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Design, Synthesis, Characterization and Cancer Cell Growth-Inhibitory Properties of Novel Derivatives of 2-(4-Fluoro-phenyl)-5-(5-Aryl Substituted-1, 3, 4-Oxadiazol-2-yl) Pyridine

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Authors' contributions

This work was carried out in collaboration between all authors. Author AV designed the study, Synthesis, characterization purification and studies of anticancer activity. Author MS wrote the protocol and wrote the first draft of the manuscript. Author AHJ Managed the Proton and Carbon NMR studies. Author KSL managed the literature searches, analyses of the study performed the spectroscopy analysis and managed the experimental process. All authors read and approved the final manuscript.

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ABSTRACT

Aims: A series of novel 2-(4-Fluoro-phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine derivatives 7a-7e were synthesized and evaluated for their cancer cell growth inhibitory properties.
Study Design: Designed and synthesized the novel derivatives of pyridine containing 1, 3, 4-oxadiazole ring based on the initial SAR studies of pyridine and their diversified biological properties.
Place and Duration of the Study: Synthesis, purification was performed at centre for scientific research and advanced learning, Mount Carmel College, Autonomous, Bangalore, India (between June 2013 to March 2014), characterization using ^{1}H-NMR and ^{13}C NMR was done at Indian Institute of Science (IISc), Bangalore, India. Cytotoxic evaluation was performed at Genelon Institute of Life Sciences, Yalahanka, Bangalore, India.
Methodology: A mixture of 6-Bromo-nicotinic acid ethyl ester with 4-Fluoro-phenyl boronic acid in ethyl alcohol afforded the 6-(4-Fluoro-phenyl)-nicotinic acid ethyl ester (4). Subsequent reaction of (4) with hydrazine hydrate in ethanol afforded 6-(4-Fluoro-phenyl)-nicotinic acid hydrazide. Reaction with compound (5) and various aldehydes furnished the novel Schiff base derivatives (6a-6e). Schiff base derivatives were cyclized using Chloramine-T under microwave irradiation and obtained novel derivatives 7a-7e. All the reagents, chemicals and solvents were purchased from S-d fine and spectro chem Ltd. Bengaluru. India. ^{1}H-NMR and ^{13}CNMR were recorded by Brucker 400 MHz spectrophotometer. Melting points were determined using Buchi melting point 545. Mass spectra were recorded by Agilent 1200 series. TLC was done on F254 grade silica 60 from Merck. IR spectra were recorded by using FTIR (1800S) series. Microwave used was whirlpool semi-automated specially designed. The human cancer cell lines were purchased from NCCS (National Centre for Cell Science), Pune, India.
Results: Compounds 7b and 7d showed better activity than 5-Fluorouracil against MCF7 cell line. Title compounds 7a-7e is prepared by the oxidative cyclization reaction of the corresponding Schiff base compounds 6a-6e using chloramine-T as promoter. Cytotoxicity of the 1, 3, 4-oxadiazole compounds were obtained by screening the compounds against human cancer cell lines using MTT assay. Among the various derivatives (7a-7e), compounds bearing 5-bromo-2-fluoro-phenyl ring and 3-methyl thiophene ring demonstrated potent antiproliferative activity. In this research work compounds 7b and 7d showed good cytotoxicity against MCF7 cell line.
Conclusion: Novel derivatives of 1, 3, 4-oxadiazole compounds namely, 5-[5-(5-Bromo-2-fluoro phenyl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-fluoro-phenyl)-pyridine (7b) and 2-(4-Fluoro-phenyl)-5-[5-(3-methyl-thiophen-2-yl)-[1, 3, 4] oxadiazol-2-yl]-pyridine (7d) showed better anticancer properties against MCF7 cell line with IC_{50} of 6.9 µM and 3.8 µM respectively. Cytotoxicity of the compounds (7c), (7d) against Caco-2 cell line with IC_{50} of 23.6 µM and 56.5 µM respectively. Rest all the compounds showed more resistance against all the cell lines.

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

1. INTRODUCTION

Several novel derivatives of pyridine containing 1, 3, 4-oxadiazole moiety have been studied for their antiinflammatory [1], antimicrobial [2], antitumor [3] and anticancer [4,5] properties. Especially the groups attached at C2 position of the pyridine ring containing 1, 3, 4-oxadiazole moiety at C3 position (Fig. 1.1, A) showed cancer cell growth inhibitory properties. It was envisaged that by synthesizing novel derivatives of 1, 3, 4-oxadiazole compounds at C5 position of the pyridine ring (Fig. 1.1, B) containing 4-Fluoro-phenyl group at C2 position could enhance the cytotoxicity. The novel 1, 3, 4-oxadiazole compounds has been obtained by the oxidative cyclization reaction of the intermediate Schiff base compounds [6] using Chloramine-T as promoter. The synthesis follows the conversion of 6-bromo nicotinic acid into 6-(4-fluoro-phenyl)-nicotinic acid ethyl ester which is further converted into reactive carbohydrazide [7,8]. The carbohydrazide thus obtained was converted into corresponding novel Schiff base derivatives (6a-6e) by treating with different aldehydes (a-e) in presence of catalytic amount of acetic acid. The obtained Schiff base compounds [9,10] were cyclized and obtained the desired novel derivatives of 2-(4-fluoro-phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine

(Fig. 1.1, C). It was envisaged that the novel 1, 3, 4-oxadiazole derivatives at the C5 position of the pyridine (Fig. 1.1, B) ring may enhance the potency of the molecules and may possess anticancer properties. The synthesized compounds were characterized by LCMS, ^{1}H-NMR, ^{13}C NMR spectral analysis. The novel 1, 3, 4-oxadiazoles were screened for their antiproliferative activity [10] against five different cancer cell lines namely HeLa, MCF7, Caco-2, HepG2 *and* SK-N-SH. The results (Table 1) of (IC_{50}) MTT assay of the compounds were compared with 5-FU.

2. EXPERIMENTAL DETAILS

2.1 Synthesis of 6-Bromo-nicotinic Acid (2)

A stirred mixture of 2-bromo-5-methyl-pyridine (15 g, 0.0873 mol, 1 equiv), KOH (14.65 g, 0.0261 mol) and $KMnO_4$ (68.96 g, 0.0436 mol) in pyridine-water (100 mL: 10 mL) was refluxed for 12 h. The reaction mixture was allowed to cool to room temperature and insoluble material was collected by filtration. Filtrate was acidified with 1N Hydrochloric acid. Precipitates that are separated out was filtered, washed with ice cold water and dried. Yield 10 g (66 % yield); m. p.109-115°C; MS (ESI): *m/z*: [M-H] 201; ^{1}H-NMR (400 MHz, $CDCl_3$): *δ* ppm 7.04 (dd, 1H, phenyl), 9.47 (dd, *J* = 12.3Hz, 1H, phenyl), 9.76 (m, 1H), 10.8 (bs,1H, hydroxy).

2.2 Synthesis of 6-Bromo-nicotinic Acid Ethyl Ester (3)

A mixture of 6-bromo-nicotinic acid (**2**) (10g, 0.0434 mol, 1equiv) and 10 drops of conc. H_2SO_4 in EtOH (100 mL) was refluxed for 8h. TLC (Thin layer chromatography) was monitored to check the completion of the reaction. Solvent was removed under vacuum, residue was diluted with ice cold water and neutralized with 10% $NaHCO_3$ solution. The aqueous was extracted with ethyl acetate (25 mL × 2), organic layer was washed with brine (10 mL) and dried using Na_2SO_4. Purification by column-chromatography afforded the analytically pure product 6-bromonicotinic acid ethyl ester as pale yellow syrup. Yield 8.5 g (85 % yield); MS (ESI): *m/z*: 231 $[M+H]^{+}$; ^{1}H-NMR (400MHz, $CDCl_3$): *δ* ppm 1.18 (t, 3H, ester CH_3), 3.85 (q, 2H, ester CH_2), 7.04 (dd, *J* = 7.8 Hz,1H, phenyl), 9.47 (dd, *J* = 12.2 H, 1H), 9.76 (m,1H, phenyl).

2.3 Synthesis of 6-(4-Fluoro-phenyl)-nicotinic Acid Ethyl Ester (4)

To a mixture of 6-bromo-nicotinic acid ethyl ester (**3**) (8.5 g, 0.0369 mol, 1 equiv), K_2CO_3 (15.27 g, 0.1107 mol), Tetrakis (triphenyl phosphine) palladium (0) (0.213 g, 0.000185 mol), 4-Fluoro-phenyl boronic acid (5.166 g, 0.0369 mol) in EtOH (100 mL) was refluxed for 10 h. The reaction mixture was cooled to room temperature, ethanol was removed under reduced pressure. Residue was diluted with water and the aqueous was extracted with ethyl acetate (25 mL × 3), washed with brine (10 mL) and dried using Na_2SO_4. Purification by column-chromatography afforded the analytically pure product 6-(4-Fluoro-phenyl)-nicotinic acid ethyl ester (4) as yellow syrup. Yield 5.2g (61 % yield); m. p.124-127°C; MS (ESI) *m/z*: 246 $[M+H]^{+}$; ^{1}H-NMR (400MHz, $CDCl_3$); *δ* ppm 2.18 (t, 3H, *J* = 13.2 Hz, ester CH_3), 3.97 (q, 2H, ester CH_2), 7.14 (dd, 2H, *J* = 8.5 Hz), 8.47 (dd, 2H, phenyl), 8.76 (m,3H, phenyl).

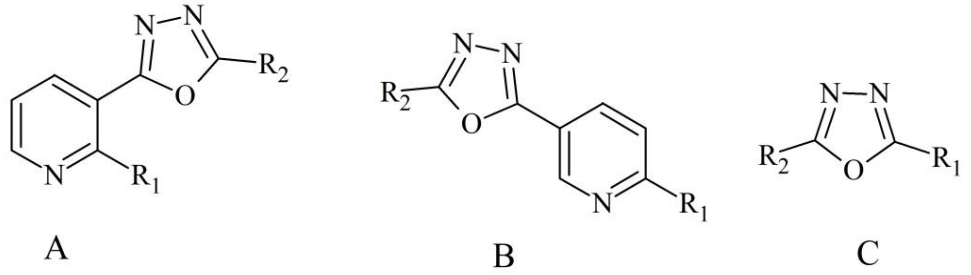


Fig. 1.1. Structures of pyridine containing 2-substituted-3-[1, 3, 4] oxadiazoles analogues (A); pyridine containing 2-substituted-5-[1, 3, 4] oxadiazole derivatives (B); General structure of 2, 5-disubstituted 1, 3, 4-oxadiazole (C)

2.4 Synthesis of 6-(4-Fluoro-phenyl)-nicotinic Acid Hydrazide (5)

To a mixture of 6-(4-Fluoro-phenyl)-nicotinic acid ethyl ester (**4**) (5.2 g, 0.0211 mol, 1 equiv) and hydrazine hydrate (10 mL) in EtOH (50 mL) was refluxed for 10h. Reaction mixture was cooled to room temperature. Solvent was completely removed under vacuum, residue was added with ice cold water and stirred, precipitate that are separated out was filtered, washed with ice cold water and air dried. Yield 3.1 g (59% yield); m. p. 167-168°C; MS (ESI) *m/z*: 232 $[M+H]^+$; ^{1}H-NMR (400MHz, $CDCl_3$): *δ* ppm 2.18 (t, 3H, ester CH_3),3.97 (q, 2H, ester CH_2), 4.15 (bs, 2H, amine), 7.14 (dd, 2H, phenyl), 8.47 (dd, *J* = 11.6Hz, 2H, phenyl), 8.76 (m, 3H, phenyl).

2.5 General Procedure for the Synthesis of Schiff Base Derivatives of 6-(4-Fluoro-phenyl)-nicotinic Acid Hydrazide (6)

A mixture of appropriate carbohydrazide (**6**)(1mmol, 1equiv) and different aldehydes (**a-e**) (1.1 equiv) and 3-5 drops of acetic acid in EtOH (10 mL) was refluxed for 1-3 h. TLC was monitored to check the completion of the reaction, after completion, solvent was completely removed under vacuum. The residue was poured over ice cold water and the precipitate separated out was filtered, washed with water (10 mL) and air dried.

2.6 General Procedure for the Synthesis of 2-(4-Fluoro-phenyl)-5-(5-Aryl substituted-[1, 3, 4] oxadiazol-2-yl)-pyridine (7)

To a mixture of appropriate Schiff base compounds 7a-7e and chloramine-T (1.5 equivalent) in EtOH (10 mL) was irradiated with microwaves in microwave oven for a period of 2 min, TLC was monitored to check the completion of the reaction, reaction mixture was diluted with ice cold water and aqueous was extracted with ethyl acetate (10 mL × 3), washed with brine (10 mL) and evaporated under reduced pressure. Purification by column-chromatography afforded using (silica gel 100-200mesh, solvent 100% n-hexane 50% ethyl acetate) afforded the analytically pure compounds (Fig 1.2) of 7a-7e.

2.7 Reaction Scheme

Linear synthesis of 2-(4-Fluoro-phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine 7a-7e.

Reagents and conditions: i) KOH, $KMnO_4$, H_2O Reflux; ii) Conc.H_2SO_4, Ethanol Reflux; iii) 4-Fluoro-phenyl boronic acid, K_2CO_3, Tetrakis (triphenyl phosphine) palladium (0), ethanol Reflux ;iv) Hydrazine hydrate, ethanol Reflux ;v) Acetic acid, ethanol reflux.

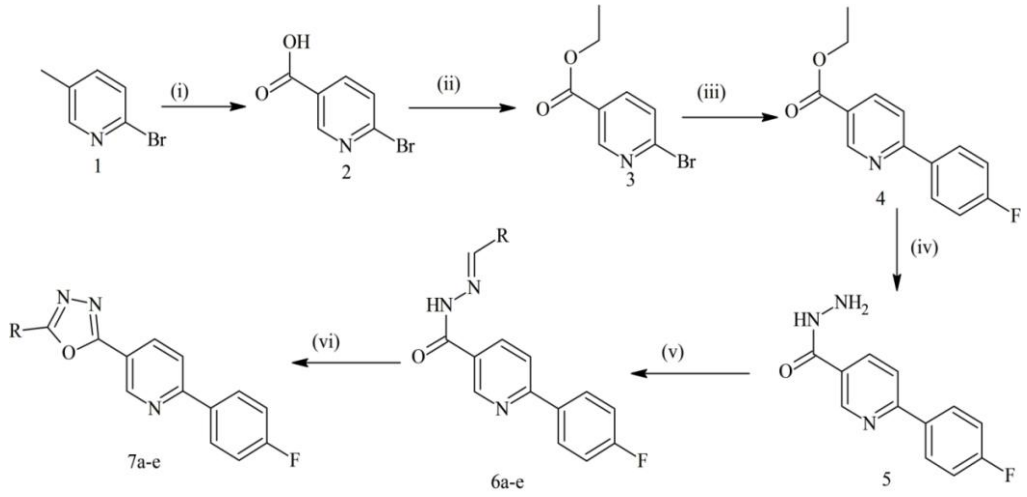


Fig. 1.2. Synthesis of Novel Pyridine 2-(4-Fluoro-phenyl)-5-1, 3, 4- Oxadiazoles

2.8 Analytical Data of the Novel Derivatives of 2-(4-Fluoro-phenyl)-5-(5-Aryl Substituted-1, 3, 4-Oxadiazol-2-yl) Pyridine

2.8.1 5-[5-(2, 5-Dimethoxy-phenyl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-fluoro-phenyl)-pyridine (Fig. 1.5, 7a)

The mixture was separated by column-chromatography [EtOAc: Hexane (5:5)] to afford 7(b) as pale brown solid (34 mg, 73% yield); m.p.121-124^{0}C;^{1}H-NMR (400MHz, $CDCl_3$): δ ppm 2.5 (dd, 3H, J = 13.6 Hz, methoxy), 3.51(dd, 3H, J = 13.6 Hz, methoxy), 3.76 (s, 2H, phenyl), 6.66 (d, 2H, J = 7.2 Hz, phenyl), 7.42 (m, 2H, Ar-H), 7.45 (m, 2H, phenyl), 7.54 (m, 2H, phenyl), 8.8 (dd, 2H, phenyl); ^{13}C NMR (100 MHz, $CDCl_3$): 45.1, 52, 77, 107.8, 115.3, 130, 138.6, 152, 157, 164 ;IR (KBr, ν_{max}/cm^{-1}): 896, 1280, 2786, 2945, 3276,3450; MS (ESI) *m/z*: 378 $[M+H]^+$; $C_{20}H_{14}FN_3O_2^+$: 347.3 (67%), $C_{13}H_8FN_3O^+$ (43%): 241.2, $C_{11}H_8FN^+$: 173.1 (8%); Anal. Calculated for $C_{21}H_{16}FN_3O_3$; C, 66.84; H, 4.27; F, 5.03; N, 11.14; O, 12.72; found C, 66.86; H, 4.29; F, 5.07; N, 11.15; O, 12.74.

2.8.2 5-[5-(5-Bromo-2-fluoro-phenyl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-fluoro-phenyl)-pyridine (7b)

The mixture was separated by column-chromatography [EtOAc: Hexane (7:3)] to afford 7(d) as white solid (66 mg, 86% yield); m. p 163-165^{0}C; ^{1}H-NMR (400MHz, $CDCl_3$): δ ppm 7.22 (dd, 2H, phenyl), 7.38 (dd, 2H, phenyl), 7.549 (m, 2H, J = 8.4 Hz, phenyl), 7.75 (dd, 1H, phenyl), 8.15 (dd,2H, phenyl), 9.31(s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): 77.1,117.3, 119.7, 121.7, 129.6, 135.8, 144.3, 147, 158.8, 162.9; IR (KBr ν_{max}/cm^{-1}): 861, 1290, 2785, 2895,3350, 3496; MS (ESI) *m/z*: 414.2 $[M+H]^+$;$C_{19}H_{11}F_2N_3O^+$: 335.3 (72%), $C_{13}H_8FN_3O$+ (22%): 241.2, $C_{11}H_8FN^+$: 173.1(9%) : Anal. Calculated for $C_{19}H_{10}BrF_2N_3O$; C, 55.09; H, 2.43; Br, 19.29; F, 9.17; N, 10.14; O, 3.86; Found C, 55.12; H, 2.46; Br, 19.34; F, 9.21; N, 10.17; O, 3.91.

2.8.3 5-[5-(4'-Fluoro-biphenyl-2-yl)-[1, 3, 4] oxadiazol-2-yl]-2-(4-fluoro-phenyl)-pyridine 7(c)

The mixture was separated by column-chromatography [EtOAc: Hexane (6:4)] to afford 7(a) as off white solid (28 mg, 63% yield); m.p.189-192°C; ^{1}H-NMR (400MHz, $CDCl_3$): δ ppm 7.2 (m, 3H, J = 7.5 Hz, phenyl), 7.62-7.72 (m, 3H, phenyl), 7.78 (m, 2H, Hphenyl),7.81 (m, 2H, Phenyl), 7.94 (dd, J = 7.6 Hz, 2H, phenyl), 8.15 (dd, 2H, phenyl), 8.59 (s, 1H, phenyl), 9.43 (s, 1H, NH); ^{13}C NMR (100MHz, $CDCl_3$): 21.6, 29.8, 77.1, 116.3, 124.07, 126.54, 128.9, 129.9, 130.6, 136.5, 141.5, 143, 146, 165; IR (KBr, ν_{max}/cm^{-1}): 894, 1168, 2765, 2935, 3320, 3346; MS (ESI) *m/z*: 412.5 $[M+H]^+$; $C_{19}H_{12}FN_3O^+$: 317.3 (65%), $C_{13}H_8FN_3O^+$ (43%): 241.2, $C_{11}H_8FN^+$: 173.1(11%); Anal. Calculated for $C_{25}H_{15}F_2N_3O$; C, 72.99; H, 3.68; F, 9.24; N, 10.21; O, 3.89; found C, 72.97; H, 3.69; F, 9.27; N, 10.24; O, 3.92.

2.8.4 2-(4-Fluoro-phenyl)-5-[5-(3-methyl-thiophen-2-yl)-[1, 3, 4] oxadiazol-2-yl]-pyridine (7d)

The mixture was separated by column-chromatography [EtOAc: Hexane (8:2)] to afford 7(c) as off white solid solid (44 mg, 58% yield); m. p 130-134°C; ^{1}H-NMR (400MHz,$CDCl_3$): δ ppm 1.8 (s, 3H), 7.03 (dd, 1H, ArH), 7.22 (m, 2H, J =8.4 Hz, phenyl), 7.49 (dd, 2H, phenyl), 8.10 (dd, 2H, phenyl),7.47(dd, 2H, phenyl), 9.35 (s, 1H, NH);^{13}CNMR (100MHz, $CDCl_3$): 16.19, 77.1, 116.3, 118.7, 119.1, 129.3, 133.8, 142, 147, 158.4, 161.9; IR (KBr, ν_{max}/cm^{-1}) :894, 2875, 2995, 3376, 3390; MS (ESI) *m/z*: 338 $[M+H]^+$; $C_{17}H_{10}FN_3OS^+$: 323.3 (88%), $C_{11}H_8FN^+$: 173.1(9%); Anal. Calculated for $C_{18}H_{12}FN_3OS$; C, 64.08; H, 3.59; F, 5.63; N, 12.46; O, 4.74; S, 9.50;Found C, 64.09; H, 3.61; F, 5.65; N, 12.47; O, 4.75; S, 9.53.

2.8.5 5-[5-(4-Chloro-phenyl)-[1,3,4] oxadiazol-2-yl]-2-(4-fluoro-phenyl)-pyridine (7e)

The mixture was separated by column-chromatography [EtOAc: Hexane (7:3)] to afford **7(e)** as off white solid (27 mg, 48% yield); m. p. 120-121°C; ^{1}H-NMR (400 MHz, $CDCl_3$): δ ppm 7.20 (dd, 2H, phenyl), 7.41 (dd, 2H, phenyl), 7.49 (m, 2H, J = 8.4 Hz, phenyl), 7.65 (dd, 2H, phenyl), 8.18 (dd, 2H, phenyl), 9.31 (s, 1H, NH);^{13}C NMR (100 MHz, $CDCl_3$): 77.1,118.3, 122.7, 124.7, 127.6, 135.9, 142.3, 146,147, 158.8, 164.9; IR (KBr, ν_{max}/cm^{-1}): 859, 1280, 2785, 2895,3289, 3486; MS (ESI) *m/z*: 352.2 $[M+H]^+$; $C_{19}H_{12}FN_3O^+$: 317.3 (65%), $C_{11}H_8FN^+$: 173.1(19%): Anal. Calculated for $C_{19}H_{11}ClFN_3O$; C, 64.87; H, 3.15;Cl, 10.08; F, 5.40; N, 11.95; O, 4.55; Found C, 64.88; H, 3.18;Cl, 10.09; F, 5.43; N, 11.98; O, 4.57.

2.8.6 Inhibition pictures of HeLa untreated and MCF7 treated with compound 7(b)

Fig. 1.3 shows the inhibition pictures of HeLa and MCF7. Compound 7(b) showed the better inhibition as compared with the standard 5-FU. (Inhibition pictures of HeLa untreated and MCF7 treated with compound 7(b))

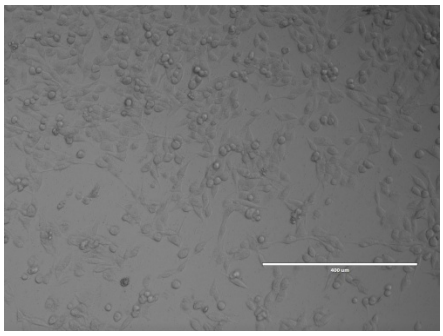
2.8.7 Inhibition Pictures of MCF7 Treated with compounds 7(d) and 7(e)

Fig. 1.4 shows the inhibition pictures of MCF7 against active compounds 7(d) and 7(e). Both the compounds showed better cytotoxicity as compared with the standard drug 5-FU (Inhibition Pictures of MCF7 Treated with compounds 7(d) and 7(e))

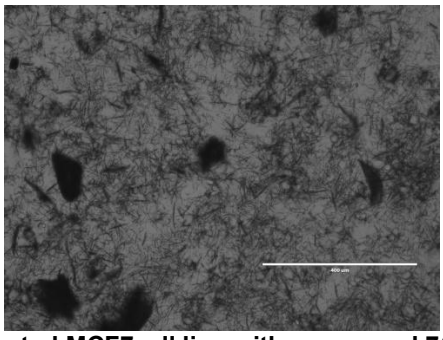
2.9 Cyto.toxic Evaluation

2.9.1 MTT assay

The *in vitro* anticancer activity assay was analysed by MTT method. Five human carcinoma cell lines were used for the evaluation namely HeLa, MCF7, Caco-2, HepG2 and SK-N-SH cell lines. All the cell lines were grown in

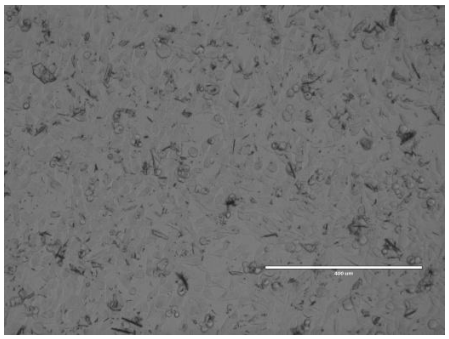


Untreated HeLa cell line

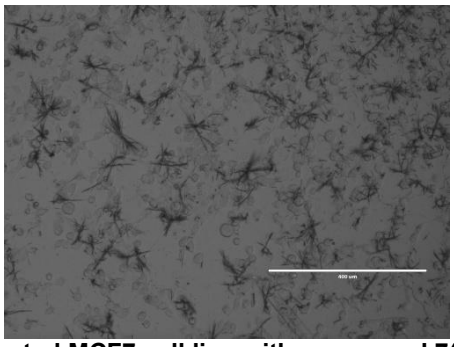


Treated MCF7cell line with compound 7(b)

Fig. 1.3. Pictures of untreated HeLa and MCF7 against compound 7(b)



Treated MCF7 cell line with active compound 7(b)



Treated MCF7 cell line with compound 7(e)

Fig. 1.4. Pictures of MCF7 treated with compounds 7(d) and 7(e)

Table 1. Containing the physiochemical properties of the intermediate compounds 6a-6e

Sl. No (6a-6e)	Compounds Mol. Wt	Molecular Formula	%Yield	M. P	Physical State
6a	380.6	$C_{21}H_{18}FN_3O_3$	87%	102-108°C	Off white Solid
6b	416.2	$C_{19}H_{12}BrF_2N_3O$	68%	123-128°C	White Solid
6c	414.2	$C_{25}H_{17}F_2N_3O$	75%	110-112°C	Off white Solid
6d	340.7	$C_{18}H_{14}FN_3OS$	69%	134-136°C	Yellow Solid
6e	354.7	$C_{19}H_{13}ClFN_3O$	77%	178-179°C	Off White Solid

DMEM-HG supplemented with 10% heat-inactivated FBS. Penicillin-Streptomycin (2%) and 2.5 µg/mL Amphotericin-B solution (All from HI Media Labs, Mumbai, India) were added to the cultured medium. Cell lines were incubated at 37°C in a humidified atmosphere of 95% air, 5% CO_2. Following 24-48 h. of incubation period, the adherent monolayer cells were detached using Trypsin-EDTA solution (HI Media Labs, Mumbai, India) to make single cell suspensions. The viable cells were counted using Luna automated cell counter. Cytotoxicity of the novel 1, 3, 4-oxadiazoles (Table 2) have been determined using MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay.

2.9.2 Cell viability assay (MTT assay)

The anticancer activity was carried out at Genelon Institute of Life Sciences Pvt. Ltd. 200 µL cell suspension was seeded in 96-well micro plates (Corning®, USA**)** at a density of 25,000 cells/well and incubated for 24 h, all the cells were seeded in duplicates with novel compounds of 7a-7e in a range of concentrations from 50 µM-500 µM. The micro plate was then incubated in a CO_2 incubator at 37°C. Treated cells were thereafter incubated with 10% MTT (5 mg/mL; HI Media Labs, Mumbai, India) for 3 h. The culture medium was then aspirated with 200 µL dimethyl sulfoxide. 5-Fluoro uracil (5-FU) was used as standard. Cell viability was determined by measuring the absorbance on a micro plate reader (BMG labtech, Germany) at 570 nm. MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring converting MTT to an insoluble purple formazan. Therefore, the amount of formazan is directly proportional to number of viable cells. Cell viability was calculated as a percentage of viable cells at different test concentrations relative to the control (5-FU).

[% cell viability = (A_{570} of treated cells / A_{570} of control cells) ×100%].

3. RESULTS AND DISCUSSION

3.1 Chemistry

The strategies adapted for the synthesis of the intermediates and target compounds are depicted in Fig. 1.2. Novel derivatives of 2-(4-Fluoro-phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine (7a-7e) were synthesized, characterized and evaluated for their cytotoxicity [10] against HeLa, MCF7, Caco-2, HepG2 and SK-N-SH cell lines. Synthetic chemistry involves the conversion of 2-Bromo-5-methyl-pyridine into 6-bromo nicotinic acid (**2**) (shifting of the C=O in IR~ 1180 vmax cm^{-1}). The compound (**2**) was further converted into ester (**3**) by refluxing with concentrated sulphuric acid and ethanol. The ester (3) was coupled with 4-Fluoro-phenyl boronic acid by Suzuki-Mayora coupling reaction [11,12]. The 4-Fluoro-phenyl group was introduced at C-2 position of the pyridine ring in order to increase the bioavailability of the 1, 3, 4-oxadiazoles. The compound thus obtained (4) was converted into corresponding carbohydrazide [13] by refluxing with hydrazine hydrate and ethanol (IR absorbance of NH~ 3385 vmax cm^{-1}) and (appearance of broad NH_2 peak at δ 4.15). The intermediate 6-(4-Fluoro-phenyl)-nicotinic acid hydrazide was reacted with different aldehydes a-e and afforded the novel Schiff base compounds 6a-6e. The Schiff base compounds were cyclized in presence of chloramine T [14,15] as promoter and obtained a series of novel 1, 3, 4-oxadiazole derivatives 7a-7e. In this research work the author has synthesized novel derivatives of 2-(4-Fluoro-phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine and

Table 2. IC_{50} values of the synthesized novel 2-(4-Fluoro-phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine 7a-7e

Compounds 7a-7e	Ar (R)	IC_{50} Values of 1, 3, 4-Oxadiazoles in µM				
		HeLa	Caco-2	MCF7	HepG2	SK-N-SH
7a	2,5-OCH_3 C_8H_3	118.3 ± 0.054	123.1±0.12	26.9±0.013	689.9±0.13	908±0.24
7b	2-F-5-Br C_6H_3	122.4 ± 0.08	56.5±0.05	6.9±0.075	789.9±0.083	-
7c	4-F-$C_{12}H_8$	96.5 ± 0.05	23.6±0.06	23.5±0.032	243.5±0.43	305.8±0.22
7d	C_5H_5S	132.4 ± 0.065	56.6±0.04	3.8±0.11	129±0.043	632.9±0.01
7e	4-ClC_6H_4	22.7 ± 0.08	123.6±0.13	89.9±0.76	234±0.05	379±0.018
5-FU		5.6 ± 0.02	8.8±0.023	7.8±0.032	7.8±0.034	9.8±0.017

5-FU: 5-fluorouracil-standard used in the MTT assay; IC_{50}- inhibitory concentration at 50% of the cells inhibited

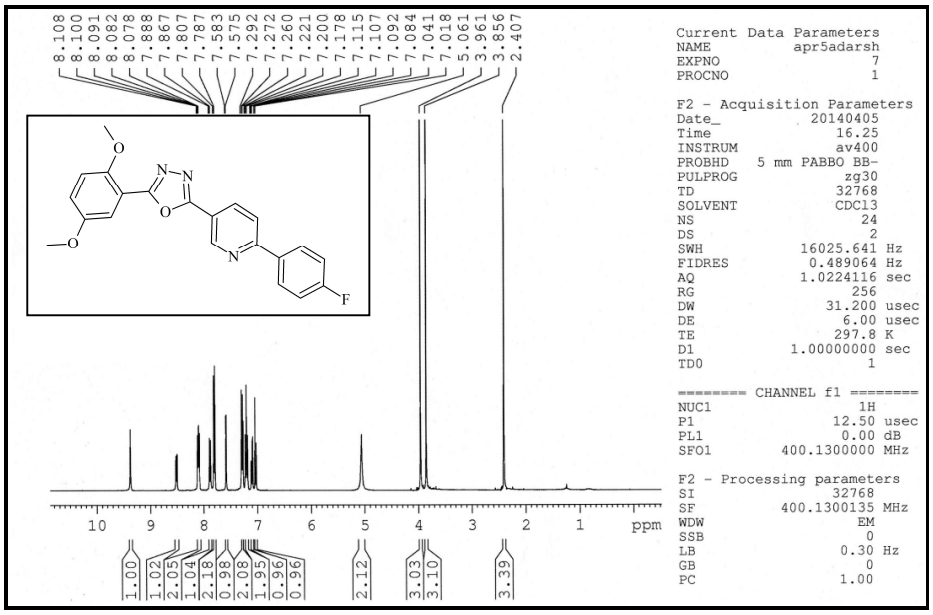


Fig. 1.5 ^{1}H-NMR spectra of the compound 7(a)

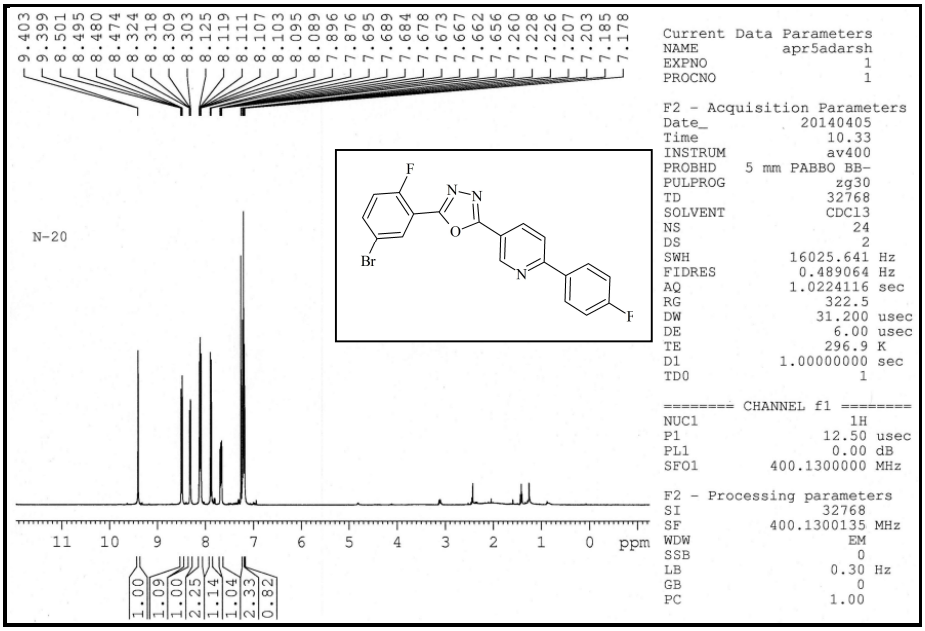


Fig. 1.6. ^{1}H-NMR spectra of the compound 7(b)

screened for its cytotoxicity against five human cancer cell lines (MTT assay) [16]. The different substituted 1, 3, 4-oxadiazole [17,18] derivatives of pyridine showed wide range of cell viability. The result of the *in vitro* cytotoxic evaluation [19,20] of these compounds were expressed in the form of minimum inhibitory concentration (IC_{50}). Upon substituting 4-Fluoro-phenyl group at the C-2 position of the pyridine and constructed the 1, 3, 4-oxadiazole derivatives at C-5 position of pyridine ring.

3.2 Biology

Initially these 1, 3, 4-oxadiazoles compounds were screened for their cytotoxicity against

human cancer cell lines using MTT assay. The results were expressed in the form of concentration of the compound that is required to inhibit the growth of 50% of the viable cells. The IC_{50} of the compounds were compared with the IC_{50} of the standard used (5-FU). Compounds 7b and 7d showed very good cytotoxicity against MCF7 cell lines with IC_{50} of 6.9 µM and 3.8 µM respectively (Fig 1.4) which is comparable with the cytotoxicity of the standard used (5-FU). The compounds 7c, 7d were showed more resistance against Caco-2 cell line with IC_{50} of 23.6 µM, and 56.5 µM respectively (Fig 1.3). Overall the synthesized1, 3, 4- oxadiazoles derivatives showed more resistance than the cytotoxicity against all the five cell lines used for the evaluation.

4. CONCLUSION

In our present study we have synthesized novel derivatives of 2-(4-Fluoro-phenyl)-5-(5-aryl substituted-1, 3, 4-oxadiazol-2-yl) pyridine. The synthesized novel 1, 3, 4-oxadiazole compounds showed good cytotoxicity against MCF7 cell line. They exhibited more resistance with the heterocyclic compounds as compared with the cytotoxicity of 5-FU. The IC_{50} value of compound **7b** (Fig 1.6) above and **7d** against MCF7 was found to be 6.9 µM and 3.8 µM respectively. The compounds **7c**, **7d** were showed moderate cytotoxicity against Caco-2 cell line with IC_{50} of 23.6 µM and 56.5 µM respectively. The author has further planed the various biological activity and apoptosis of these compounds in his further research.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors declared that they do not have any competing interest.

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