantages of high atom efficiency, simplified isolation of product easy recovery, and recyclability of the catalyst in several organic transformations (Babu and Ramasamy, 2011; Rout *et al*., 2012; Kantam *et al*., 2008).

Inspired by the recent advantages of Cu-catalyzed C–H functionalization processes, we hypothesized that inexpensive CuO nano-catalyst should also be capable of promoting C–O cyclization reactions of *N*-aroyl-*N*-arylidinehydrazine derivatives. Below, we describe a new process for the preparation of 2,5-disubstituted 1,3,4-oxadiazoles through aerobic imine $C(sp^2)$–H functionalization starting with *N*-aroyl-*N*-arylidinehydrazines. The methodology relies on a simple reaction system and inexpensive CuO nano as a catalyst.



Scheme 1 Synthesis of *N*-aryl-*N*-arylidinehydrazine derivatives from salicylate or methyl thiosalicylate. Reagents and conditions: (*i*) allyl (or) benzyl chloride, KOH, MeOH, 6 h reflux; (*ii*) $NH_2NH_2.H_2O$, MeOH, 5 h reflux; (*iii*) R^2-CHO, AcOH, EtOH, 2 h reflux



Scheme 2 CuO nanoparticle catalyzed synthesis of 2,5-disubstituted 1,3,4-oxadiazoles **8–11**(**a–e**). Reaction conditions: *N*-aryl-*N*-arylidinehydrazines **4–7**(**a–e**) (1.0 mmol), CuO nanoparticles (0.1 mmol), Cs_2CO_3 (1.0 mmol), in DMSO, 9–10 h at 80 °C

Results and discussion

Chemistry

Considering the biological significance of 1,3,4-oxadiazoles in particular derived from salicylic acid and thiosalicylic acid, we have developed a straightforward and versatile protocol with CuO nano for the synthesis of a novel series 2,5-disubstituted 1,3,4-oxadiazoles. CuO nano-catalyst successfully performs the C–H functionalization followed by an intra-molecular C–O cross-coupling to afford cyclized products from *N*-aroyl-*N*-arylidinehydrazine derivatives.

N-aroyl-*N*-arylidinehydrazine derivatives were synthesized according to Scheme 1. Reaction of methyl salicylate or methyl thiosalicylate **1** with appropriate allyl or benzyl chloride methanolic KOH solution afforded corresponding products **2**(**a–d**). Acid hydrazides **3**(**a–d**) were readily prepared by treatment of **2**(**a–d**) with hydrazine hydrate in methanol (Zarghi *et al*. 2005b). Acid hydrazides **3**(**a–d**) were refluxed with aryl aldehydes in the presence of acetic acid afforded the pure *N*-aroyl-*N*-arylidinehydrazines **4–7**(**a–e**) (Okimoto and Chiba, 1990; Prakash *et al*., 2010).

In a typical procedure, *N*-aroyl-*N*-arylidinehydrazines **4–7**(**a–e**) (1.0 mmol) was carried out with Cs_2CO_3 (1.0 mmol) and commercially available CuO nano (<50 nm particle size) (10 mol %) in DMSO by stirring at 80 °C for 9–10 h, under an air atmosphere. After completion of the reaction (monitored by TLC), expected products **8–11**(**a–e**) were formed in good yields (75–90 %). The structures of the products **8–11**(**a–e**) (Scheme 2, Table 1) were confirmed by NMR and HRMS spectral data.

The occurrence of the above reaction is based on both the catalyst and base. In the absence of either catalyst or base, the reaction did not yield any product even after long reaction times, which suggests that a metal/base combination is required for the reaction to occur.

Optimization of reaction conditions

Initially, the reaction conditions such as solvent, base, amount of base, amount of catalyst, and temperature were optimized. The data are listed in Table 2.

From the data given in Table 2, it is concluded that among the five different solvents such as acetonitrile (CH_3CN), *N*,*N*-dimethylformamide (DMF), dimethyl sulfoxide (DMSO), ethanol (EtOH), and water (H_2O), the solvent dimethyl sulfoxide is an efficient one (Table 2, entries 1–5). Similarly among the various carbonate salts like Na_2CO_3, K_2CO_3 and Cs_2CO_3, Cs_2CO_3 is found to be a better one (Table 2, entries 3, 6 and 7). A 10 mol% amount of CuO nanoparticles is found to be the optimum amount of catalyst (Table 2, entries 3, 10, 11 and 12). Excess or less than 10 mol% CuO decreases the yield of product. It is noted that temperature has significant effect on the efficiency of present catalytic system. The product **11a** was obtained in 90 % yield after stirring at 80 °C in DMSO.

Table 1 Synthesis of 2,5-disubstituted 1,3,4-oxadiazoles **8–11(a–e)**[b,c]

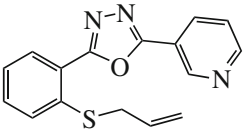
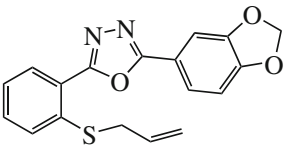
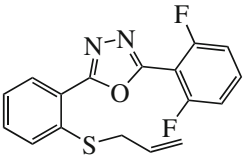
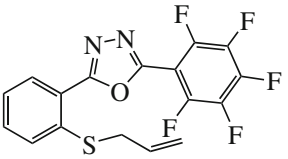
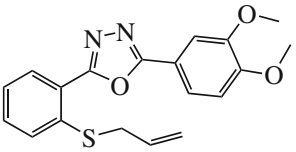
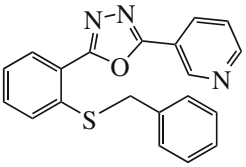
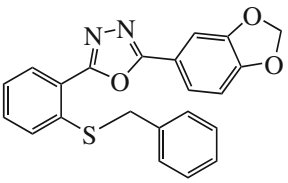
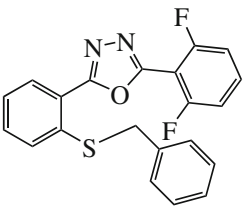
Entry	Compound	Time (h)	Yield (%)[a]	mp (°C)
8a		9.5	83	98–100
8b		10	81	127–129
8c		10	75	76–78
8d		10	77	82–84
8e		10	79	102–104
9a		9	88	176–178
9b		9.5	82	188–190
9c		9	81	118–120

Table 1 continued

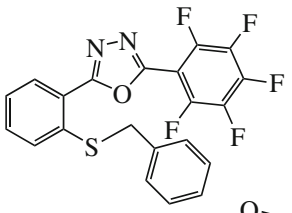
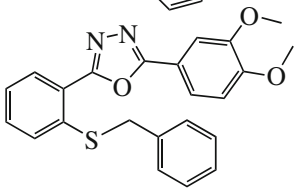
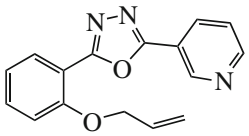
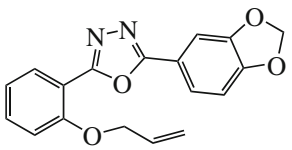
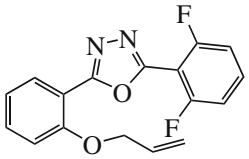
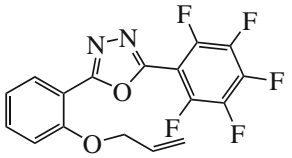
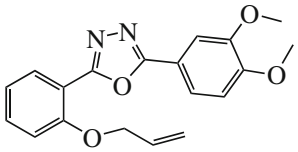
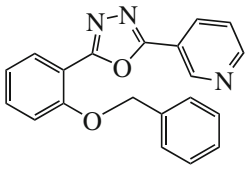
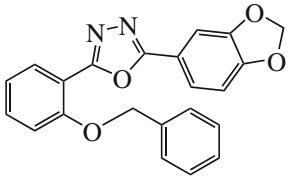
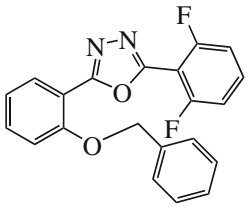
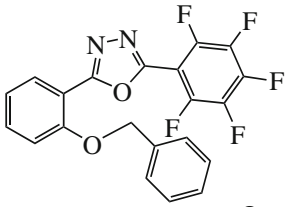
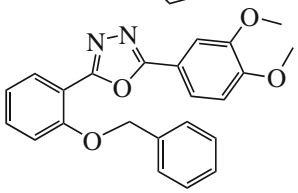
Entry	Compound	Time (h)	Yield (%)[a]	mp (°C)
9d		10	84	138–140
9e		9.5	86	157–159
10a		9.5	85	103–105
10b		10	80	144–146
10c		10	79	79–81
l0d		10	78	117–119
l0e		10	77	109–111
11a		9	90	101–103
11b		9.5	83	177–179

Table 1 continued

Entry	Compound	Time (h)	Yield (%)[a]	mp (°C)
11c		9	85	130–132
11d		10	88	134–136
11e		9.5	84	143–145

[a] Isolated yield

[b] Reaction conditions: *N*-aroyl-*N*-arylidinehydrazines **4–7(a–e)** (1.0 mmol), CuO nano (10 mol%), Cs_2CO_3 (1.0 mmol), in DMSO

[c] Structures of synthesized compounds was confirmed by NMR and HRMS spectral data

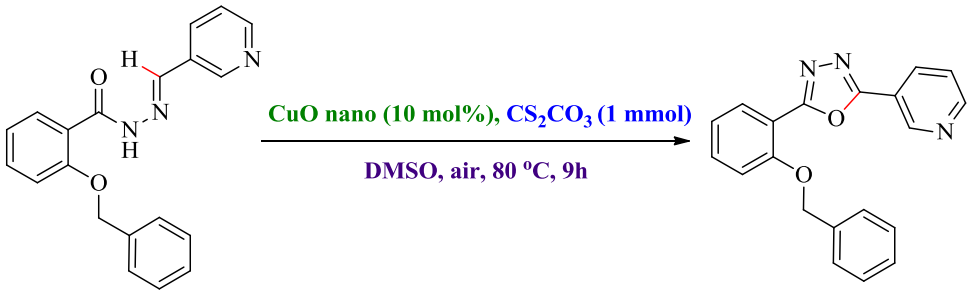
Lower or higher temperature makes the reaction slower (Table 2, entries 13, 14, 15 and 16). When the reaction was operated in nitrogen, the cyclizations were considerably less efficient, suggesting that O_2 played a vital role in the catalytic cycle.

With these results in hand, we sought to examine the scope and generality of the method. *N*-aroyl-*N*-arylidinehydrazines derived from arylaldehydes possessing di-OMe as electron-donating group gave substituted 1,3,4-oxaziazoles **8e**, **9e**, **10e,** and **11e** in good yields. Similarly substrates possessing F as electron-withdrawing group underwent C–H functionalization to give 1,3,4-oxaziazoles **8c**, **8d**, **9c**, **9d**, **10c**, **10d**, **11c**, and **11d** in better yields. *N*-aroyl-*N*-arylidinehydrazines substrates originating from heterocyclic aldehyde like pyridine gave excellent yields of the products **8a**, **9a**, **10a**, and **11a**. Also it was observed that electron-withdrawing substituent on the arylaldehydes improved the yields as in the case of **10c**, **10d**, **11c,** and **11d** probably through an increment of the acidity of the imine proton thereby facilitating the metalation step.

On the basis of observation, a typical mechanism proposed for CuO nanoparticles-catalyzed oxidative C–O coupling by direct C–H bond activation of *N*-aroyl-*N*-arylidinehydrazines is given in Scheme 3. CuO nanoparticles **(A)** bearing high reactive morphologies expectedly form an active five coordinated intermediate **(B)** (Guin *et al*., 2011; Rout *et al*., 2012) under basic condition, which on oxidative addition may give intermediate **(C)** (Zhang, 2010; Guin *et al*., 2011). The intermediate **(C)** undergoes an intra-molecular C–O bond formation via reductive elimination to afford the product and regenerating the catalyst under oxygen atmosphere.

In the next step, the recycling ability of the catalyst was studied, since it is very important for industrial applications. After completion of the first reaction, the reaction mixture was centrifuged to separate the catalyst and washed several times with ethyl acetate and oven dried at 80 °C overnight. A new second reaction was then performed with fresh solvent and reactant under identical conditions. Using this approach, our catalyst could be reused for three times with almost consistent activity (Table 3). After three cycles, the fresh and used catalysts were well characterized by powder XRD, SEM, and TEM. The crystallinity of CuO nanoparticles (NPs) was confirmed by powder X-ray diffraction (Fig. 2), and the observed diffraction peak positions and intensities are in good agreement with the reported values (JCPDS file No. 05-661) and could be indexed with lattice planes. These NPs retained its monoclinic structure with lattice parameters: $a = 4.688$ Å, $b = 3.422$ Å, $c = 5.131$ Å, $\beta = 99.506$, and $V = 82.31$ Å. SEM and TEM techniques were employed to study the

Table 2 Screening of reaction conditions for compound **11a**

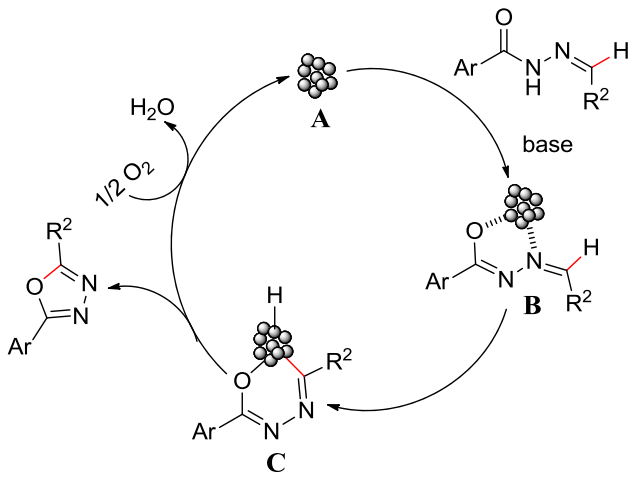


7a **11a (90%)**

S.NO	Solvents	Base	Amount of base (mmol)	Amount of catalyst (mol%)	Temp (°C)	Yield (%)[a]
1	CH_3CN	Cs_2CO_3	1	10	80	45
2	DMF	Cs_2CO_3	1	10	80	82
3	DMSO	Cs_2CO_3	1	10	80	90
4	EtOH	Cs_2CO_3	1	10	80	30
5	H_2O	Cs_2CO_3	1	10	80	Trace
6	DMSO	Na_2CO_3	1	10	80	75
7	DMSO	K_2CO_3	1	10	80	80
8	DMSO	Cs_2CO_3	2	10	80	39
9	DMSO	Cs_2CO_3	3	10	80	26
10	DMSO	Cs_2CO_3	1	0	80	No reaction
11	DMSO	Cs_2CO_3	1	5	80	50
12	DMSO	Cs_2CO_3	1	15	80	48
13	DMSO	Cs_2CO_3	1	10	40	23
14	DMSO	Cs_2CO_3	1	10	60	40
15	DMSO	Cs_2CO_3	1	10	100	32
16	DMSO	Cs_2CO_3	1	10	120	18

All the reactions were performed with 1.0 mmol of 2-(benzyloxy)-*N*'-(pyridin-3-ylmethylene)benzohydrazide

[a] Isolated yield



Scheme 3 Proposed mechanism for CuO nanoparticles-catalyzed oxidative C–O coupling by direct C–H bond activation of *N*-aryl-*N*-arylidinehydrazines

Table 3 Recycling of the catalyst[a]

No of cycle	Yield (%)[b]	recovery (%)
1	90	96
2	87	94
3	85	90

[a] Reactions were monitored by TLC

[b] Isolated yield

morphologies and particle size of the fresh and the recovered catalysts after three cycles as represented in Figs. 3 and 4. Selected Area Electron Diffraction (SAED) patterns shown as inset in Fig. 4 are also in consonance with the power XRD patterns reconfirming that the crystalline nature of the CuO nanoparticles is intact even after three cycles and has excellent reusability and chemical stability.

Pharmacology

The aim of the present study was to illustrate the effect of newly synthesized 2,5-disubstituted 1,3,4-oxadiazoles on the human cell lines, some bacterial and fungal strains in comparison with some standard drugs in a trial to get more potent and less toxic agents.

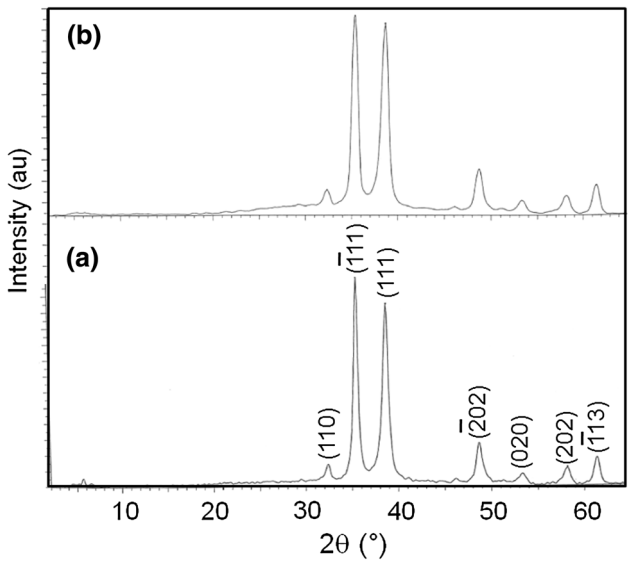


Fig. 2 Powder XRD patterns **a** fresh and **b** after third cycle of CuO nanoparticles

Antiproliferative activity

The prepared oxadiazoles were evaluated to determine their antiproliferative activities against a panel of four different human tumor cells from cervix(HeLa), breast(MCF-7), lung(A549), and neuroblastoma(IMR32) (HeLa, MCF7, A549, and IMR32 were obtained from American Type culture collection) using the sulforhodamine B (SRB) assay method (Reddy *et al*., 2011). Combretastatin A-4 was used as standard drug. The IC_{50} values are listed in Table 4.

From the screening results in Table 4, it was observed that compounds **8–11**(**a–e**) exhibited moderate to good antiproliferative activity. Two of the most active compounds are **8d** and **10d**, with IC_{50} values against the four tested human cancer cell lines ranging from 3.66 to 7.03 μM and 3.89 to 4.86 μM, respectively. Compound **10d** was more cytotoxic than CA4 against all tested four human cancer cell lines, while **8d** was less active than CA4 only against HeLa cells. We have also observed that compound **9d** was more potent against MCF-7 with IC_{50}

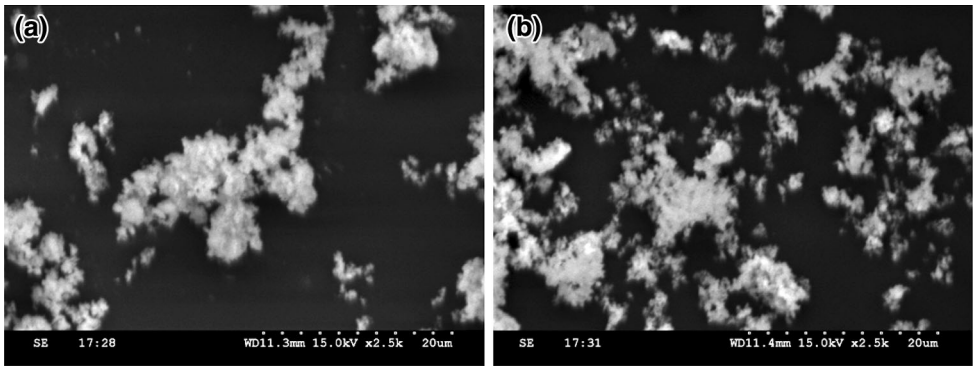


Fig. 3 Scanning Electron Microscope (SEM) images **a** fresh and **b** after third cycle of CuO nanoparticles

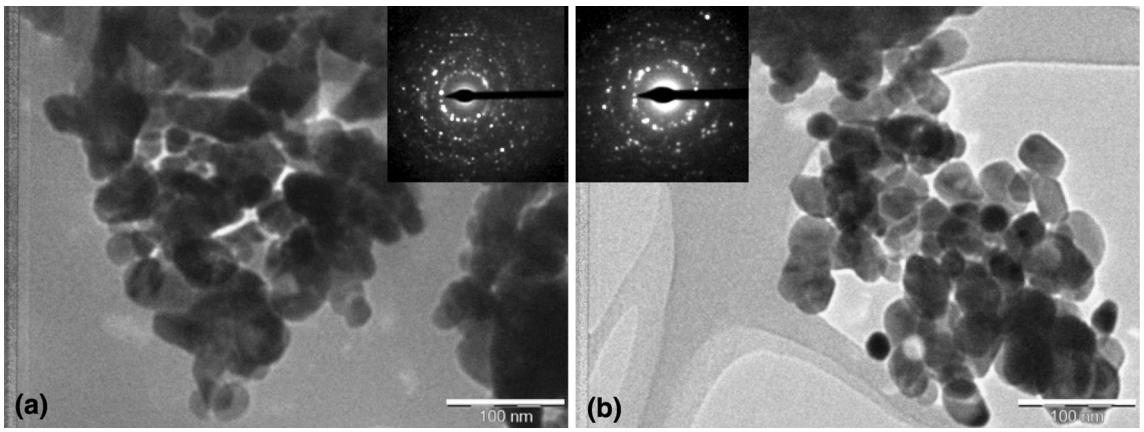


Fig. 4 Transmission Electron Microscope (TEM) images **a** fresh and **b** after third cycle of CuO nanoparticles

Table 4 Cytotoxic effects of compounds **8–11**(**a–e**) on HeLa, MCF7, A549, and IMR32 human cancer Cells[a]

Entry	IC_{50} (μM)[b]			
	HeLa	MCF-7	A549	IMR32
8a	12.69 ± 1.5	11.66 ± 1.9	33.64 ± 1.9	19.61 ± 1.0
8b	21.16 ± 2.5	15.09 ± 2.7	13.32 ± 1.0	29.15 ± 1.6
8c	19.09 ± 2.1	27.3 ± 2.8	12.72 ± 1.2	42.23 ± 9.5
8d	7.03 ± 3.2	**3.66 ± 2.7**	**6.85 ± 2.0**	**4.93 ± 3.9**
8e	32.6 ± 2.3	>100 ± 2.5	10.68 ± 1.3	>100 ± 2.5
9a	27.96 ± 4.4	18.08 ± 1.2	15.04 ± 4.3	>100 ± 6.2
9b	17.6 ± 1.6	28.35 ± 1.6	16.36 ± 1.4	42.58 ± 1.4
9c	30.46 ± 9.2	36.76 ± 1.2	20.03 ± 8.3	80.8 ± 4.8
9d	11.48 ± 2.4	**4.51 ± 2.5**	18.77 ± 1.4	9.22 ± 3.5
9e	25.95 ± 2.7	18.7 ± 1.7	23.03 ± 4.9	55.14 ± 1.5
10a	13.78 ± 1.3	12.92 ± 1.7	24.16 ± 9.8	10.53 ± 6.5
10b	13.4 ± 8.6	9.83 ± 5.8	36.2 ± 3.7	10.2 ± 6.4
10c	9.55 ± 2.0	6.82 ± 2.6	13.58 ± 2.1	8.74 ± 6.5
l0d	**4.46 ± 3.0**	**3.89 ± 3.6**	**4.86 ± 2.1**	**4.28 ± 3.6**
l0e	14.02 ± 2.7	10.02 ± 1.4	29.3 ± 5.2	11.2 ± 3.3
11a	9.55 ± 2.3	8.73 ± 3.6	17.04 ± 1.2	11.95 ± 2.1
11b	18.32 ± 2.3	8.44 ± 2.0	24.96 ± 1.2	13.21 ± 2.1
11c	12.97 ± 1.1	8.14 ± 9.6	23.02 ± 9.3	19.46 ± 1.0
11d	**5.44 ± 2.7**	21.81 ± 4.0	**6.14 ± 2.2**	9.08 ± 2.0
11e	8.75 ± 1.8	6.91 ± 2.0	17.39 ± 1.3	15.13 ± 4.2
CA4[c]	5.36 ± 0.12	4.63 ± 0.15	6.45 ± 0.19	5.72 ± 0.13

The compounds which show good activity are in bold

[a] Cell lines were treated with different concentration of compounds for 48 h as mentioned in materials and methods section. Cell viability was measured employing SRB assay

[b] IC_{50} values (in μM) are indicated as mean ± SD (standard deviation) of three independent experiments

[c] Combretastatin A-4 (CA4) was used as reference drug

Table 5 In vitro Antibacterial activities of oxadiazoles **8–11**(**a–e**) using well-plate method

Entry	Diameter of growth of inhibition zone (mm)[a]			
	E. coli	*B. subtilis*	*M. luteus*	*K. pnemoniae*
8a	24	20	25	23
8b	18	15	17	18
8c	21	14	18	16
8d	17	13	17	13
8e	23	22	25	23
9a	17	16	19	20
9b	0	12	17	13
9c	0	0	15	11
9d	0	0	16	0
9e	14	17	20	18
10a	0	0	14	0
10b	15	20	14	22
10c	16	12	17	14
l0d	15	12	15	14
l0e	20	18	15	17
11a	20	14	15	17
11b	18	15	15	14
11c	14	13	16	13
11d	16	13	17	16
11e	14	13	16	13
Streptomycin	36	33	33	35

[a] Values, including diameter of the well, means of three replicates

value 4.51 μM, and compound **11d** was active against A549, HeLa with IC_{50} value 6.14, 5.44 μM, respectively. In case of **11**(**a–e**) series, all the compounds showed significant cytotoxicity; compounds **11a** and **11e** are showed IC_{50} value ranging from 6.91 to 9.55 μM against MCF7 and HeLa cell lines, while the compounds **11b** and **11c** are showed IC_{50} value 8.44 and 8.14 μM only on MCF7 cell line. All other remaining compounds **8**(**a–c**), **8e**, **9**(**a–c**), **9e**, **10**(**a–c**), **10c,** and **10e** showed moderate activity. Among the synthesized compounds, the compounds having **perfluorophenyl** (**8d**, **9d**, **10d**, **11d**) substitution at 5-position to 1,3,4-oxadiazole ring have shown significant effect against all the cell lines screened. The increased activity is attributed to the presence of **fluorine** atoms (highly electronegative) in the molecules, which increases the lipophilicity and affects the partitioning of molecules into membranes and facilitates hydrophobic interactions of the molecules with specific binding sites on either receptors or enzymes. It has been evident from that fluorine-substituted heterocycles have got significant place in modern medicinal chemistry (Pushpan *et al*., 2012).

Antibacterial activity

All the synthesized compounds **8–11**(**a–e**) were also evaluated for their in vitro antibacterial activity against two gram-positive bacteria namely, *Bacillus subtilis* (MTCC 736), *Micrococcus luteus* (MTCC 106), two gram-negative bacteria namely, *Escherichia coli* (MTCC 40) and *Klebsiella pneumoniae* (MTCC 3384) by using broth dilution method (Prakash *et al*., 2011). Streptomycin was used as a control drug for antibacterial activity. The results of the antibacterial studies are presented in Tables 5 and 6. In general, the antibacterial activity profile differed with type of bacterial strain and nature of compounds. For example, compounds **8a** and **8e** displayed moderate inhibitory activity on both gram-positive and gram-negative bacteria. In case of gram-positive bacteria, **8b**, **9a**, **9e**, **10e,** and **11b** compounds, while in the case of gram-negative bacteria, **8b**, **8c**, **9a**, **10b**, **10e**, **11a**, and **11d** compounds showed

Table 6 Minimum inhibitory concentrations (MIC) (in μg/mL) of compounds **8–11**(**a–e**) using broth dilution method

Entry	MIC (μg/mL)[a]			
	E. coli	*B. subtilis*	*M. luteus*	*K. pnemoniae*
8a	37.5	37.5	37.5	37.5
8b	50	100	75	37.5
8c	50	100	75	75
8d	75	100	75	100
8e	37.5	37.5	37.5	37.5
9a	75	100	50	50
9b	na	100	75	100
9c	na	na	150	150
9d	na	na	150	na
9e	100	75	50	50
10a	na	na	150	na
10b	150	75	100	37.5
10c	150	150	100	100
l0d	150	150	150	100
l0e	75	100	150	75
11a	75	150	75	75
l1b	75	75	75	150
11c	150	150	100	150
l1d	100	150	100	100
l1e	100	150	150	150
Streptomycin	10	12	12	10

[a] Mean of three replicates, na not active

Table 7 In vitro antifungal activities of oxadiazoles **8–11**(**a–e**) using well-plate method

8aEntry	Diameter of growth of inhibition zone (mm)[a]		
	A. niger	*A. terreus*	*A. fumigatus*
8a	40	30	30
8b	15	12	20
8c	0	0	21
8d	15	13	17
8e	0	0	0
9a	0	0	0
9b	0	0	0
9c	0	0	0
9d	0	0	0
9e	0	0	0
10a	**35**	**35**	**30**
10b	15	12	14
10c	14	14	16
10d	15	12	15
10e	20	18	15
11a	20	14	15
11b	18	15	15
11c	14	13	16
11d	16	13	17
11e	14	13	16
Cycloheximide	20	22	25

[a] Values, including diameter of the well, means of three replicates

antibacterial activity ranging from 15 to 20 mm zone, and the compounds **8a**, **8e**, **9a**, **10b**, **10e**, **11a** exhibited efficient zone of inhibition (Table 5). The compounds **8d**, **10c**, **10d**, **11c**, **11e** were possessed moderate efficiency in inhibiting the growth of all tested strains. Further *E.coli* growth was not inhibited by **9b**, **9c**, **9d**, and **10a**. Among all the newly synthesized compounds **8a**, **8e** showed broad spectrum antibacterial activity (for all tested strains MIC 37.5 μg/mL), and compounds **8b**, **10b** showed antibacterial activity against *Klebsiella pneumoniae* (MIC 37.5 μg/mL) (Table 6). Critical evaluation of the above data suggested that each of the synthesized compounds is specific in nature especially in regulating the microbial growth.

Antifungal activity

The in vitro antifungal activity of synthesized compounds **8–11**(**a–e**) was determined against three fungal strains belonging to *Aspergillus genus* by using well-plate method (Prakash *et al*., 2011; Zovko *et al*., 2012). The results are summarized in Table 7. It is evident that among all the compounds **8a**, **8b**, **8d**, **10a**, **10d**, **10e**, **11a**, **11b**, and **11d** showed maximum zone of inhibition. Among these **8a** and **10a**, compounds showed superior antifungal activity as compared with standard drug cycloheximide (Table 7). This manifested that an allyloxy and allylthio group-containing oxadiazole derivatives (**8a** and **10a**) exhibited excellent antifungal activity rather than benzyloxy and benzylthio group containing oxadiazole derivatives (**9a** and **11a**), while the compounds **10b** and **10c** showed promising activity. Compounds **8e**, **9a–e** did not exhibit antifungal activity. The compound **8c** depicted more efficient zone of inhibition against *A. fumigatus* and is not effective against *A. niger* and *A. terreus*. From the data, the compound **8a** revealed 40 mm of inhibitory zone against *A. niger*, while standard antifungal drug cycloheximide showed only 20 mm of inhibitory zone for the same fungal strains. Similar trend has been noticed with compound **10a** (Table 7). The evaluation data indicated that the presence of pyridyl group on the 5-position of oxadiazole moiety and the allyl substituent attached to phenolic or thiophenolic functionality are more effective for antifungal activity. To the best our knowledge, such type of work has not been reported so far.

Experimental protocols

General

Analytical thin-layer chromatography (TLC) was carried out using silica gel 60 F254 pre-coated plates. Visualization was accomplished with UV lamp or I_2 stain. All products were characterized by their NMR and HRMS spectra. The ^{1}H NMR (300 and 500 MHz) spectra were recorded on Bruker Avance spectrometer, and ^{13}C NMR (75 MHz) spectra were recorded on Bruker Avance spectrometers using TMS as an internal standard; Chemical shifts were reported in parts per million (ppm, δ) downfield from tetra methyl silane. ESI and HRMS were recorded on "High Resolution QSTAR XL hybrid MS/MS system, Applied bio systems" under Electron Spray Ionization conditions preparing sample solutions in methanol. Powder X-ray diffraction (XRD) analyses were performed using a Make Bruker, Model: D8-Advance, Detector: Lynx-Eye. Scanning Electron Microscopy (SEM) analyses were performed using a Make: Hitachi S- 3000 N scanning electron microscope. Transmission Electron Microscope (TEM) analyses were performed using Philips TECNAI F12 FEI transmission electron microscope (TEM). Melting points were recorded on Buchi R-*535* apparatus and are uncorrected.

A typical procedure for synthesis of 2,5 disubstituted 1,3,4-oxadiazoles **8–11**(**a**–**e**)

In a typical experiment, the reaction of *N*-aroyl-*N*-arylidinehydrazines **4–7**(**a**–**e**) (1.0 mmol) was carried out with Cs_2CO_3 (1.0 mmol), CuO nanoparticles (10 mol%) in DMSO by stirring at 80 °C for 9–10 h. After completion of the reaction (as monitored by thin-layer chromatography (TLC)), the reaction mixture was centrifuged to separate the catalyst and washed several times with ethyl acetate. The filtrate was concentrated, and the residue was purified by column chromatography on silica gel (hexane/ethyl acetate, 80/20) to afford pure product 2,5-disubstituted 1,3,4-oxadiazoles (75–90 %).

2-(2-(allylthio)phenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole **(8a)**

Light yellow solid; Yield: 83 %; mp 98–100 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.37 (s, 1H, Ar–H), 8.80 (d, $J = 3.7$ Hz, 1H, Ar–H), 8.46 (m, 1H, Ar–H), 8.04 (d, $J = 7.7$ Hz, 1H, Ar–H), 7.54–7.47 (m, 3H, Ar–H), 7.38–7.30 (m, 1H, Ar–H), 5.99–5.84 (m, 1H, =CH), 5.29 (dd, $J = 16.9$, 1.5 Hz, 1H, $=CH_2$), 5.17 (dd, $J = 10.0$, 1.5 Hz, 1H, $=CH_2$), 3.69 (d, $J = 6.6$, 2H, $S–CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.2, 162.3, 152.3, 147.9, 138.1, 134.2 (6 ArC), 132.4 (=CH), 131.5, 130.2, 128.5, 125.8, 125.4, 123.8, 122.5 (7 ArC), 118.7 ($=CH_2$), 36.4 ($S–CH_2$): HRMS : 296.0845 $[M+H]^+$ Calcd for $C_{16}H_{13}N_3OS$: 296.0852.

2-(2-(allylthio)phenyl)-5-(benzo[d][1,3]dioxol-5-yl)-1,3,4-oxadiazole **(8b)**

Light gray solid; Yield: 81 %; mp 127–129 °C; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.0 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.70 (d, $J = 8.0$ Hz, 1H, Ar–H), 7.60 (d, $J = 2.0$ Hz, 1H, Ar–H), 7.50–7.43 (m, 2H, Ar–H), 7.33–7.28 (m, 1H, Ar–H), 6.97–6.92 (m, 1H, Ar–H), 6.07 (s, 2H, $O–CH_2–O$), 5.96–5.86 (m, 1H, =CH), 5.27 (dd, $J = 17.0$, 1.0 Hz, 1H, $=CH_2$), 5.15 (dd, $J = 10.0$, 1.0 Hz, 1H, $=CH_2$), 3.67 (d, $J = 6.0$ Hz, 2H, $S–CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 164.1, 163.3, 150.5, 148.1, 137.8, 132.5 (=CH), 131.0, 129.8, 128.1, 125.1, 122.0, 118.5 ($=CH_2$), 108.7, 106.9, 106.7, 101.7 ($O–CH_2–O$), 36.2 ($S–CH_2$): HRMS : 339.0792 $[M+H]^+$ Calcd for $C_{18}H_{14}N_2O_3S$: 339.0797.

2-(2-(allylthio)phenyl)-5-(2,6-difluorophenyl)-1,3,4-oxadiazole **(8c)**

Light red solid; Yield: 75 %; mp 76–78 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.00 (dd, $J = 7.5$, 3.7 Hz, 1H, Ar–H), 7.57–7.46 (m, 2H, Ar–H), 7.41–7.29 (m, 1H, Ar–H), 7.17–7.07 (m, 3H, Ar–H), 6.00–5.84 (m, 1H, =CH), 5.28 (dd, $J = 16.6$, 1.5, Hz, 1H, $=CH_2$), 5.16 (dd, $J = 9.8$, 1.5 Hz, 1H, $=CH_2$), 3.68 (d, $J = 6.7$ Hz, 2H, $S–CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): 162.4, 158.9 (d, $J = 4.3$ Hz, ArC–F), 133.4, 132.5 (=CH), 131.8, 131.4, 130.5, 130.2, 128.9, 128.3, 126.0, 125.2, 118.5 ($=CH_2$), 112.4, 112.2, 36.3 ($S–CH_2$): HRMS : 331.0705 $[M+H]^+$ Calcd for $C_{17}H_{12}F_2N_2OS$: 331.0711.

2-(2-(allylthio)phenyl)-5-(perfluorophenyl)-1,3,4-oxadiazol **(8d)**

Light yellow solid; Yield: 77 %; mp 82–84 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.03–7.95 (m, 1H, Ar–H), 7.58–7.46 (m, 2H, Ar–H), 7.42–7.28 (m, 1H, Ar–H), 5.99–5.83 (m, 1H, = CH), 5.28 (dd, $J = 16.6$, 1.5, Hz, 1H, $=CH_2$), 5.17 (dd, $J = 9.0$, 1.5 Hz, 1H, $=CH_2$), 3.69 (d, $J = 6.7$ Hz, 2H, $S–CH_2$); ^{13}C NMR ($CDCl_3$, 75 MHz): 160.0, 139.8, 138.6 (d, $J = 4.4$ Hz, ArC–F), 132.4 (=CH), 131.8, 130.3, 128.6, 125.4, 122.0, 118.6 ($=CH_2$), 36.4 ($S–CH_2$): HRMS : 385.0424 $[M+H]^+$ Calcd for $C_{17}H_9F_5N_2OS$: 385.0428.

2-(2-(allylthio)phenyl)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole **(8e)**

Light gray solid; Yield: 79 %; mp 102–104 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.00 (d, $J = 7.5$ Hz, 1H, Ar–H), 7.75–7.65 (m, 2H, Ar–H), 7.47 (t, $J = 2.2$ Hz, 2H, Ar–H), 7.36–7.28 (m, 1H, Ar–H), 6.99 (dd, $J = 8.3$, 2.2 Hz, 1H, Ar–H), 6.00–5.81 (m, 1H, =CH), 5.27 (dd, $J = 18.1$, 1.5 Hz, 1H, $=CH_2$), 5.15 (dd, $J = 11.3$, 1.5 Hz, 1H, $=CH_2$),

4.0 (s, 3H, O–CH_3), 3.97 (s, 3H, O–CH_3), 3.67 (dd, $J = 6.7$, 1.5 Hz, 2H, S–CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.1, 151.7, 148.9, 137.6, 132.3 (=CH), 130.9, 129.6, 127.9, 124.9, 120.2, 118.3 (=CH_2), 116.1, 110.8, 109.1, 55.8 (O–CH_3), 55.7 (O–CH_3), 36.0 (S–CH_2): HRMS : 355.1103 $[M+H]^+$ Calcd for $C_{19}H_{18}N_2O_3S$: 355.1110.

2-(2-(benzylthio)phenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole **(9a)**

Light yellow solid; Yield: 88 %; mp 176–178 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.35 (dd, $J = 2.0$, 0.7 Hz, 1H, Ar–H), 8.79 (dd, $J = 4.9$, 1.7 Hz, 1H, Ar–H), 8.46–8.41 (m, 1H, Ar–H), 8.02 (dd, $J = 7.3$, 1.1 Hz, 1H, Ar–H), 7.53–7.42 (m, 5H, Ar–H), 7.39–7.28 (m, 4H, Ar–H), 4.24 (s, 2H, S–CH_2–Ph); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.3, 162.3, 152.3, 147.9, 138.5, 136.0, 134.2, 131.6, 130.1, 128.8, 128.7, 128.5, 127.4, 125.5, 123.8, 122.5, 120.4 (ArC), 38.4 (S–CH_2–Ph) : HRMS : 346.1001 $[M+H]^+$ Calcd for $C_{20}H_{15}N_3OS$: 346.1008.

2-(benzo[d][1,3]dioxol-5-yl)-5-(2-(benzylthio)phenyl)-1,3,4-oxadiazole **(9b)**

Light Yellow solid; Yield: 82 %; mp 188–190 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.64 (d, $J = 8.1$ Hz, 2H, Ar–H), 7.57–7.45 (m, 4H, Ar–H), 7.40–7.23 (m, 4H, Ar–H), 6.95 (d, $J = 8.1$ Hz, 2H, Ar–H), 6.09 (s, 2H, O–CH_2–O), 4.24 (s, 2H, S–CH_2–Ph); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.1, 163.3, 150.2, 148.1, 137.8, 132.5, 131.0, 129.8, 128.1, 125.1, 122.0, 121.7, 108.7, 107.5, 106.9 (ArC), 101.7 (O–CH_2–O), 36.2 (S–CH_2–Ph) : HRMS : 389.0946 $[M+H]^+$ Calcd for $C_{22}H_{16}N_2O_3S$: 389.0954.

2-(2-(benzylthio)phenyl)-5-(2,6-difluorophenyl)-1,3,4-oxadiazole **(9c)**

White solid; Yield: 81 %; mp 118–120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.99 (d, $J = 7.5$ Hz, 1H, Ar–H), 7.62–7.22 (m, 9H, Ar–H), 7.19–7.06 (m, 2H, Ar–H), 4.23 (s, 2H, S–CH_2–Ph); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.2, 162.3 (d, $J = 4.3$ Hz, ArC–F), 158.8, 156.4, 138.5, 136.0, 133.3, 131.4, 131.2, 130.0, 128.8, 128.4, 127.2, 125.3, 122.2, 112.4, 112.1 (ArC), 38.1 (S–CH_2–Ph): HRMS : 381.0863 $[M+H]^+$ Calcd for $C_{21}H_{14}F_2N_2OS$: 381.0867.

2-(2-(benzylthio)phenyl)-5-(perfluorophenyl)-1,3,4-oxadiazole **(9d)**

Light yellow solid; Yield: 84 %; mp 138–140 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.98 (d, $J = 7.5$ Hz, 1H, Ar–H), 7.49–7.42 (m, 2H, Ar–H), 7.39–7.21 (m, 6H, Ar–H), 4.23 (s, 2H S–CH_2–Ph); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.7, 154.3, 146.9 (d, $J = 6.0$ Hz, ArC–F), 143.3 (d, $J = 3.8$ Hz, ArC–F), 139.7 (d, $J = 6.6$ Hz, ArC–F), 138.9, 135.8, 131.8, 130.1, 128.8, 128.4, 127.3, 125.4, 121.5 (ArC), 38.2 (S–CH_2–Ph): HRMS : 435.0576 $[M+H]^+$ Calcd for $C_{21}H_{11}F_5N_2OS$: 435.0585.

2-(2-(benzylthio)phenyl)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole **(9e)**

White solid; Yield: 86 %; mp 157–159 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.02 (d, $J = 7.3$ Hz, 1H, Ar–H), 7.71 (dd, $J = 8.3$, 1.8 Hz, 1H, Ar–H), 7.64 (d, $J = 1.7$ Hz, 1H, Ar–H), 7.50–7.27 (m, 8H, Ar–H), 6.98 (d, $J = 8.4$ Hz, 1H, Ar–H), 4.23 (s, 2H, S–CH_2–Ph), 3.96 (s, 3H, O–CH_3), 3.93 (s, 3H, O–CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): 151.9, 149.2, 138.2, 136.1, 131.2, 130.0, 128.9, 128.5, 127.3, 125.4, 122.9, 120.5, 116.4, 111.0, 109.5 (ArC), 56.0(O–CH_3), 38.4(S–CH_2–Ph): HRMS : 405.1260 $[M+H]^+$ Calcd for $C_{23}H_{20}N_2O_3S$: 405.1267.

2-(2-allyloxy)phenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole **(10a)**

Yellow solid; Yield : 85 %; mp 103–105 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.35 (d, $J = 1.5$ Hz, 1H, Ar–H), 8.79 (dd, $J = 4.5$, 1.5 Hz, 1H, Ar–H), 8.47–8.42 (m, 1H, Ar–H), 8.09 (dd, $J = 7.5$, 1.5 Hz, 1H, Ar–H), 7.57–7.46 (m, 2H, Ar–H), 7.17–7.05 (m, 2H, Ar–H), 6.20–6.07 (m, 1H, =CH), 5.58 (dd, $J = 17.3$, 1.5 Hz, 1H, =CH_2), 5.36 (dd, $J = 10.5$, 1.5 Hz, 1H, = CH_2), 4.73 (d, $J = 5.2$ Hz, 2H, O–CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.0, 162.2, 156.7, 152.0, 147.7, 133.9 (6 ArC), 133.2 (=CH), 132.3, 130.5, 123.7, 120.9 (4 ArC), 117.8 (=CH_2), 113.1 (ArC), 69.3 (O–CH_2): HRMS : 280.1079 $[M+H]^+$ Calcd for $C_{16}H_{13}N_3O_2$: 280.1080.

2-(2-allyloxy)phenyl)-5-(benzo[d][1,3]dioxol-5-yl)-1,3,4-oxadiazole **(10b)**

Light yellow solid; Yield : 80 %; mp 144–146 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.04 (dd, $J = 7.7$, 1.7 Hz, 1H, Ar–H), 7.68 (dd, $J = 8.1$, 1.5 Hz, 1H, Ar–H), 7.58 (d, $J = 1.5$ Hz, 1H, Ar–H), 7.54–7.45 (m, 1H, Ar–H), 7.14–7.02 (m, 2H, Ar–H), 6.93 (d, $J = 8.3$ Hz, 1H, Ar–H), 6.19–6.08 (m, 1H, =CH), 6.06 (s, 2H, O–CH_2–O), 5.59 (dd, $J = 17.1$, 1.1 Hz, 1H, =CH_2), 5.34 (dd, $J = 10.5$, 1.1 Hz, 1H, =CH_2), 4.71 (d, $J = 4.9$ Hz, 2H, O–CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.1, 163.0, 156.6, 150.3, 148.1, 132.7 (=CH), 132.4, 130.4, 121.8, 120.9, 117.6 (=CH_2), 113.1, 108.7, 106.9, 101.7 (O–CH_2–O), 69.3 (O–CH_2): HRMS : 323.1022 $[M+H]^+$ Calcd for $C_{18}H_{14}N_2O_4$: 323.1026.

2-(2-(allyloxy)phenyl)-5-(2,6-difluorophenyl)-1,3,4-oxadiazole **(10c)**

Light yellow solid; Yield : 79 %; mp 79–81 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.07 (dd, J = 7.5, 2.2 Hz, 1H, Ar–H), 7.62–7.47 (m, 2H, Ar–H), 7.17–7.03 (m, 4H, Ar–H), 6.18–6.03 (m, 1H, =CH), 5.57 (dd, J = 17.3, 1.5 Hz, 1H, =CH_2), 5.31 (dd, J = 10.5, 1.5, 1H, =CH_2), 4.72 (d, J = 4.5 Hz, 2H, O–CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.1, 162.3 (d, J = 4.9 Hz, Ar<u>C</u>–F), 158.9 (d, J = 4.9 Hz, Ar<u>C</u>–F), 156.8, 133.1, 132.3 (=<u>C</u>H), 130.6, 120.8, 120.4, 117.6 (=<u>C</u>H_2), 113.3, 112.4, 112.1, 69.5 (O–<u>C</u>H_2): HRMS : 315.0937 $[M+H]^+$ Calcd for $C_{17}H_{12}F_2N_2O_2$: 315.0939.

2-(2-(allyloxy)phenyl)-5-(perfluorophenyl)-1,3,4-oxadiazole **(10d)**

Light gray solid; Yield : 78 %; mp 117–119 °C; ^{1}H NMR ($CDCl_3$ 300 MHz): δ 8.06 (dd, J = 7.7, 1.5 Hz, 1H, Ar–H), 7.57–7.50 (m, 1H, Ar–H), 7.15–7.04 (m, 2H, Ar–H), 6.17–6.03 (m, 1H, =CH), 5.54 (dd, J = 17.1, 1.3 Hz, 1H, =CH_2), 5.33 (dd, J = 10.5, 1.3 Hz, 1H, =CH_2), 4.72 (d, J = 5.0 Hz, 2H, O–CH_2); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.7, 157.0, 146.9 (d, J = 12.6 Hz, Ar<u>C</u>–F), 133.6, 132.3(=<u>C</u>H), 130.7, 121.0, 117.9(=<u>C</u>H_2), 113.3, 112.4, 69.6(O–<u>C</u>H_2): HRMS : 369.0653 $[M+H]^+$ Calcd for $C_{17}H_{19}F_5N_2O_2$: 369.0657.

2-(2-(allyloxy)phenyl)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole **(10e)**

Light yellow solid; Yield : 77 %; mp 109–111 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.06 (dd, J = 7.4, 2.1 Hz, 1H, Ar–H), 7.70 (dd, J = 8.4, 2.1 Hz, 1H, Ar–H), 7.66 (d, J = 2.1 Hz, 1H, Ar–H), 7.52–7.46 (m, 1H, Ar–H), 7.13–7.03 (m, 2H, Ar–H), 6.98 (dd, J = 8.4, 1.0 Hz, 1H, Ar–H), 6.17–6.08 (m, 1H, =CH), 5.60 (dd, J = 16.9, 2.1 Hz, 1H, =CH_2), 5.32 (dd, J = 10.6, 2.1 Hz, 1H, =CH_2), 4.71 (d, J = 4.2 Hz, 2H, O–CH_2), 3.98 (s, 3H, O–CH_3), 3.96 (s, 3H, O–CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.3, 163.0, 156.5, 151.6, 149.1, 132.7, 132.4 (=<u>C</u>H), 130.4, 120.8, 120.2, 117.5 (=<u>C</u>H_2), 113.0, 110.9, 109.2, 69.2 (O–<u>C</u>H_2), 55.9 (O–<u>C</u>H_3): HRMS : 339.1335 $[M+H]^+$ Calcd for $C_{19}H_{18}N_2O_4$: 339.1339.

2-(2-(benzyloxy)phenyl)-5-(pyridine-3-yl)-1,3,4-oxadiazole **(11a)**

Light gray solid; Yield: 90 %; mp 101–103 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 9.37 (s, 1H, Ar–H), 9.22 (s, 1H, Ar–H), 8.83 (d, J = 4.1 Hz, 1H, Ar–H), 8.75 (d, J = 4.1 Hz, 1H, Ar–H), 8.48–8.41 (m, 1H, Ar–H), 8.22–8.13 (m, 1H, Ar–H), 7.88 (dd, J = 7.7, 1.3 Hz, 1H, Ar–H), 7.61–7.35 (m, 4H, Ar–H), 7.21–7.03 (m, 2H, Ar–H), 5.29 (s, 2H, O–CH_2–Ph); ^{13}C NMR ($CDCl_3$, 75 MHz): 164.6, 161.1, 157.7, 152.7, 147.9, 134.1, 133.3, 130.6, 128.6, 128.1, 127.4, 126.5, 123.8, 121.1, 120.0, 117.7, 113.2 (Ar<u>C</u>), 70.7 (O–<u>C</u>H_2–Ph): HRMS : 330.1234 $[M+H]^+$ Calcd for $C_{20}H_{15}N_3O_2$: 330.1237.

2-(benzo[d][1,3]dioxol-5-yl)-5-(benzyloxy)phenyl)-1,3,4-oxadiazole **(11b)**

Light yellow solid; Yield : 83 %; mp 177–179 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.13 (dd, J = 8.3, 1.5 Hz, 1H, Ar–H), 7.66 (dd, J = 8.3, 1.5 Hz, 1H, Ar–H), 7.59–7.53 (m, 3H, Ar–H), 7.49–7.36 (m, 3H, Ar–H), 7.18–7.11 (m, 2H, Ar–H), 6.94 (d, J = 8.3 Hz, 2H, Ar–H), 6.07 (d, J = 3.7 Hz, 2H, O–CH_2–O), 5.23 (s, 2H, O–CH_2–Ph); ^{13}CNMR ($CDCl_3$, 75 MHz) : 163.9, 156.5, 150.4, 148.2, 136.2, 132.9, 130.5, 128.5, 128.0, 127.4, 121.9, 121.8, 121.0, 113.0, 108.7, 108.6, 106.8 (Ar<u>C</u>), 101.7 (O–<u>C</u>H_2–O), 70.5 (O–<u>C</u>H_2–Ph) : HRMS : 395.1001 $[M+Na]^+$ Calcd for $C_{22}H_{16}N_2O_4$: 395.1002.

2-(2-(benzyloxy)phenyl)-5-(2,6-difluorophenyl)-1,3,4-oxadiazole **(11c)**

White solid; Yield: 85 %; mp 130–132 °C; ^{1}H NMR (CDCl3, 300 MHz): δ 7.59–7.52 (m, 12H, Ar–H), 5.28 (s, 2H, O–CH_2–Ph); ^{13}C NMR ($CDCl_3$, 75 MHz): 161.8 (d, J = 5.4 Hz, Ar<u>C</u>–F), 159.7 (d, J = 4.5 Hz, Ar<u>C</u>–F), 157.5, 133.8, 133.7, 133.6, 130.8, 128.4, 127.7, 126.9, 121.1, 112.5, 112.3, 102.8 (Ar<u>C</u>), 70.5 (O–<u>C</u>H_2–Ph): HRMS : 365.1093 $[M+H]^+$ Calcd for $C_{21}H_{14}F_2N_2O_2$: 365.1096.

2-(2-(benzyoxy)phenyl)-5-(perfluorophenyl)-1,3,4-oxadiazole **(11d)**

Light yellow solid; Yield : 88 %; mp 134–136 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.36 (dd, J = 7.9, 1.8 Hz, 1H, Ar–H), 7.57–7.53 (m, 2H, Ar–H), 7.51–7.48 (m, 3H, Ar–H), 7.43 (m, 1H, Ar–H), 7.18 (t, J = 7.9 Hz, 1H, Ar–H), 7.14 (d, J = 8.2 Hz, 1H, Ar–H), 5.23 (s, 2H, O–CH_2–Ph); ^{13}C NMR ($CDCl_3$, 75 MHz): 161.6, 156.6, 134.9, 134.4 (d, J = 6.5 Hz, Ar<u>C</u>–F), 133.9, 133.0, 129.4, 129.1, 128.5, 122.1, 112.5 (Ar<u>C</u>), 70.5(O–<u>C</u>H_2–Ph): HRMS : 419.0804 $[M+H]^+$ Calcd for $C_{21}H_{11}F_5N_2O_2$: 419.0818.

2-(2-(benzyloxy)phenyl)-5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazole **(11e)**

Light orange solid; Yield: 84 %; mp 143–145 °C; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 8.11 (dd, J = 7.5, 1.5 Hz, 1H, Ar–H), 7.61 (d, J = 1.7 Hz, 1H, Ar–H), 7.58–7.47 (m, 4H, Ar–H), 7.43–7.30 (m, 2H, Ar–H), 7.20–7.08 (m, 2H, Ar–H),

7.06–6.95 (m, 1H, Ar–H), 6.92–6.86 (m, 1H, Ar–H), 5.25 (s, 2H, O–CH_2–Ph), 3.96 (s, 3H, O–CH_3), 3.88 (s, 3H, O–CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): 132.9, 130.6, 128.5, 127.9, 127.1, 121.9, 121.1, 120.3, 113.2, 110.9, 109.3, 108.8, 106.9, 101.8 (Ar<u>C</u>), 70.4(O–<u>C</u>H_2–Ph), 56.0(O–<u>C</u>H_3), 55.9(O–<u>C</u>H_3): HRMS : 389.1499 Calcd for $C_{23}H_{20}N_2O_4$: 389.1495.

In vitro cytotoxic activity studies

The cell lines used in this study (HeLa, MCF-7, A549, and IMR32) were purchased from the American Type Culture Collection (ATCC, United States). All these cell lines were grown in Dulbecco's modified Eagle's medium (containing 10 % fetal bovine serum (FBS) in a humidified atmosphere of 5 % CO_2 at 37 °C). Cells were trypsinized when sub-confluent from T25 flasks/60-mm dishes and seeded in 96-well plates. The synthesized oxadiazoles were evaluated for their in vitro cytotoxicity in four different human cancer cell lines. A protocol of 48 h continuous drug exposure was used, and a SRB cell proliferation assay (Reddy *et al*., 2011) was used to estimate cell viability or growth. The cell lines were grown in the media containing 10 % fetal bovine serum and were seeded into 96-well microtiter plates in 100 µL aliquots at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5 % CO_2, 95 % air, and 100 % relative humidity for 24 h prior to addition of experimental drugs and were incubated for 48 h with different doses (1, 10, 100 µM) of prepared derivatives. After 48 h incubation at 37 °C, cell monolayers were fixed by the addition of 100 µL of 10 % (wt/vol) cold trichloroacetic acid and incubated at 4 °C for 1 h. The supernatant was discarded, and the cells were then stained with 0.057 % SRB dissolved in 1 % acetic acid for 30 min at room temperature. Unbound SRB was washed away with four washes of 1 % acetic acid and the bound SRB stain, representing surviving cells, was dissolved in 50 µL of Tris base (10 mM). The optical density was determined at 510 nm using a microplate reader (Enspire, Perkin Elmer, USA).

Microorganisms and growth conditions

Antimicrobial activity for all synthesized compounds was evaluated using four different bacterial strains viz., *Escherichia coli* (MTCC 40), *Bacillus subtilis* (MTCC 736), *Klebsiella pneumonia* (MTCC 3384), and *Micrococcus luteus* (MTCC 106) and fungal *Aspergillus niger* (MTCC 281), *Aspergillus terreus* (MTCC 1782), and *Aspergillus fumigatus (*MTCC 343) strains. All bacterial strains were grown in nutrient broth (NB), while fungal strains were grown in potato dextrose broth (PDB) for 24 and 48 h, respectively. The inoculated flasks were incubated at 150 rpm at 37 °C for bacterial strains and at 30 °C for fungal strains for active cultures.

The synthesized compounds were dissolved in DMSO to get a final concentration of one mg/ml. In order to ensure that the solvent had no effect on bacterial and fungal growth, a control test was also performed containing inoculated broth supplemented with only DMSO at the same dilution used in the experiments.

Zone of inhibition plate tests

Well-plate method (Prakash *et al*., 2011; Zovko *et al*., 2012) was used for both antibacterial and antifungal activities for measuring the zone of inhibition. All twenty compounds **8–11(a–e)** were used for antimicrobial activity studies. Sterile NB and PDB plates were prepared, and the 30 µL of above test organism fermentation broth was poured on the separate plates and uniformly spread using sterile spreader. Wells were made with sterile cork borer, and each well was loaded with exactly 50 µL of sample. Control (only sterile broth without inoculation) and standard (drug i.e., streptomycin in case of bacterial strains and cycloheximide in case of fungal strains) also placed in separate wells in the similar concentration as that of synthesized compounds i.e., 50 µg per well. The plates were first incubated for 20–30 min at 4 °C to allow the compounds to diffuse into the agar and then subsequently incubated for 24 h at 37 °C for bacteria and 48 h at 30 °C for fungi. After incubation, the zone of growth inhibition was measured using calibrated scale and expressed in mm. Experiment was performed triplicate to minimize the deviations, and average values were reported.

Minimum inhibitory concentration (MIC) (in µg/mL)

The selected bacterial strains viz., *Escherichia coli* (MTCC 40), *Bacillus subtilis* (MTCC 736), *Klebsiella pneumonia* (MTCC 3384), and *Micrococcus luteus* (MTCC 106) were used for this study using broth dilution method (Prakash *et al*., 2011). Exponentially growing cultures of above strains in one ml volume (OD equal to the turbidity of a Mac Farland 0.5 standard tube) were inoculated in one ml NB for bacterial strains. These tubes were supplemented with final compound at a concentration range from 9.37 to 300 µg/mL broth. The tubes were incubated for 12–16 h at 37 °C, and the turbidity of each tube was measured with respect to control. MIC values (the lowest concentration of compound at which growth is completely inhibited) were determined based on the turbidity data.

Conclusion

In conclusion, we have developed an efficient synthetic strategy toward the synthesis of unsymmetrical 2,5-disubstituted 1,3,4-oxadiazoles via oxidative C–O coupling by direct C–H bond activation of *N*-aroyl-*N*-arylidinehydrazines mediated by CuO nano. The catalyst CuO nanoparticle is air-stable, recyclable, and used for three cycles with almost consistent activity. All the synthesized compounds were subjected to in vitro antiproliferative, antibacterial, and antifungal activity. In case of cytotoxic studies, the results revealed that the compounds **8d** and **10d** are the most promising cytotoxic agents with IC_{50} value of 3.66 and 3.89 μM in MCF-7. In case of antimicrobial studies, compounds **8a**, **8e** showed broad spectrum antibacterial activity (for all tested strains MIC 37.5 μg/mL), and the compounds **8a** and **10a** displayed potent activity and exerted antifungal activity better than standard drug. These results are therefore conclusive in showing that 1,3,4-oxadiazoles derived from salicylic acid and thiosalicylic acid bearing **perfluorophenyl** group at 5-position to oxadiazole act as antiproliferative and pyridyl groups at 5-position to oxadiazole act as antimicrobial agents.

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