Design, Synthesis and Characterization of Novel Amine Derivatives of 5-[5-(Chloromethyl)-1, 3, 4-Oxadiazol-2-yl]-2-(4-Fluorophenyl)-Pyridine as a New Class of Anticancer Agents

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ABSTRACT: A linear strategy was adopted in synthesizing the novel amine derivatives **7(a-h)** of 5-[5-(chloromethyl)-1, 3, 4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine **(6)** and screened these compounds for *in vitro* anticancer activity against three human cancer cell lines (*HeLa*,*Caco-2* and *HepG2*). The synthesised novel compounds were characterized by ^{1}H NMR, MS and ^{13}C NMR spectroscopic evidences. Microwave irradiation of compound (**5**) in presence of chloroacetyl chloride and phosphoryl oxychloride yielded the dehydrated cyclized key intermediate 5-[5-(chloromethyl)-1,3,4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine which upon treatment with various primary or secondary amines (**a-h**) resulted into the corresponding amine derivatives. The IC_{50} values of the final compounds were compared with that of 5-fluorouracil (5-FU) taken as the standard. Compounds **7a** and **7d** were found to be highly cytotoxic against *HepG2* cell lines with IC_{50} values of 2.6 µM (IC_{50} = 34.0 ± 0.5 µM) and 5.8 µM (IC_{50} = 112 ± 1.4 µM) respectively. The compound **(7f)** alone was found to have high cytotoxicity against *Caco-2* cell lines with IC_{50} value of 2.3 µM (IC_{50} = 87 ± 2.6 µM).

Key words: Anticancer, *HeLa*, 1, 3, 4-Oxadiazoles, *HepG2*, cytotoxicity

INTRODUCTION

As per literature review, various analogues of 2, 5-disubstituted-1, 3,4-oxadiazole derivatives of pyridine (Figure 1,C) have been studied for their analgesic, anti-inflammatory[1], antimicrobial[2,3], antitumor[4] and anticancer[5] properties. Substitution of 4-fluorophenyl group at second position of the pyridine containing 1,3,4-oxadiazolesmoiety[6] has shown very good cytotoxicity against human cancer cell lines[7]. In view of this, novel amine derivatives of 5-[5-(chloromethyl)-1,3,4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine (Figure 1, A) have been designed and synthesised. These novel 1,3,4-oxadia-zole amine compounds **7(a-h)** were obtained from compound (**6**) which was prepared from (**5**) by the *in situ* dehydration and cyclization reaction using phosphoryl oxychloride and chloroacetic acid under microwave irradiation. The intermediate carbohydrazide (**5**) was completely converted into product **(6)** in the form of a solid by subsequent neutralization. The starting material used for this synthetic pathway was 6-bromonicotinic acid **(1)** which was converted to 6-(4-fluorophenyl) nicotinic acid ethyl ester[8] (**4**) by treating the ethyl ester (**3**) with 4-fluorophenyl boronic acid in presence of tetra bis (triphenyl phosphine) palladium (0) in ethanol. The ester (**4**) thus obtained was converted into the corresponding hydrazide (**5**) by treating with hydrazine hydrate.[9] The novel amine derivatives **7**(**a-h**) obtained by refluxing compound (**6**) with primary or secondary amines in deoxan and TEA were purified by column chromatography (silica gel 100-

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200 mesh) and characterized by ^{1}H NMR, ^{13}C NMR and MS spectroscopic analyses. The percentage yield of all the final compounds was found to be in the range 48-88% and purity in between 94-97%. The scheme of reactions from compound **1-6** is shown in figure 2. The conversion of compound (**6**) to amine derivatives, 7(a-h) have been given in figure 3. It was envisaged that the novel amine derivatives of 1, 3, 4-oxadiazole (Figure 1, B) would enhance the solubility, total polar surface area (TPSA) and bioavailability. With this view the compounds have been subjected to MTT assay[10] for testing their cytotoxic activity using 5-fluorouracil as the standard drug. Three human carcinoma cell lines were employed for the screening and the activity was calculated in terms of the IC_{50} values. Some of the synthesized 1,3,4-oxadiazoles were found to exhibit better cytotoxicity against *HepG2* and *Caco*-2cell lines.

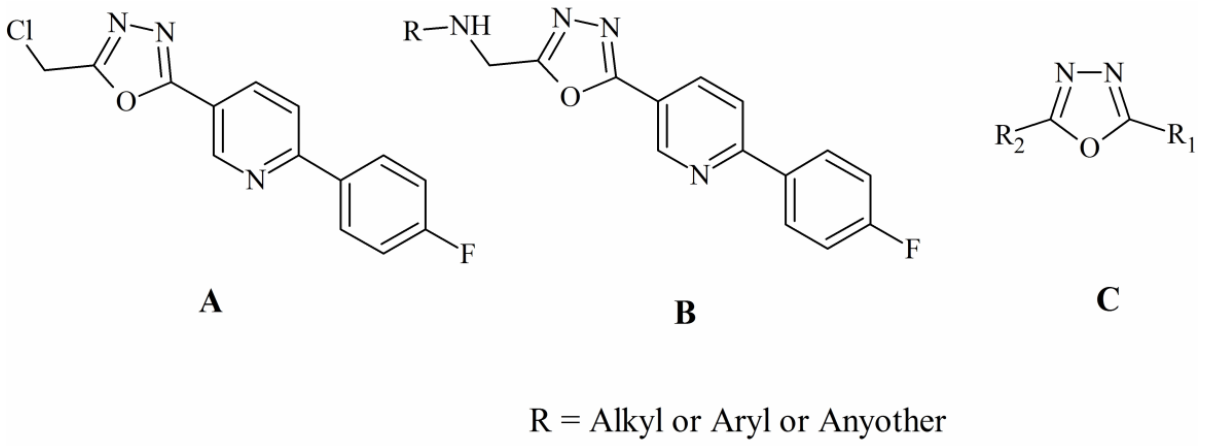

Figure 1**.** 5-[5-(chloromethyl)-1, 3, 4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine(**A**); Novel amine analogues of 5-[5-(chloromethyl)-1, 3, 4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine (**B**); General structure of 2,5-disubstituted 1,3,4-oxadiazole moiety (**C**).

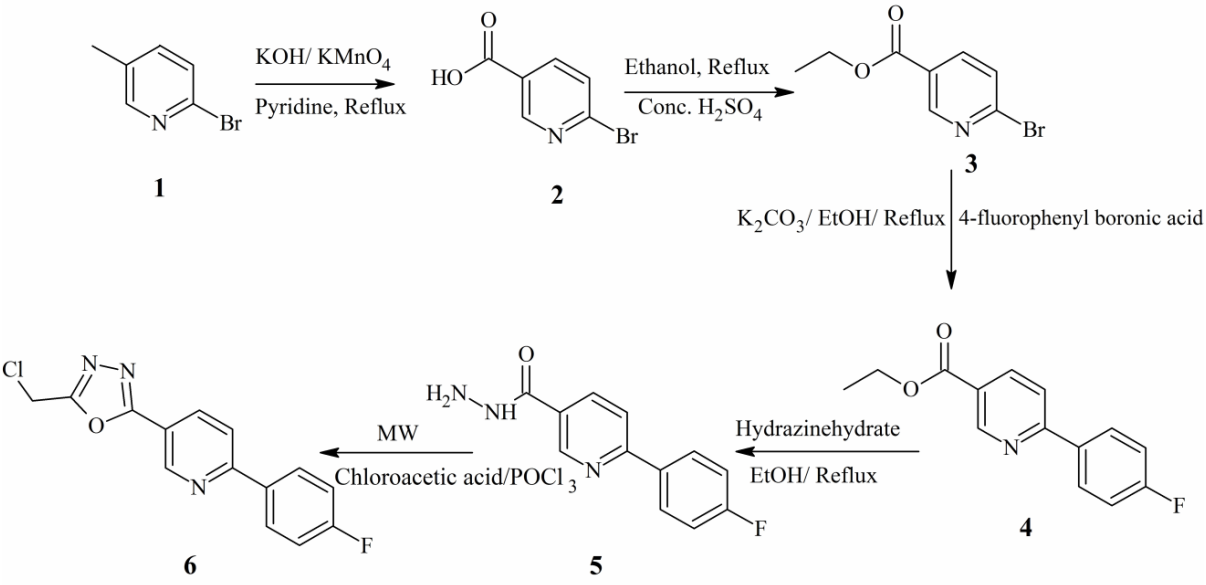

Figure 2. Scheme of synthetic pathway of the compound 5-[5-(chloromethyl)-1, 3, 4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine (**6**).

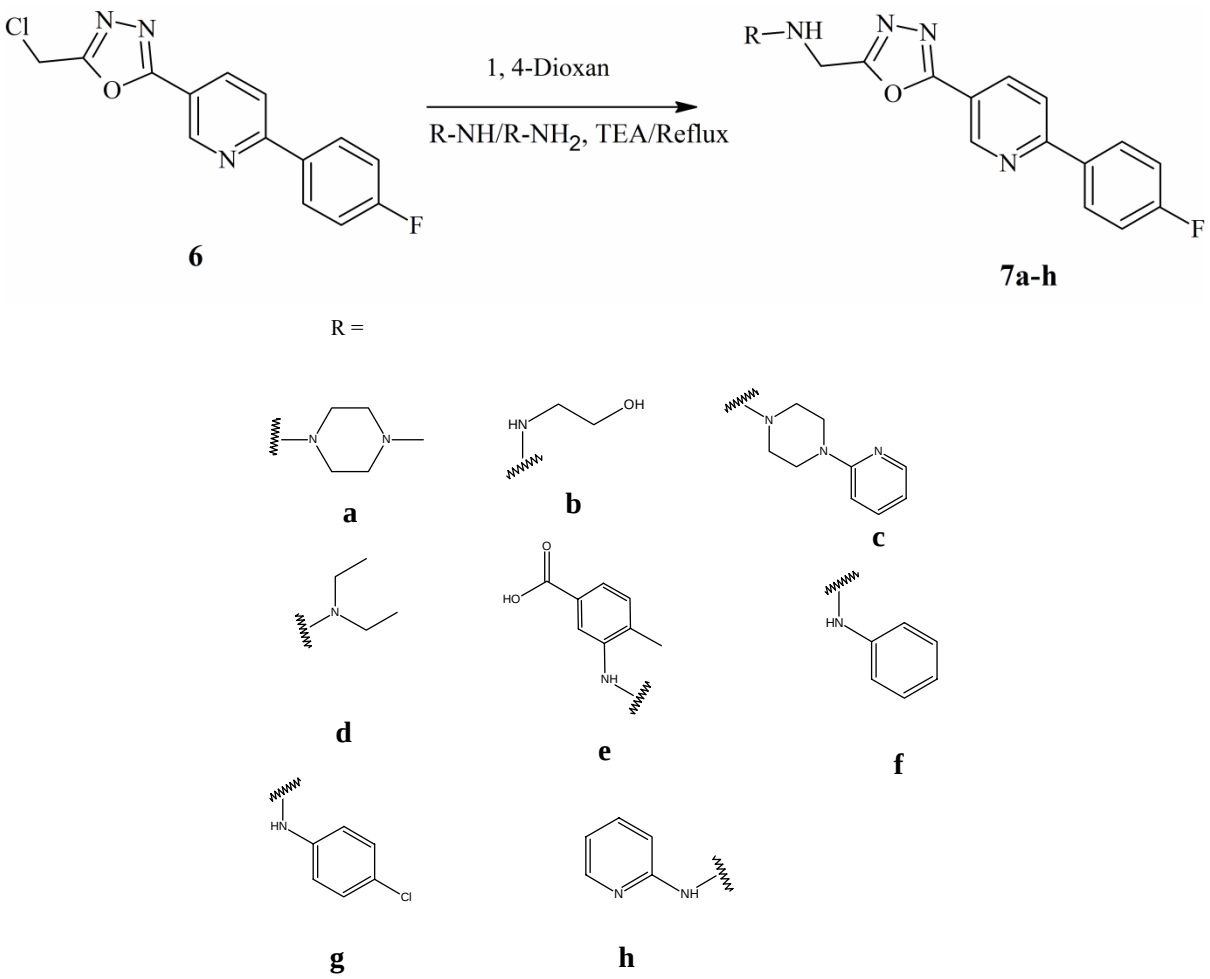

Figure 3. Synthetic route of the novel amine derivatives of 5-[5-(chloromethyl)-1, 3, 4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine **7** (a-h).

MATERIALS AND METHODS

All reagents, chemicals and solvents were purchased from S-D fine and Spectrochem Ltd., Bangalore, India. ^{1}H-and ^{13}C-NMR were recorded by Brucker 400 MHz spectrophotometer. Melting points were determined using Buchi melting point apparatus 545. Mass spectra were recorded by Agilent 1200 series. TLC was done on F_{254} grade silica 60 from Merck. All the final and intermediate compounds were purified by normal glass column using silica gel 100-200 mesh. IR spectra were recorded by FTIR (1800S) series. Microwave reaction was carried out using Whirlpool semi-automated microwave.

Synthesis of 6-bromo-nicotinic acid (2). 2-Bromo-5-methyl-pyridine (15 g, 0.0873 mol) was taken in 1L RB flask containing 100 ml of water and 100 ml of pyridine. KOH (14.65 g, 0.0261 mol) and $KMnO_4$ (68.96 g, 0.0436 mol) were added to this reaction mixture and it was refluxed at 85°C overnight. After completion of the reaction as observed by TLC, solvent was removed, residue was diluted with water (100-150 ml) and filtered. The filtrate was neutralized with 1 N HCl and the solid that separated out was filtered, washed with water and dried. Yield 66.66%; m.p. 108-112°C; MS (ESI): $[M-H]^+$ 201; ^{1}HNMR (400 MHz, $CDCl_3$): δ7.03 (dd,1H, J = 12.2Hz, Ar-H), 9.49 (dd, J = 7.2 Hz, 1H), 9.78 (m, J = 15.6Hz, 1H), 10.85 (bs,1H, OH).

Synthesis of 6-bromo-nicotinic acid ethyl ester (3). 6-Bromo-nicotinic acid (**2**) (10 g, 0.0434 mol) was taken in 100 ml ethanol. To this solution 10 drops of conc. H_2SO_4 were added and the mixture

was refluxed at 80 °C for 8 hrs. TLC was used to check the completion of the reaction. After completion of the reaction, solvent was removed, residue was diluted with water and neutralised with 10% $NaHCO_3$ solution. The product was extracted with ethyl acetate (15 ml × 3), washed with brine (10 ml) and dried over Na_2SO_4. Ethyl acetate was concentrated to get pale yellow syrup. Yield 85%; MS (ESI): $[M+H]^+$ 231; ^{1}HNMR (400MHz, $CDCl_3$): δ1.19 (t, 3H, CH_3), 3.88 (q, 2H, CH_2), 7.14 (dd, 1H, J = 12.2 Hz, Ar-H), 9.50 (dd, J = 8.4Hz, 1H), 9.82 (m, J = 14.3 Hz, 1H).

Synthesis of 6-(4-fluorophenyl)-nicotinic acid ethyl ester (4). A mixture of 6-bromonicotinic acid ethyl ester (**3)** (8.5 g, 0.0369 mol), K_2CO_3 (15.27 g, 0.1107 mol), tetrabis triphenyl palladium (0) (0.213 g, 0.000185 mol) and 4-fluorophenylboronic acid (5.166 g, 0.0369 mol) were refluxed at 85°C in ethanol for 3-5 hrs (Suzuki-Mayora coupling reaction). After completion of the reaction as per the TLC monitoring, solvent was removed, residue was diluted with water and the product was extracted with ethyl acetate (25 ml × 4), washed with brine (10 ml) and dried over Na_2SO_4. Ethyl acetate was concentrated to afford 6.3 g of crude product. The crude product was purified by column chromatography (silica gel 100-200 mesh). Purification process started with 100% *n*-hexane and solvent polarity was increased to up 28% ethyl acetate. Yield 61.1%; m.p. 128-127°C; MS (ESI): $[M+H]^+$ 246; ^{1}H NMR (400 MHz, $CDCl_3$) : δ 2.19 (t,3H, CH_3), 3.96 (q, 2H, CH_2), 7.24 (dd, 2H, J = 7.8Hz, Ar-H), 8.54 (dd, J = 8.8Hz, 2H), 8.88 (m, J = 14.2 Hz, 3H).

Synthesis of 6-(4-fluorophenyl)-nicotinic acid hydrazide (5). A mixture of 6-(4-fluorophenyl) nicotinic acid ethyl ester (**4**) (5.2 g, 0.0211 mol), 10 ml of hydrazine hydrate and 50 ml of ethanol was refluxed for overnight at 80°C. After completion of the reaction, solvent was removed and few ice pieces and saturated brine solution were added to the residue. Solid that separated out was filtered, washed with 10 ml of water and dried. Yield 59.6%; m.p. 172-178°C; MS (ESI): $[M+H]^+$ 232; ^{1}H NMR (400 MHz, $CDCl_3$) : δ2.19 (t, 3H, CH_3), 3.97 (q, 2H, CH_2), 4.21 (bs, 2H, NH_2), 7.32 (dd, 2H, J = 11.8 Hz, Ar-H), 8.49 (dd, J = 8.7 Hz, 2H), 8.92 (m, J = 12.4 Hz, 3H).

Synthesis of 5-[5-(chloromethyl)-1,3,4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine (6). A 200 ml flask containing compound (**4**) (3.1 g, 1 mmol), phosphoryl oxychloride and chloroacetic acid was irradiated with microwave for a period of 5 minutes. TLC was monitored to check the completion. After completion of the reaction ice cold water was added to the mixture and neutralised with a saturated solution of Na_2CO_3. The separated solid was filtered, washed with 10 ml of water and dried. The product was used without any further purification. Yield 64.5%; MS (ESI): $[M+H]^+$; m.p. 98-104°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ4.56 (s, 2H, CH_2), 7.31 (dd, 2H, J = 8.2Hz, Ar-H), 8.54 (dd, J = 12.2 Hz, 2H), 8.86 (m, J = 13.2 Hz, 3H).

General procedure for the synthesis of amine derivatives of 5-[5-(chloromethyl)-1,3,4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine(7a-h). Compound (**6**) (200 mg, 1 mmol) was added to a mixture of 1, 4-dioxin (10 ml), TEA (2 ml) and the corresponding amines (a-h, 1.1 mmol) refluxed for 3-8 hrs. TLC was used to check the completion of the reaction. After completion, the solvent was removed, residue was diluted with water (10 ml) and the product was extracted with ethyl acetate (10 ml × 2), washed with brine (10 ml) and dried over anhydrous Na_2SO_4. The crude product was purified by column chromatography (silica gel 100-200 mesh) using ethyl acetate-hexane (50 : 50).

Analytical data of the novel amine derivatives of 5-[5-(chloromethyl)-1,3,4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine (7a-h). 1-{5-[6-(4-Fluoro-phenyl)-pyridin-3-yl]-[1,3,4] oxadiazol-2-yl-methyl}-4-methyl-piperazine **7**(**a**).

Brown syrup; yield 68%; ^{1}H NMR (400 MHz, $CDCl_3$): δ2.27 (s, 3H, CH_3), 2.46 (bs, 4H, CH_2), 2.48 (bs, 4H, CH_2), 3.2(s, 2H, CH_2), 7.2 (dd, 2H, J = 7.4 Hz), 7.34 (dd, 2H, J = 11.4 Hz, ArH), 7.58(s, 1H, ArH), 7.71 (m, 2H, J = 14.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): 38.7, 53.6, 55.1, 58, 116.0, 122.6, 124.8, 128.7, 130.3, 135.3, 142.2, 155.7, 161.7; IR

(KBr, ν_{max}/cm^{-1}): 2905, 3326, 1108, 894, 2715, 3311; MS (ESI): $[M+H]^+$ 354, anal. calculated for $C_{19}H_{20}FN_5O$; C, 64.57; H, 5.70; F, 5.38; N, 19.82; O, 4.57; Found C, 64.59; H, 5.73; F, 5.39; N, 19.83; O, 4.61.

2-({5-[6-(4-Fluorophenyl)-pyridin-3-yl]-[1,3,4] oxadiazol-2-yl-methyl}-amino)-ethanol (**7b**). Pale brown syrup; yield 53%; ^{1}H NMR (400 MHz, $CDCl_3$): δ2.74 (s, 2H, CH_2), 3.65 (s, 2H, CH_2), 3.81 (s, 2H, CH_2), 4.2 (bs, 1H, OH), 7.47 (dd, 2H, *J* = 7.2 Hz, Ar-H), 7.54 (dd, *J* = 8.9 Hz, 2H), 7.64 (m, *J* = 13.3 Hz, 2H), 8.14 (s, 1H), 10.8 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 49.5, 52, 64.9, 116, 120.8, 122.3, 124.8, 128.7, 131.1, 135.3, 142.3, 148.8, 149.6, 152.3, 160.7; IR (KBr ν_{max}/cm^{-1}): 2935, 3236, 1210, 873, 2885, 3420; MS (ESI) : $[M-H]^+$ 312; anal. calculated for $C_{16}H_{15}FN_4O_2$; C, 61.14; H, 4.81; F, 6.04; N, 17.83; O, 10. 19; found C, 61.16; H, 4.84; F, 6.06; N, 17.85; O, 10.29.

1-{5-[6-(4-Fluorophenyl)-pyridin-3-yl]-[1,3,4] oxadiazol-2-yl-methyl}-4-pyridin-2-yl-piperazine (**7c**). Off white solid; yield 52%; m. p. 203-204^{0}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ2.59 (bs, 4H, CH_2), 3.16 (bs, 4H, CH_2), 3.62 (s, 2H, CH_2), 7.13 (dd, *J* = 4.9 Hz, 2H, ArH), 7.32 (dd, *J* = 7.2 Hz, 2H), 7.54 (m, *J* = 12.8 Hz, 2H),7.81 (m, *J* = 14.2 Hz, 2H), 7.84 (dd, *J* = 8.4 Hz, 2H), 8.10 (s, 1H), 9.35 (s, 1H,NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 48.7, 53.2, 55, 57.9, 61.1, 108.8, 112, 113.9, 116.3, 122.7, 125.5, 128.7, 132.2, 135.4, 138.8, 142.3, 148.9, 153.2, 155.8, 161.9, 168.9; IR(KBr,ν_{max}/cm^{-1}): 2913, 3326, 2865, 3380; MS (ESI): $[M+H]^+$ 418; anal. calculated for $C_{23}H_{21}FN_6O$; C, 66.33; H, 5.08; F, 4.56; N, 20.18; O, 3.84; found C, 66.35; H, 5.09; F, 4.58; N, 20.19; O, 3.85.

Diethyl-{5-[6-(4-fluorophenyl)-pyridin-3-yl]-[1,3,4] oxadiazol-2-yl-methyl}-amine (**7d**). Pale semisolid; yield 71%; ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.1 (s, 6H, $2CH_3$), 2.40 (s, 4H, CH_2), 3.62 (s, 2H, CH_2), 7.32 (dd, *J* = 14.2 Hz, 2H), 7.48 (dd, *J* = 7.8 Hz, 2H), 7.54 (m, *J* = 14.8 Hz, 1H, Ar-H), 7.75 (dd, *J* = 8.8 Hz, 2H), 9.81 (s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 13.7, 24.5, 46.3, 53.7, 116.7, 118.8, 122.2, 128.6, 130.5, 135.4, 142.3, 149.3, 151.8,158.8, 162.9; IR (KBr, ν_{max}/cm^{-1}): 2983, 3486, 1250, 881, 2795, 3311; MS (ESI): $[M+H]^+$ 327; anal. calculated for $C_{18}H_{19}FN_4O$; C, 66.24; H, 5.87; F, 5.82; N, 17.17; O, 4.90; found C, 66.25; H, 5.89; F, 5.84; N, 17.18; O, 4.98.

3-({5-[6-(4-Fluorophenyl) pyridin-3-yl]-[1,3,4] oxadiazol-2-yl-methyl}-amino)-4-methyl- benzoic acid (**7e**). White solid; yield 48%;m.p. 143-148^{0}C; ^{1}H NMR (400 MHz, $CDCl_3$): δ2.35 (s, 3H, CH_3), 4.32 (s, 2H, CH_2), 4.8 (s, 1H, NH), 7.33 dd, *J* = 5.2 Hz, 2H), 7.41 (dd, *J* = 7.3 Hz, 2H), 7.49 (m, *J* = 14.2 Hz, 2H, Ar-H), 7.65 (dd, *J* = 4.2 Hz, 1H), 8.18 (m, *J*= 14.2 Hz, 3H), 10.8 (bs, 1H, OH); ^{13}C NMR (100 MHz $CDCl_3$): 12.1, 51.9, 116.2, 118.9, 119.3, 122.3, 122.6, 127.8, 128.7, 133.4, 135.3, 140.5, 144.1, 149.6, 152.3, 160.7, 162.3, 169.8, 170.8, 174.2; IR (KBr, ν_{max}/cm^{-1}): 2865, 3476, 1288, 849, 2795, 3279; MS (ESI): $[M-H]^+$ 403; anal.calculated for $C_{22}H_{17}FN_4O_3$; C, 65.34; H, 4.24; F, 4.70; N, 13.85; O, 11.87; found C, 65.36; H, 4.25; F, 4.72; N, 13.87; O, 11.89.

{5-[6-(4-Fluorophenyl)-pyridin-3-yl]-[1,3,4] oxadiazol-2-yl-methyl}-phenyl-amine(**7f**). Brown coloured solid; yield 38%; m. p. 96-102°C; ^{1}HNMR (400 MHz, $CDCl_3$): δ 4.32 (s, 2H, CH_2), 4.78 (bs, 1H, NH), 7.28(dd, *J* = 14.1 Hz, 2H), 7.36 (dd, *J* = 8.7 Hz, 2H), 7.48 (m, *J* = 13.2 Hz, 3H, Ar-H), 7.65 (dd, *J*= 7.8 Hz, 2H), 7.76 (m, *J* = 12.2 Hz,3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.6, 112.3, 114.5, 116.7, 120.6, 122.4, 129.3, 131.7, 135.8, 142.2, 149.6, 155.7, 160.8, 164.5; IR (KBr, ν_{max}/cm^{-1}): 2815, 3426, 1360, 889, 2755, 3359; MS (ESI) $[M+H]^+$ 347; anal. calculated for $C_{20}H_{15}FN_4O$; C, 69.35; H, 4.37; F, 5.49; N, 16.18; O, 4.62; Found C, 69.37; H, 4.39; F, 5.51; N, 16.19; O, 4.64.

(4-Chlorophenyl)-{5-[6-(4-fluorophenyl)-pyridin-3-yl]-[1,3,4] oxadiazol-2-yl-methyl}-amine **(7g)**. White coloured solid; yield 64%; m. p. 128-129°C; ^{1}HNMR(400 MHz, $CDCl_3$): δ7.31 (dd, *J* = 7.2 Hz, 2H), 7.43 (dd, *J* = 12.5 Hz, 2H), 7.48 (m, *J*= 14.2 Hz, 2H, Ar-H), 7.65 (m, *J* = 14.3Hz, 2H), 7.88 (dd, *J* = 8.8 Hz, 2H), 8.35 (s, 1H), 9.5(s, 1H, NH); ^{13}C NMR (100 MHz, $CDCl_3$): δ 51.6, 112.0, 113.7, 122.2, 124.5, 128.6, 130.3, 134.5, 142.2, 149.6,

155.7, 161.8, 162.7; IR (KBr, ν_{max}/cm^{-1}): 2834, 3446, 1480, 879, 2795, 3329; MS (ESI): $[M+H]^+$ 382; anal. calculated for $C_{20}H_{14}ClFN_4O$; C, 63.08; H, 3.71; Cl, 9.31; F, 4.99; N, 14.71; O, 4.20; Found C, 63.09; H, 3.74; Cl, 9.33; F, 5.01; N, 14.72; O, 4.22.

{5-[6-(4-Fluorophenyl)-pyridin-3-yl]-[1,3,4] oxadiazol-2-yl-methyl}-pyridin-2-yl-amine (**7h**). Pale yellow coloured solid; yield 48%; m. p. 132-136°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ7.31 (dd, J = 7.2 Hz, 2H), 7.43 (dd, J = 14.2 Hz, 2H), 7.48 (m, J = 12.8Hz, 3H, Ar-H), 7.65 (m, J = 12.8 Hz, 2H), 7.88 (dd, J = 5.2 Hz,1H), 9.5(s, 1H, NH); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 51.6, 52.2, 108.3, 112.0, 113.7, 120.8, 122.6, 130.8, 132.2, 141.2, 144.5, 149.6, 160.7, 164.3; IR (KBr, ν_{max}/cm^1): 2855, 3486, 1480, 884, 2795, 3379; MS (ESI): $[M+H]^+$ 347; anal. calculated for $C_{19}H_{14}FN_5O$; C, 65.70; H, 4.06; F, 5.47; N, 20.16; O, 4.61; Found C, 65.72; H, 4.08; F, 5.49; N, 20.18; O, 4.63.

Cytotoxic evaluation

MTT assay. Cytotoxicity of the novel amine derivatives of 1, 3, 4-oxadiazoles has been determined using 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay which was carried out at Genelon Institute of Life Sciences Pvt. Ltd. The *in vitro* anti-proliferative MTT assay was performed against three human carcinoma cell lines namely, *HeLa*, *Caco-2* and *HepG2*. All the cell lines were grown in DMEM-HG supplemented with 10% heat-inactivated FBS, 2% penicillin-streptomycin and 2.5 µg/ml amphotericin-B solutions (all from HI Media Labs, Mumbai, India). Cell lines were incubated at 37°C in a humidified atmosphere with 95% air, 5% CO_2. Following 24-48 hrs of incubation period, the adherent cells were detached using Trypsin-EDTA solution. Cell count was done using the Luna automated cell counter (Logos Bio systems, India) based on trypan blue dye exclusion method.

Cell viability assay. The MTT assay was carried out using the following procedure. Cell suspension (200 µl) was seeded in 96-well micro plates (Corning®, USA**)** at a density of 25,000 cells/well and incubated for 24 hrs. All cells were seeded in duplicates with novel compounds **7a-7h** having range of concentrations from 50-500 µM, incubated in a CO_2 incubator at 37°C. Treated cells were there after incubated with 10% MTT (5 mg/ml; HI Media Labs, Mumbai, India) for 3 hrs. The culture medium was then aspirated and 200 µl dimethyl sulfoxide (DMSO; Sigma-Aldrich, India) was added to it. 5-Fluorouracil (5-FU) was used as the standard. Cell viability was determined by measuring the absorbance on a micro plate reader (BMG Labtech, Germany) at 570 nm and 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%].

RESULTS AND DISCUSSION

A series of novel derivatives of 2-(4-fluorophenyl)-5-(5-aryl substituted-1,3,4-oxadiazol-2-yl) pyridine (**7a-h**) were synthesized, characterized and evaluated for their cytotoxic effect[11] against *HeLa*, *Caco-2* and *HepG2* cell lines. The synthesis started with 2-bromo-5-methyl-pyridine which was converted into 6-bromonicotinic acid (**2)**[12,13] (the shifting of the CO in IR was 1180 cm^{-1}). Compound **2** was converted into corresponding ethyl ester which was further reacted with 4-fluorophenyl boronic acid by Suzuki-Mayora coupling reaction. Compound **4** thus obtained was converted into corresponding carbohydrazide by refluxing with hydrazine hydrate and ethanol (IR absorbance of NH at 3395 ν_{max}/cm^{-1}) and appearance of broad NH_2 peak at δ 4.23). The intermediate 6-(4-fluorophenyl) nicotinic acid hydrazide were reacted with phosphorous oxychloride in presence of monochloroacetic acid to yield cyclised product (**6)**. The intermediate **6** was reacted with various primary and secondary amines to afford the final compounds (**7a-h)**. The compounds were screened against three cancer cell lines (MTT assay). The *in vitro* anticanceractivity[14,15] of these compounds was expressed in the form of inhibitory concentration (IC_{50}). The different substituted 1,3,4-oxadiazoles derivatives of pyridine[16,17] moiety showed broad range of viability against *Caco-2* and *MCF7* cell lines.

Table 1. **IC_{50} values of the synthesized novel amine derivatives of 5-[5- (chloromethyl)-1, 3, 4-oxadiazol-2-yl]-2-(4-fluorophenyl)-pyridine (7a-h).**

Compound No	IC_{50}# values of **7**(a-h) in (μM)		
7a-h	*HeLa*	*Caco-2*	*HepG2*
7a	212.4 ± 1.2	203.6 ± 2.3	2.6 ± 0.5
7b	85.6 ± 0.8	112.5 ± 1.2	45.6 ± 1.1
7c	34.8 ± 1.3	123.8 ± 1.4	128.9 ± 3.5
7d	112.9 ± 0.4	145.6 ± 0.4	5.8 ± 1.6
7e	118.4 ± 0.5	212.3 ± 0.4	32.2 ± 0.3
7f	78.3 ± 5.4	2.3 ± 0.5	23.5 ± 4.6
7g	56.4 ± 3.4	56.8 ± 1.2	156.7 ± 2.3
7h	88.6 ± 1.2	34.6 ± 0.9	176.4 ± 1.6
5-FU	7.6 ± 0.3	8.8 ± 0.6	7.6 ± 0.2

Inhibitory concentration at 50% of the viable cells

± Average value of the two independent experiments

Table 2. **CC_{50} of novel amine derivatives of 5-[5- (chloromethyl)-1,3,4-o xadiazol-2-yl]-2-(4-fluorophenyl)-pyridine (7a-h).**

Compound no.	CC_{50}* of the compounds **7**(a-h)		
7a-h	*HeLa*	*Caco-2*	*HepG2*
7a	120 ± 1.2	112 ± 1.3	34 ± 0.5
7b	76 ± 0.6	145 ± 1.1	129 ± 0.3
7c	200	178 ± 2.3	102 ± 1.1
7d	450	100 ± 2.6	112 ± 1.4
7e	56 ± 2.4	62 ± 1.2	76 ± 3.4
7f	127 ± 3.4	87 ± 2.6	77 ± 0.4
7g	200	23 ± 1.5	91 ± 4.3
7h	123 ± 2.3	156 ± 0.4	73 ± 1.1
5-FU	57 ± 0.3	69 ± 2.3	52 ± 1.8

Concentration of compound at 50% of the remaining viable cells.

Table 3. Selectivity index (SI) of the novel amine derivatives.(7a-h).

Compound no.	SI of the novel amine derivatives **7** (a-h)		
7a-h	*HeLa*	*Caco-2*	*HepG2*
7a	0.566	0.551	13.06
7b	0.887	1.288	2.828
7c	5.747	1.437	0.791
7d	3.985	0.686	19.31
7e	0.472	0.292	0.236
7f	1.621	37.8	3.276
7g	3.546	0.404	0.580
7h	1.388	4.508	0.413
5-FU	7.5	7.84	6.84

Compounds **7a** and **7d** showed very good cytotoxicity[18-20] with selectivity index (SI) against *HepG2* cell lines having IC_{50} of 2.6 µM (**SI-13.06**) and 5.8 µM (**SI-19.31**), respectively. The compound **7f (SI-37.8)** showed cytotoxicity against *Caco-2* cell line having IC_{50} of 2.3 µM. Overall the synthesized 1, 3,4-oxadiazoles derivatives showed better cytotoxicity against *HepG2* and *Caco-2* cell lines.

CONCLUSION

In the present study novel amine derivatives of 2-(4-fluorophenyl)-5-(5-aryl substituted-1,3,4-oxadiazol-2-yl) pyridine have been synthesised. These compounds have showed good cytotoxicity against *HepG2* and *Caco-2* cell lines. The IC_{50} values of the compounds**7a**and **7d** against *HepG2* was found to be 2.6 µM (SI 13.06) and 5.8 µM (SI 19.31), respectively. On *Caco-2* cell lines the compound **7f** exhibited good cytotoxicity having IC_{50} 2.3µM and CC_{50} 87µM (SI 37.8).

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