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Design, Synthesis, Characterization, and Anticancer Properties of Novel Derivatives of 2-(4-t-Butyl)-5-(5-Aryl Substituted-1, 3, 4-Oxadiazol-2-yl) Pyridine

Vinayak Adimule [1,*], Sheetal R Batakurki [2], Basappa C Yallur [3], Rangappa Keri [4]

[1] Department of Chemistry, Angadi Institute of Technology and Management (AITM), Savagaon Road, Belagavi-590009, Karnataka, India; adimulevinayak@yahoo.in (V.A.);
[2] Department of Chemistry, MS Ramaiah University of Applied Sciences, MSR Nagar, New BEL Road, Bangalore, Karnataka, India; sheetalrb@gmail.com (S.R.B.);
[3] Department of Chemistry, M S Ramaiah Institute of Technology, Bangalore-560054, Karnataka, India; yallurbc@gmail.com (B.C.Y.);
[4] Department of Organic and Medicinal Chemistry, Centre for Nano and Material Sciences, Jain University, Bangalore-562112, Karnataka, India; sk.rangappa@jainuniversity.ac.in (R.K.);
* Correspondence: adimulevinayak@yahoo.in (V.A.); researcher@vinayakadimule.in (V.A.);
Scopus Author ID 57203170233

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Abstract: In the present investigation, novel derivatives of 1, 3, 4-oxadiazoles were synthesized by a linear synthetic method and characterized by LCMS, ^{1}H-NMR, and ^{13}C spectroscopic analysis. The compound 2-(4-t-butyl-phenyl)-5-(5-aryl substituted-[1, 3, 4] oxadiazol-2-yl)-pyridine has been synthesized by oxidative cyclization reaction, and all the aryl derivatives were purified by column chromatography. The key precursor 6-(4-t-butyl-phenyl)-nicotinic acid hydrazide reacted with various substituted aldehydes (a-f) and obtained Schiff base derivatives. The Schiff base derivatives (7a-7f) were screened for MTT assay against HeLa, MCF7and Caco-2 cell lines. IC_{50} (inhibitory concentration at 50) was obtained by plotting the percentage viability and concentration of the compounds graph. Among the synthesized novel derivatives of 1, 3, 4-oxdiazoles, two compounds, 7b, and 7d exhibited better cytotoxicity against MCF7 cell lines with IC_{50} of 6.74 µM and 3.69 µM. Compounds 7c, 7d, and 7f showed moderate cytotoxicity against Caco-2 with IC_{50} of 24.6 µM, 55.5 µM, and 24.8µM, respectively.

Keywords: MCF7; 1, 3, 4-oxadiazoles; HeLa; cytotoxicity; Caco-2.

1. Introduction

In recent years, heterocyclic compounds, especially 1,3,4-oxadiazole, attracted much attention from medicinal chemists for their unique physical and chemical properties having medicinal applications [1]. Among various oxadiazoles, 1,2,4 and 1,3,4 have been of prime importance in pharmaceutical industries such as antibacterial, antifungal, antitumoral, neuroprotective, antidiabetics, etc. [2-6]. Various derivatives of 1,3,4 oxadiazole compounds were synthesized to understand the chemical properties, which enrich the biological activity of the parent oxadiazole moiety [7-9]. It was observed that introducing a sulfur-containing group, or thiazole [10-12] adjacent to the nitrogen or oxygen in1,3,4 oxadiazole resulted in enhanced biological activity. Pyridine containing 1, 3, 4-oxadiazoles derivatives have been studied for their anti-inflammatory [13], antimicrobial [14,15], anti-inflammatory and chemopreventive

effects [16], antitumor [17] and anticancer [18,19] properties. Additionally, the magnetic anisotropy and G-quadruplex binding property of 1,3,4-oxadiazole linked to pyridine have been studied [20,21]. Specifically, the groups attached at a second position of the pyridine ring and 3rd position containing 1, 3, 4-oxadiazole moiety exhibited enhanced (Fig. 1, (A)) [22,23] anticancer properties. Based on the above literature evidence author has synthesized novel derivatives of 1, 3, 4-oxadiazoles at the fifth position of the pyridine ring (Fig. 1, (B)) containing 4-tertiary butyl phenyl group. An oxidative cyclization reaction was performed to synthesize novel 1, 3, 4-oxadiazole compounds using a catalytic amount of Chloramine T. The synthetic pathway consists of 6- bromo nicotinic acid, which is converted into 6-(4-tertiary butyl phenyl)-nicotinic acid ethyl ester. Thus obtained ester was further treated with hydrazine hydrate and obtained key intermediate 6-(4-tertiary butyl-phenyl)-nicotinic acid hydrazide. The reaction of hydrazide with various aldehydes (in the presence of a catalytic amount of acetic acid) resulted in the formation of novel Schiff base derivatives (6a-6f). The obtained Schiff base compounds were cyclized, and desired novel derivatives of 2-(4-t-butyl-phenyl)-5-(5-aryl substituted-[1, 3, 4] oxadiazol-2-yl)-pyridine (Fig. 1, (C)) 7a-7f were obtained in good yield. The author envisaged that novel 1, 3, 4-oxadiazole derivatives at the fifth position of the pyridine [6,7] (Fig. 1, (B) rings may enhance the anticancer properties. The synthesized compounds were characterized by LCMS, ^{1}HNMR, and ^{13}C spectroscopic analysis. The novel derivatives of 1, 3, 4-oxadiazoles have been studied for their anti-proliferative activity [24] against three different cell lines: HeLa, MCF-7, and Caco-2. The standard drug used for screening was 5-FU[1]. The results of (IC_{50}), the compounds' MTT assay [25], were compared with standard drug 5-FU.

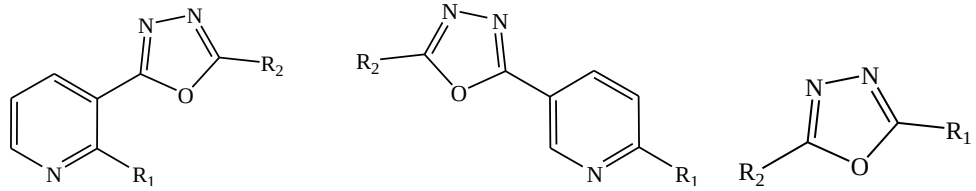


Figure 1. Structures of pyridine containing 2-substituted-3-[1, 3, 4] oxadiazoles analogues (A); pyridine containing 2-substituted-5-[1,3,4] oxadiazole derivatives (B);2,5-disubstituted pyridine (C).

[1] 5-fluorouracil

2. Materials and Methods

2.1. Step 1: Synthesis of 6-bromo-nicotinic acid (2).

The 2-bromo-5-methyl-pyridine (15g, 0.0873mol) and pyridine:water mixture (100 ml:100 ml) were taken in a 1L round bottom flask (RB) flask. KOH (14.65g, 0.0261mol) and $KMnO_4$ (68.96g, 0.0436 mol) were added, and the reaction mixture was refluxed overnight. After completing the reaction, the solvent was removed, and the residue was dissolved in 100 mL of water, stirred, and filtered. The filtrate was treated with concentrated HCl to $p^H = 2$, and the separated precipitate was filtered, washed with cold water, and dried. Yield 10g; m.p-109-115 °C; MS (ESI) *m/z* [M-H] 201; ^{1}H-NMR (400 MHz, $CDCl_3$- δ) 7.04 (dd, 1H, Ar-H), 9.47(dd, 1H), 9.76(m, 1H), 10.8 (broad singlet, 1H, OH).

2.2. Step 2: Synthesis of 6-bromo-nicotinic acid ethyl ester (3).

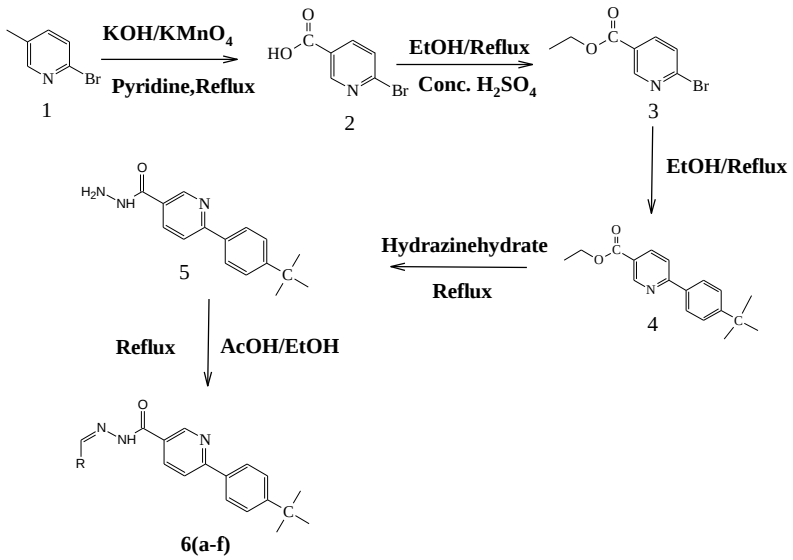
The synthesis involved using 6-bromo-nicotinic acid (2) (10g, 0.0434 mol), ethanol (100 ml), conc. H_2SO_4 (10 drops) were added to 500 mL RB flask. The RM was refluxed for 8h. After 8h, TLC was monitored to confirm the completion of the reaction. The contents of the flask evaporated to dryness, diluted with 10 ml of ice-cold water, and added sodium bicarbonate (NaHCO3) solution (10%). The product was extracted with 25 ml of ethyl acetate two times, followed by 10 ml of brine wash to the ethyl acetate. The moisture content in ethyl acetate extract was removed by adding 1g of sodium sulfate, and the ethyl acetate layer evaporated completely under the vacuum color of the crude product-pale yellow syrup. Yield 8.5g; MS (ESI); m/z-231[M+H] +; ^{1}H-NMR (400MHz, $CDCl_3$- δ_H) 1.18 (t, 3H, CH_3), 3.85 (q, 2H, CH_2), 7.04 (dd, 1H, Ar-H), 9.47 (dd, 1H), 9.76 (m, 1H).

2.3 Step 3: Synthesis of 6-(4-tertiary butyl-phenyl)-nicotinic acid ethyl ester (4)

The 6-bromo-nicotinic acid ethyl ester (8.5 g, 0.0369), K_2CO_3(15.27 g, 0.1107), tetrakis triphenyl palladium (0) (0.213 g, 0.000185 mol),4-tertiary butyl phenylboronic acid (5.166g, 0.0369mol) were added to 500 ml RB flask, the RM was refluxed for overnight TLC monitored for completion of starting material, entire contents were evaporated to dryness, 20 ml of ice cold water was added, the product was extracted with 25 ml of ethyl acetate for 2 times. The ethyl acetate layer was given 10 ml of brine wash, dried over 1g of sodium sulfate, and evaporated completely under a vacuum. A pale yellow-colored crude product was purified by using silica gel (100-200 mesh) (column chromatography). The solvent system used was 100 % n-hexane and increased up to 25 % ethyl acetate. Yield 5.2g; m.p-124-127 °C.

2.4. Step 4: Synthesis of 6-(4-tertiary butyl-phenyl)-nicotinic acid hydrazide: (5).

The 6-(4-tertiary butyl-phenyl)-nicotinic acid ethyl ester (4) [26] (5.2g, 0.0211mol), and10 ml of hydrazine hydrate, 50ml of ethanol were added in 100 ml RB flask and contents refluxed overnight. TLC was monitored for completion of starting material, and the solvent was evaporated to dryness. The residue was treated with ice-cold water under constant stirring. Solids were separated, filtered, and dried. Yield 3.1g; m.p-167-168°C.



Scheme 1. Synthetic reaction pathway of intermediate Schiff bases derivatives (6a-6f).

2.5. General procedure for the synthesis of Schiff base derivatives of 6-(4-tertiary butyl -phenyl)- nicotinic acid hydrazide: (6).

Typical synthesis involves a reaction between hydrazide (6) and aldehydes (a-f) under stirring using absolute ethanol as solvent. To the RM, 3-5 drops of acetic acid were added, and RM was refluxed for 1-3 h. TLC showed completion of the reaction, and the solvent was completely removed under vacuum. The residue was poured over ice-cold water, precipitate thus separated was filtered and dried (Scheme 1).

2.6. Analytical data of the compounds (6a-6f).

5-[5-(4-fluoro biphenyl-phenyl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-fluoro-phenyl)-pyridine (6a):

White solid; yield 49 %; m.p-111-115 ^{0}C; MS (ESI) m/z- 449.5 $[M+H]^+$; anal. Calculated for $C_{29}H_{24}FN_3O$; C, 77.49; H, 5.38; F, 4.23; N, 9.35; O, 3.56; ^{1}H NMR: δ 7.31 (2H, ddd, *J* = 8.7, 1.4, 0.5 Hz), 7.44 (2H, ddd, *J* = 8.5, 1.4, 0.5 Hz), 7.53-7.88 (5H, 7.60 (ddd, *J* = 8.7, 1.5, 0.5 Hz), 7.72 (ddd, *J* = 8.2, 7.6, 0.4 Hz), 7.82 (ddd, *J* = 8.5, 1.7, 0.5 Hz)), 8.07-8.24 (4H, 8.13 (dt, *J* = 7.6, 1.6 Hz), 8.15 (dt, *J* = 8.2, 1.6 Hz), 8.16 (dd, *J* = 8.3, 0.5 Hz), 8.18 (dd, *J* = 8.3, 1.6 Hz)), 8.47 (1H, td, *J* = 1.5, 0.4 Hz), 9.36 (1H, dd, *J* = 1.6, 0.5 Hz).

5-[5-(2, 5-Dimethoxy-phenyl)-[1,3,4]oxadiazol-2-yl]-2-(4-teriary butyl -phenyl)-pyridine: (6b)

Pale brown solid; yield 79 %; m.p-147-152^{0}C; MS (ESI) m/z- 415.4 [M+H]+; anal. Calculated for C_{25} H_{25} N_3O_3; C, 72.84; H, 6.27; N, 10.14; O, 11.72; 1H NMR: δ 1.36 (9H, s), 3.76 (3H, s), 3.94 (3H, s), 6.97 (1H, dd, J = 8.6, 1.5 Hz), 7.12 (1H, dd, J = 8.6, 0.4 Hz), 7.36 (2H, ddd, J = 8.1, 1.5, 0.4 Hz), 7.50 (1H, dd, J = 1.5, 0.4 Hz), 7.74 (2H, ddd, J = 8.1, 1.6, 0.4 Hz), 8.12-8.34 (2H, 8.18 (dd, J = 8.3, 0.5 Hz), 8.27 (dd, J = 8.3, 1.5 Hz)), 9.05 (1H, dd, J = 1.5, 0.5 Hz).

2-(4-tertiary butyl -phenyl)-5-[5-(3-methyl-thiophen-2-yl)-[1, 3, 4] oxadiazol-2-yl]-pyridine :(6c)

Off white solid; yield 59 %; m.p-165-166 ^{0}C; MS (ESI) m/z-375.4 $[M+H]^+$; anal. calculated for $C_{22}H_{23}N_3OS$; C, 70.08; H, 5.59; N, 11.46; O, 4.74; S, 8.50; 1H NMR: *δ* 1.27 (9H, s), 2.30 (3H, s), 7.24-7.42 (3H, 7.30 (d, J = 5.1 Hz), 7.36 (ddd, J = 8.2, 1.4, 0.4 Hz)), 7.73 (1H, d, J = 5.1 Hz), 7.93 (2H, ddd, J = 8.2, 1.6, 0.4 Hz), 8.12-8.35 (2H, 8.18 (dd, J = 8.3, 0.5 Hz), 8.29 (dd, J = 8.3, 1.5 Hz)), 9.07 (1H, dd, J = 1.5, 0.5 Hz).

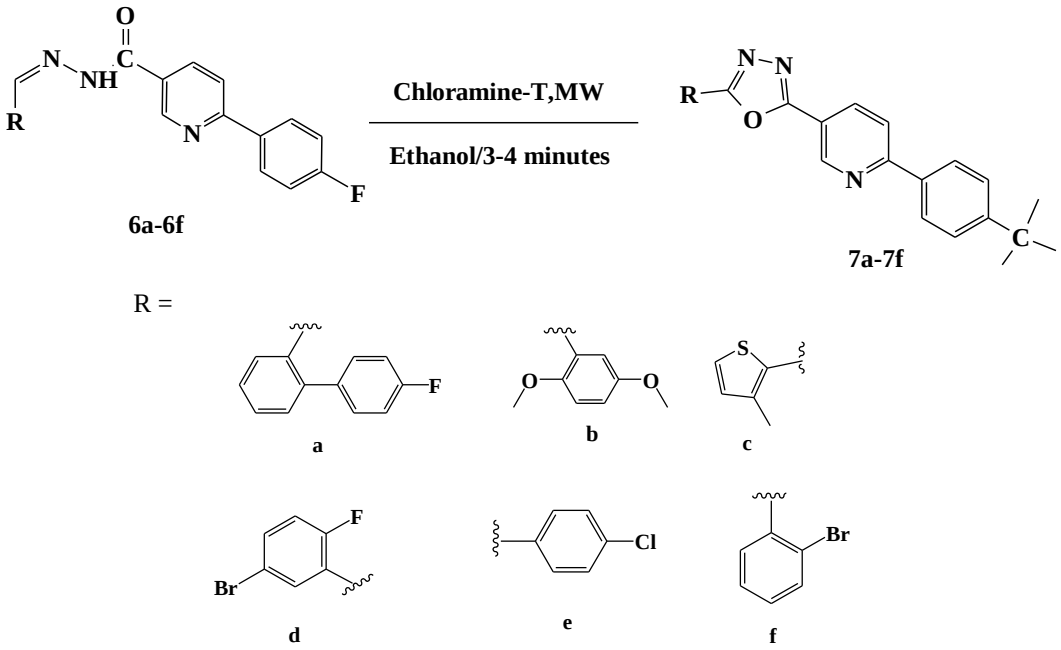
5-[5-(5-bromo-2-fluoro-phenyl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-tertiary butyl -phenyl)-pyridine :(6d)

White solid; yield 88 %; m.p-194-195 ^{0}C; MS (ESI) m/z- 452.3 $[M+H]^+$; anal. Calculated for $C_{23}H_{19}BrF$ N_3O; C, 61.09; H, 4.43; Br, 17.29; F, 4.17; N, 09.14; O, 3.86; ^{1}H NMR: *δ* 1.27 (9H, s), 2.30 (3H, s), 7.24-7.42 (3H, 7.30 (d, J = 5.1 Hz), 7.36 (ddd, J = 8.2, 1.4, 0.4 Hz)), 7.73 (1H, d, J = 5.1 Hz), 7.93 (2H, ddd, J = 8.2, 1.6, 0.4 Hz), 8.12-8.35 (2H, 8.18 (dd, J = 8.3, 0.5 Hz), 8.29 (dd, J = 8.3, 1.5 Hz)), 9.07 (1H, dd, J = 1.5, 0.5 Hz).

5-[5-(4-chloro-phenyl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-fluoro-phenyl)-pyridine (6e): White solid; yield 48%; m.p110-114 0C; MS (ESI) m/z-389.8 $[M+H]^+$; anal. Calculated for $C_{23}H_{20}ClN_3O$; C, 70.87; H, 5.15; Cl, 9.08; N, 10.95; O, 4.55; ^{1}H NMR: δ 1.35 (9H, s), 7.34 (2H, ddd, J = 8.1, 1.5, 0.4 Hz), 7.88-8.08 (6H, 7.94 (ddd, J = 8.1, 1.7, 0.4 Hz), 8.00 (ddd, J = 8.3, 1.6, 0.4 Hz), 8.02 (ddd, J = 8.3, 1.8, 0.4 Hz)), 8.16 (1H, dd, J = 8.3, 0.5 Hz), 8.29 (1H, dd, J = 8.3, 1.5 Hz), 9.32 (1H, dd, J = 1.5, 0.5 Hz).

5-[5-(2-bromo-phenyl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-tertiary butyl-phenyl)-pyridine (6f):

Brown liquid; yield 89 %; m.p-187-195 °C; MS: (ESI) m/z- 434.3 $[M+H]^+$; anal. Calculated for $C_{23}H_{20}BrN_3O$; C, 63.60; H, 4.80; Br, 18.17; N, 9.61; O, 3.04 ; 1 H NMR: δ 1.35 (9H, s), 7.35 (2H, ddd, J = 8.1, 1.5, 0.4 Hz), 7.43-7.58 (2H, 7.50 (ddd, J = 8.1, 7.6, 1.6 Hz), 7.51 (td, J = 7.6, 1.1 Hz)), 7.81-7.94 (3H, 7.87 (ddd, J = 8.1, 1.7, 0.4 Hz), 7.88 (ddd, J = 8.1, 1.1, 0.4 Hz)), 8.03 (1H, ddd, J = 7.6, 1.6, 0.4 Hz), 8.10-8.34 (2H, 8.16 (dd, J = 8.3, 0.5 Hz), 8.28 (dd, J = 8.3, 1.5 Hz)), 9.32 (1H, dd, J = 1.5, 0.5 Hz)



Scheme 2. Synthesis of 2-(4-t-butyl-phenyl)-5-(5-aryl substituted-[1, 3, 4] oxadiazol-2-yl)-pyridine (7a-7f).

2.7. General procedure for the synthesis of 2-(4-t-butyl-phenyl)-5-(5-Aryl substituted-[1, 3, 4] oxadiazol-2-yl)-pyridine :(7).

The Schiff base derivatives (7a-7f) and a catalytic amount of chloramine-T were taken in 10mL ethanol, and RM was irradiated with microwave radiation in an interval of 30 seconds up to 2 minutes, TLC monitored the reaction; after completion of the reaction, RM was diluted with ice cold water and the product was extracted with ethyl acetate (10ml × 3 times), washed with brine (10ml) and concentrated under reduced pressure. The crude product was purified by column chromatography using silica gel 100-200mesh, solvent 100 % n-hexane was used, and the polarity was increased up to 50 % using ethyl acetate (Scheme 2).

2.7.1. Analytical data.

5-[5-(4'- tertiary butyl-biphenyl-2-yl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-fluoro-phenyl)-pyridine: 7(a). ^{1}H NMR: δ 1.47 (9H, s), 7.40-7.60 (5H, 7.46 (ddd, J = 8.1, 1.6, 0.4 Hz), 7.49 (tdd, J = 7.4, 1.8, 1.5 Hz), 7.53 (dddd, J = 7.9, 7.4, 1.7, 0.5 Hz)), 7.80 (2H, dddd, J = 7.9, 1.6, 1.5, 0.5 Hz), 7.91-8.36 (8H, 7.98 (ddd, J = 8.0, 2.2, 0.4 Hz), 8.09 (ddd, J = 8.1, 1.7, 0.4 Hz), 8.14 (ddd, J = 8.0, 1.5, 0.4 Hz), 8.25 (dd, J = 8.3, 0.5 Hz), 8.29 (dd, J = 8.3, 1.6 Hz)), 9.37 (1H, dd, J = 1.6, 0.5 Hz). Off white solid; yield %; m.p-194-196^{0}C; MS (ESI) m/z-450.1 $[M+H]^+$; anal. Calculated for $C_{29}H_{24}FN_3O$; C, 77.99; H, 5.68; F, 4.24; N, 9.21; O, 3.89.

2.8. Cytotoxic evaluation.

2.8.1. MTT assay.

The *in vitro* anti-proliferative MTT assay was carried out against three human carcinoma cell lines: HeLa, MCF-7, and Caco-2. Cell lines were grown over DMEM-HG added with 10 % FBS, Penicillin-Streptomycin (2%), and 2.5 µg/mL Amphotericin-B solution (All from HI Media Labs, Mumbai, India). The culture solution was then added, followed by incubating cells at 37 °C in a humidified atmosphere of 95 % air, 5 % CO_2 for 48hThe adherent cells were detached using Trypsin-EDTA solution (HI Media Labs, Mumbai, India). Cell count was determined using the Luna automated cell counter. Cytotoxicity of the synthesized compounds tested using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) (MTT) assay [20].

2.8.2. Cell-viability assay (MTT assay).

The 200 µL cell suspension was seeded in 96-well microplates (Corning®, USA) at a density of 25,000 cells/well and incubated for 24 h; a concentration range of 50 µM-500 µM was used in the experiment, and cell lines seeded in duplicates. The microplate was then incubated in a CO_2 incubator at 37 °C. After incubation cells were treated with 10 % MTT (5mg/ml; HI media labs, Mumbai, India) for 3 h. The culture medium was then aspirated with 200 µL dimethyl sulfoxide. 5-Fluorouracil (5-FU) was used as a standard drug. Cell viability was determined by measuring the absorbance on a microplate reader (Spectro star nano, bmg lab tech, Germany) at 570 nm. Cell viability was calculated as a percentage of viable cells at different test concentrations relative to the control (5-FU) (Fig. 2(a). [% cell viability = (A570 of treated cells / A570 of control cells) ×100%].

3. Results and Discussion

3.1. Chemistry.

Novel derivatives of 2-(4- tertiary butyl phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine [27,28] (7a-7f) were synthesized, characterized, and evaluated their cytotoxic properties against *HeLa*, *MCF7*, and *Caco-2* cell lines. Synthetic chemistry was performed with 2-Bromo-5-methyl-pyridine as starting material which was then converted into 6-chloronicotinic acid (2) (confirmed by shifting of the CO in IR ~1180 cm^{-1}). The compound (2) was further converted into an ester (3) by reaction with concentrated sulphuric acid and ethanol. An ester (3) was coupled with 4-fluoro phenyl boronic acid by the Suzuki-Mayora coupling reaction [29]. The compound thus obtained (4) [11] was converted into the corresponding carbohydrazide [29,30] by refluxing with hydrazine hydrate and ethanol [29] (confirmed by IR absorbance of NH ~3385 cm^{-1}) and (appearance of broad NH_2 peak at δ 4.15). The intermediate 6-(4-tertiary butyl-phenyl)-nicotinic acid hydrazide was reacted with aldehydes (a-f) and was converted further into Schiff base compounds (6a-6f). The Schiff base compounds were cyclized in the presence of chloramine T [31-33] and obtained final 1, 3, 4-oxadiazoles (7a-7f). In this research work, the author has synthesized novel derivatives of 2-(4- tertiary butyl phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine and screened for their percentage cell viability (anticancer activity) against three leukemic cell lines (MTT assay). The different substituted 1, 3, 4-oxadiazoles [34-36] pyridine derivatives showed a wide range of viability.

The *in vitro* anticancer activities of the compounds were expressed in the form of inhibitory concentration (IC_{50}). In this context, the author substituted the 4-fluorophenyl group at the second position of the pyridine ring and constructed the 1, 3, 4-oxadiazole derivatives at the fifth position of the pyridine ring [25]. Upon screening, these novel derivatives of oxadiazoles showed moderate to good cytotoxicity against *Caco-2* and *MCF7* cell lines.

3.2. Biology.

Initially, these 1, 3, 4-oxadiazoles compounds were screened for MTT assay studies. The results were expressed in the form of concentration of the compounds that are required to inhibit the growth of 50 % of the viable cells. The IC_{50} of the compounds was compared with the IC_{50} of the standard drug used (5-FU). Compounds 7b and 7d exhibited better cytotoxicity against *MCF7* cell lines with IC_{50} of 6.74 µM and 3.69 µM (Fig. 2 (b), (c), and (d), respectively. The compounds 7c, 7d, and 7f showed moderate cytotoxicity against *Caco-2* cell lines with IC_{50} of 24.6 µM, 55.5 µM, and 24.8 µM, respectively. Overall, the synthesized1, 3, 4-oxadiazoles derivatives showed better cytotoxicity against *MCF7* cell lines than standard drug 5-FU (Table 1).

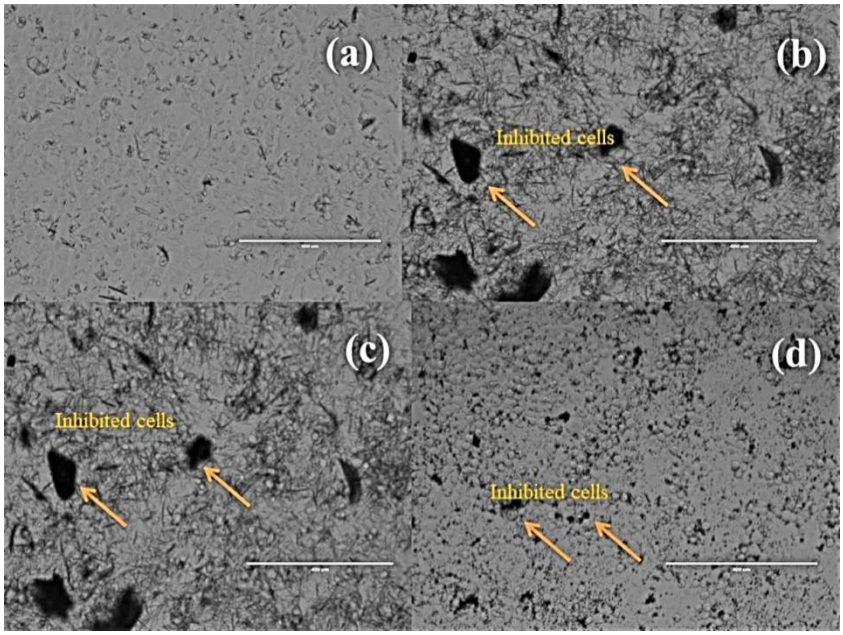


Figure 2. Inhibition cell lines (a) for blank; (b), (c), and (d) for potent compounds 7b and 7d with respect to MCF-7 cell lines.

Table 1. IC_{50} values of the synthesized novel 2-(4-tertiary butyl phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine.

Serial No.	Products	Ar (R)	IC_{50} values of 1, 3, 4-oxadiazoles (µM)		
			HeLa	***Caco-2***	***MCF7***
1	**7a**	4-F-$C_{12}H_8$	96.5	112.8	23.5
2	**7b**	2,5-OCH_3 C_8H_3	118.3	123.1	6.74[1]
3	**7c**	C_5H_5S	132.4	24.6[2]	113.8
4	**7d**	2-F-5-Br C_6H_3	122.4	55.5	3.69*
5	**7e**	4-ClC_6H_4	22.7	123.6	89.9
6	**7f**	2-BrC_6H_4	112.5	24.8	126.7
7		5-FU[a]	5.6	8.8	7.8

[a]5-Fluoro uracil (Standard) used in the screening (MTT assay), IC_{50}- The inhibitory concentration of the compounds at 50%. *Potent molecules; [1] Moderate potent

4. Conclusions

In summary, novel derivatives of 2-(4-fluorophenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine (7a-7f) were synthesized by linear synthetic method. The synthesized novel 1, 3, 4-oxadiazole compounds showed better cytotoxicity against *MCF7* and *Caco-2* cell lines. The compounds exhibited moderate cytotoxicity against all three cell lines compared to the cytotoxicity of 5-FU. The IC_{50} of compounds 7b and 7d against *MCF7* was found to be 6.74 µM and 3.69 µM, respectively. Compounds 7c, 7d, and 7f showed moderate cytotoxicity against *Caco-2* cell lines with IC_{50} of 24.6 µM, 55.5 µM, and 24.8 µM, respectively. 5-fluorouracil (5-FU) is used as a standard drug. The oxadiazole ring at the 5^{th} position of the pyridine moiety could be the reason for better water solubility and enhanced anticancer properties of the as-synthesized 1,3,4-oxadiazole derivatives.

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Conflicts of Interest

We declare that this manuscript has no conflict of financial interests (political, personal, religious, ideological, academic, intellectual, commercial, or otherwise) for publication.

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