DESIGN, SYNTHESIS, CHARACTERIZATION AND CYTOTOXIC EVALUATION OF NOVEL 2-CHLORO-N-(ARYL SUBSTITUTED)ACETAMIDE DERIVATIVES OF 5-[2-PHENYL PYRIDIN-3-YL]-1, 3, 4-OXADIAZOLE-2-SHOL.

ADIMULE VINAYAK1,3*, MEDAPA SUDHA2, KUMAR S. LALITA3, RAOPRAKASH KUMAR4

ABSTRACT
With the aim of producing new anticancer compounds a series of novel different 2-chloro N-aryl substituted acetamide derivatives of 5-[2-phenyl pyridin-3-y1]-1, 3, 4-Oxadiazole-2-Thiol have been synthesized by linear synthetic approach and screened for their cytotoxicity on PANC-1, Caco-2 and SK-N-SH cell lines and obtained the IC50 values. All the synthesized compounds were characterized by mass spectroscopy, IR, 1H and 13C (proton and Carbon 13) spectroscopies and elemental analyses. These compounds were evaluated for invitro anticancer activity on three different human cancer cell lines, namely PANC-1,Caco-2 and SK-N-SH. In total six compounds were synthesized and studied for their MTT assay. Among six synthesized novel compounds, in this series most of the compounds were highly cytotoxic against all the three cell lines used. One compound 6 (b) is moderately cytotoxic against PANC-1 cell line with IC50 of 24.3µM.

KEYWORDS
PANC-1, 1, 3, 4-Oxadiazoles, Cytotoxic, Chloroacetyl chloride, Caco-2, MTT assay.

AUTHORS AFFILIATION
*Address for correspondence
1. Mount Carmel Centre for Scientific Research and Advanced Learning, Mount Carmel College, Vasanth Nagar, Bengaluru-560 052, Karnataka, India, E-mail: adimulevinayak@yahoo.in; Mobile: +919035327313; Fax: +9108022286386.

2. Department of Chemistry, Mount Carmel College (Autonomous),Vasantha Nagar, Bengaluru- 560 052, Karnataka, India, E-mail: sudha_medapa@yahoo.com; Mobile: +919449551283.

3. Department of Chemistry, School of Sciences, IGNOU, New-Delhi, India, E-mail: Lalitaskumar@ignou.ac.in; Mobile: +919871300937.

INTRODUCTION

In this research work author synthesized the novel compounds of 2-chloro (N-aryl substituted)acetamide derivatives of 5-[2- phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-Thiol and screened these compounds for cytotoxicity\(^1\) against three different human cancer cell lines. Synthetic chemistry was started with 2-chloro nicotinic acid which is converted into ethyl ester and subsequently synthesized the carbohydrazide (4). The carbo hydrazide was cyclized using carbon disulphide and potassium hydroxide and obtained the reactive intermediate (5).This molecule is the main constituent of the integral part of the structure of the molecule (Figure1). The different chloroacetylated derivatives of aromatic amines have been synthesized and characterized by \(^1\)H-NMR, \(^13\)CNMR and LCMS. These kind of novel ring systems not yet studied but few of the derivatives of pyridine containing 1, 3, 4-oxadiazole-thiol moiety have been reported for their potent activity towards antimicrobial\(^2\), anti-inflammatory\(^3, 4\), anti-tubercular\(^5\) and anticancer\(^6\) properties. In this connection the author envisaged that by attaching different 2-chloro (N-aryl substituted)acetamide derivatives to the 1,3,4-oxadiazole-2- thiol moiety may enhance the water insolubility problem of these heterocycles and thus increasing the potency. In order to validate this hypothesis the author has synthesized six novel 2-chloro(N-aryl substituted) acetamide derivatives of 1, 3, 4-oxadiazole-2-thiol \(^7, 8\) compounds (6a-6f, Figure 1) and tested their invitro cytotoxicity against cancer cell lines. All the synthesized compounds were characterized by \(^13\)CNMR and \(^1\)H NMR spectroscopy and evaluated their anticancer activity at various concentrations. The study revealed that in this series of 1, 3, 4-oxadiazole derivatives does not exhibit cytotoxicity.

![Figure 1: Synthesis of 5-[2-phenyl pyridin-3-yl]-1, 3, 4] oxadiazole-2-thiol (Intermediate).
Scheme 2: Linear synthetic pathway of synthesis of 2-chloro (N-Aryl substituted) acetamide derivatives of 5-[2-phenyl pyridin-3-yl]-[1, 3, 4] oxadiazole-2-thiol. 6a-6f.

EXPERIMENTAL

Materials and Methods: All reagents, chemicals and solvents were purchased from S-d fine and Spectrochem Ltd. Bengaluru, India. $^1$H NMR and $^{13}$C NMR were recorded by Brucker 400 MHz spectrophotometer. Melting points are 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 was recorded by FTIR (1800S) series.

Synthesis

Synthesis of ethyl 2-chloropyridine-3-carboxylate (2)

The 2-chloro nicotinic acid (1) (10g, 1mol) was taken in a 500mL single necked round bottom flask, 150mL of ethanol and concentrated H$_2$SO$_4$ (3-5 drops) were added, reaction mixture was refluxed at 80°C for 8 hr. TLC (Thin layer chromatography) was monitored to check the completion of the reaction. Solvent was evaporated and the residue was neutralized with 10% NaHCO$_3$ solution. Aqueous was extracted with ethyl acetate (25 mL x2), washed with brine (10mL) and dried over Na$_2$SO$_4$, evaporated. The obtained pale yellow oil was recrystallized from ethanol-water as yellow needles. Yield 8.5g, MS (ESI) m/z: [M+H]- 187; HPLC purity = 96%; TLC-ethyl acetate: hexane (1:9); IR(KBr), $\nu$ max/cm$^{-1}$: 987, 1082, 2845, 3026; $^1$HNMR (CDCl$_3$, 400MHz) : δ 1.16(t, 3H), 3.87(q, 2H), 7.42(t, 1H, J 13.3Hz), 8.49(dd, 1H, J 8.5Hz), 8.88(d, 1H, J 7.8 Hz).

Synthesis of Ethyl 2-phenyl pyridine-3-carboxylate (3)

Ethyl2-chloropyridine-3-carboxylate8.5g(1mol),Na$_2$CO$_3$(1.2mol),4-phenylboronic acid (1.2mol),tetrakis (triphenyl phosphine)palladium (0)(0.05mol) were refluxed in 120mL of ethanol for 10h.TLC was monitored to check the completion of the reaction, after completion, the solvent was evaporated,aqueous was extracted with ethyl acetate (25 mL x3), washed with brine (15mL) and dried over Na$_2$SO$_4$. Ethyl acetate was evaporated to yield brown oil. The crude product was purified by column chromatography using silica gel (100 to 200mesh), gradient (0-15%) ethyl acetate in hexane as the eluent. Yield 4.6g,off white coloured solid;MS (ESI) m/z: [M+H]-228; m.p-127-129°C; IR(KBr),$\nu$max/cm$^{-1}$ : 887, 1130, 2985,3116; $^1$HNMR(CDC$_3$, 400 MHz) : δ 0.8(t, 2H), 3.52(q, 3H), 7.15(dd, J 7.8 Hz, 2H), 7.64(q,2H), 8.68(m,J 13.2Hz, 1H), 9.13(q,2H).
Synthesis of 2-phenyl nicotinic acid hydrazide (4)

Ethyl 2-phenyl pyridine-3-carboxylate (1mol) was taken in a 100mL single necked round bottom flask added with excess (15mL) of hydrazine hydrate and refluxed in ethanol overnight. TLC was monitored to check the completion of the reaction, solvent was completely removed under reduced pressure, residue was cooled to 5°C and added ice pieces and stirred. Solids that are separated out were filtered, washed with water (100mL) and dried over sodium sulphate. Yield 2.3g; white solid; TLC ed at R.T for 3

Synthesis of 5-[2-Phenyl pyridin-3-yl]-1,3,4-oxadiazole-2-thiol (5)

2-(phenyl)-nicotinic acid hydrazide (1mole) was taken in a 100mL single necked RB flask added with carbon disulphide (50mL), KOH solution (10%) and solvent ethyl acetate. RM was refluxed at 85°C overnight.TLC was monitored to check the completion of the reaction, after completion solvent was removed RM was poured over 100mL of ice cold water and neutralized with 1N HCl. Solids that are separated out was filtered and dried. The crude product was purified by column chromatography using silica gel (100 to 200mesh), gradient (0-15%) ethyl acetate in hexane as the eluent. Yield 4.6g, off white coloured solid; MS (ESI) m/z: [M+H]-272; m.p-123-128°C; IR(KBr), v_max/cm\(^{-1}\): 885, 1100, 2945, 3106, 3355; \(^1\)H NMR(CDCl\(_3\), 400MHz) δ: 4.58(bs,2H,NH\(_2\)), 7.28(dd, J 12.8Hz, 2H), 7.64(q,2H), 8.65(m,J 8.5Hz, 1H), 9.12(q,2H).

General procedure for the synthesis of 2-chloro (N-aryl substituted) acetamide derivatives a-h:

The different amines (Table 1) were taken in a 100 mL single necked RB flask to this solvent 100mL of THF was added, 5% NaOH (5-10ML) was then added under stirring and RM was cooled to 0°C. Chloroacetyl chloride was added (2.5equivalent) drop wise under stirring and RM was stirred at R.T for 3-8h.TLC was monitored to check the completion of the reaction, after completion solvent was removed under reduced pressure residue was added with few ice pieces and solid that is obtained was filtered, washed with water (50mL) and dried. These compounds were pure enough to carry to the next step.

General procedure for the synthesis of novel derivatives of 2-chloro-N-(aryl substituted) acetamide of 5-[2-(phenyl) pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol, 6a-6h:

The 5-[2-(4-Fluorophenyl) Pyridin-3-yl]-1, 3, 4-Oxadiazole-2-Thiol was taken in a 100 mL single necked RB flask to this solvent 10-15mL of aceton and K\(_2\)CO\(_3\) (1.5-2.5 equivalent) were added under stirring. RM was cooled to 0°C. Different 2-chloro (N-aryl substituted) amines (Table 1) were added (1.2 equivalent) under stirring to the RM. RM was stirred at R.T for 3h.TLC was monitored to check the completion of the reaction. If the reaction was not completed RM was warmed to 50°C for 6h.TLC was monitored again to check the completion of the reaction, after completion solvent was removed under reduced pressure residue was added with few ice pieces and aqueous was extracted with ethyl acetate,washed with brine,dried over sodium sulphate. All the final compounds6a-6f was purified by column chromatography using silica gel 100-200mesh.Eluent started with 100% n-hexane and polarity was increased to 75% using ethyl acetate.
Analytical data of the final novel derivatives of 2-chloro-(N-aryl substituted) acetamide compounds of 5-[2-Phenyl pyridin-3-yl]-1, 3, 4-Oxadiazole-2-Thiol: 6a-6f

2-[5-[2-phenyl pyridin-3-yl]-I, 3, 4] oxadiazol-2-ylsulfanyl]-N-phenyl-acetamide(6a): R = Phenyl acetamide

White coloured solid; yield 65.8% ; m.p -185-188°C; IR (KBr), νmax/cm⁻¹ : 1123, 2935, 3346, 2765, 3350; ¹H-NMR(CDCl₃, 400MHz): δ 2.9 (s, 2H, CH₂), 7.45(dd, J 8.5Hz, 3H), 7.67 (m, 4H), 7.93 (d, J 7.2Hz, 2H), 8.3 (dd, J 12.4Hz, 2H), 9.23 (dd, 2H), 10.2 (bs, 1H, NH); ³¹C NMR( CDCl₃, 100MHz): 37, 116, 124.5, 128.5, 129, 135, 137, 155, 162, 163, 173; molecular formula C₂₁H₁₅N₅O₂S; MS: (ESI) m/z:[M+H] - 388; HPLC 95.4%; anal. Calculated for C₂₁H₁₅N₅O₂S; C, 72.45; H, 3.10; N, 14.46; O, 8.26; S, 8.28; Found C, 72.65; H, 3.91; N, 14.47; O, 8.27; S, 8.29.

2-[5-[2-Phenyl pyridin-3-yl]-I, 3, 4] oxadiazol-2-ylsulfanyl]-N-pyridin-2-yl-acetamide(6b): R = Pyridin-2yl

Yellow coloured solid; yield 54.2% ; m.p -133-138°C; IR (KBr), νmax/cm⁻¹ : 1285, 2995, 3316, 2885, 3310; ¹H-NMR(CDCl₃, 400MHz): ¹H-NMR (CDCl₃, 400MHz): δ 2.9 (s, 2H, CH₂), 7.21(dd, J 8.2Hz, 2H), 7.43(dd, J 8.5Hz, 2H), 7.7(m, 4H), 7.8(dd, J 13.8Hz, 2H), 9.05(dd, J 8.5Hz, 2H), 10.03(bs, 1H, NH); ³¹C NMR( CDCl₃, 100MHz): 37, 113.5, 116, 123.2, 124, 129, 135, 136, 137, 144, 150, 155, 163, 173; molecular formula C₂₂H₂₁N₄O₂S; MS: (ESI) m/z:[M+H] - 389; HPLC 96.8%; anal. Calculated for C₂₂H₂₁N₄O₂S; C, 61.84; H, 3.63; N, 18.03; O, 8.24; S, 8.26; Found C, 61.85; H, 3.64; N, 18.05; O, 8.25; S, 8.27.

N-(5-Bromo-pyridin-2-yl)-2-[5-[2-(4-fluoro-phenyl)-pyridin-3-yl]-I, 3, 4] oxadiazol-2-ylsulfanyl]-acetamide(6c): R = 5-Bromo-pyridin-2yl

Off white coloured solid; yield 48.2%; IR (KBr), νmax/cm⁻¹ : 1215, 2965, 3376,2786, 2825, 3350; ¹H-NMR(CDCl₃, 400MHz): ¹H-NMR (CDCl₃, 400MHz): δ 3.1 (s, 2H, CH₂), 7.13(dd, J 13.2Hz, 2H), 7.3(dd, J 8.3Hz, 2H), 7.7(m, 4H), 7.82(dd, 2H), 9.2 (dd, J 6.8Hz, 2H), 10.05(bs, 1H, NH); ³¹C NMR(CDCl₃, 100MHz): 37, 115, 116, 118, 124, 129, 135, 137, 139, 148, 150, 153, 155, 162, 163, 173; molecular formula C₂₂H₁₃BrN₅O₂S; MS: (ESI) m/z:[M+H] - 467; HPLC 95.8%; anal. Calculated for C₂₂H₁₃BrN₅O₂S; C, 51.40; H, 2.80; Br, 17.10; N, 14.99; O, 6.85; S, 6.86; Found C, 51.43; H, 2.82; Br, 17.12; N, 14.92; O, 6.86; S, 6.87.

N-(5-[4-Fluoro-phenyl pyridin-3-yl]-2-[5-[2- phenyl pyridin-3-yl]-I, 3, 4] oxadiazol-2-ylsulfanyl]-acetamide(6d): R = 4-Fluoro-phenyl-pyridin-2-yl

Off white coloured solid; yield 53.8%; m.p- 102-108°C ; IR (KBr), νmax/cm⁻¹ : 1235, 2935, 3396, 2896, 2835, 3210; ¹H-NMR(CDCl₃, 400MHz): δ 2.65 (s, 2H, CH₂), 7.35(dd, 2H), 7.45(dd, J 8.5Hz, 2H), 7.51(m, 3H), 7.9(dd, J 7.8Hz, 2H), 8.3 (dd, J 12.4Hz, 2H), 9.1(dd, J 13.4, 2H), 9.3(dd, 1H), 10.05(bs, 1H, NH); ³¹C NMR( CDCl₃, 100MHz): 37, 114, 116, 124, 129, 135, 137, 144.5, 149, 151, 163, 173; molecular formula C₂₆H₁₇FN₃O₂S;MS: (ESI) m/z:[M+H] - 483; HPLC 98.3%; anal. Calculated for C₂₆H₁₇FN₃O₂S; C, 64.72; H, 3.55; F, 3.94; N, 14.51; O, 6.63; S, 6.65; Found C, 64.73; H, 3.57; F, 3.95; N, 14.52; O, 6.64; S, 6.66.

2-[5-[2-phenyl pyridin-3-yl]-I, 3, 4] oxadiazol-2-ylsulfanyl]-N-[5-[4-methoxy-phenyl]-pyridin-2-yl]-acetamide(6e): R = 4-methoxyphenyl-pyridin-2yl

White coloured solid; yield 56%; m. p: 140-141°C IR (KBr), νmax/cm⁻¹ : 1285, 2945, 3326,2916, 2835, 3240; ¹H-NMR(CDCl₃, 400MHz): δ 2.05 (s, 3H, OCH₃), 2.65 (s, 2H,

CH₂), 7.32(dd, J 12.4, 2H), 7.45 (m, 4H), 7.75(m, 3H), 7.9(dd, J 7.8Hz, 2H), 8.32 (dd, J 13.4Hz, 2H), 9.25(dd, J 13.4, 2H), 10.05(bs, 1H,NH); ¹³C NMR( CDCl₃, 100MHz): 37, 65, 114, 116, 124, 128.5, 129, 134, 135, 137, 145, 149, 150.5, 155, 159, 162, 163, 173; molecular formula C₂₇H₂₀N₅O₃S; MS: (ESI) m/z:[M+H]- 495; HPLC 96.8% ; anal. Calculated for C₂₇H₂₀N₅O₃S; C, 65.57; H, 4.08; N, 14.16; O, 9.71; S, 6.48; Found C, 65.58; H, 4.09; N, 14.17; O, 9.72; S, 6.49.

6-(2-{5-[2-phenyl pyridin-3-yl]-[1, 3, 4] oxadiazol-2-ylsulfanyl]-acetylamino})-nicotinic acid ethyl ester (6f): R = Nicotinic acid ethyl ester

Colourless liquid; yield 48.2% ; IR (KBr), νmax/cm⁻¹ : 1245, 2915, 3376,2886, 2515, 3240, 3356; ¹H-NMR(CDCl₃, 400MHz ): δ 0.3 (t, 2H, CH₂), 2.9 (s, 2H, CH₂), 3.1(q, 3H), 7.35(dd, J 13.1, 2H), 7.65(m, 3H), 7.8(dd, J 13.5, 3H), 8.2(dd, 1H), 8.8(dd, 2H),10.05(bs, 1H, NH); ¹³C NMR(CDCl₃, 100MHz): 13.5, 37, 61, 114, 116, 124, 129, 135, 137, 150, 151, 154, 155, 162.5, 163, 173, 174.; molecular formula C₂₃H₁₈N₅O₄S; MS: (ESI) m/z:[M+H]- 461; HPLC 96.3% ; anal. Calculated for C₂₃H₁₈N₅O₄S; C, 59.99; H, 3.94; N, 15.21; O, 13.90; S, 6.96; Found C, 59.98; H, 3.95; N, 15.22; O, 13.91; S, 6.97.

Table 1: Structures of amine and 2-chloro (N-aryl substituted) acetamide derivatives a-f.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amines</th>
<th>2-chloro (N-aryl substituted) acetamides a-f.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><img src="image1" alt="A1" /></td>
<td><img src="image2" alt="A" /></td>
</tr>
<tr>
<td>2.</td>
<td><img src="image3" alt="B1" /></td>
<td><img src="image4" alt="B" /></td>
</tr>
<tr>
<td>3.</td>
<td><img src="image5" alt="Cl" /></td>
<td><img src="image6" alt="C" /></td>
</tr>
<tr>
<td>4.</td>
<td><img src="image7" alt="D1" /></td>
<td><img src="image8" alt="D" /></td>
</tr>
<tr>
<td>5.</td>
<td><img src="image9" alt="E1" /></td>
<td><img src="image10" alt="E" /></td>
</tr>
</tbody>
</table>
Table 2: IC\textsubscript{50} and CC\textsubscript{50} values of the novel 2-chloro (N-aryl substituted) acetamides derivatives containing 5-[2- phenyl pyridin-3-yl]-1, 3, 4-Oxadiazole-2-Thiolmoiety.

<table>
<thead>
<tr>
<th>Compounds No.</th>
<th>IC\textsubscript{50} and CC\textsubscript{50} values of the novel 1,3,4-oxadiazole derivatives 6a-6f, PANC-1Caco-2SK-N-SH</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>29.4 (78.9) 34.6(34.8) 98.8(45.7)</td>
</tr>
<tr>
<td>6b</td>
<td>24.3 (88.9) 45.6(76.8) 32.2(56.7)</td>
</tr>
<tr>
<td>6c</td>
<td>47.5(43.3) 78.9(150) 65.5(200)</td>
</tr>
<tr>
<td>6d</td>
<td>43.2 (45.8) 122.08(34.4) 111.56(32.2)</td>
</tr>
<tr>
<td>6e</td>
<td>56.6(78.2) 32.2(23.4) 127.8(45.6)</td>
</tr>
<tr>
<td>6f</td>
<td>120.9(37.8) 45.6(34.5) 113.4\textsuperscript{1}(76.8)</td>
</tr>
<tr>
<td>5-FU</td>
<td>7.8(39.9) 6.9(36.8) 8.2(45.8)</td>
</tr>
</tbody>
</table>

IC\textsubscript{50}: Is the concentration that induces 50% of the growth inhibition as compared to untreated cells

CC\textsubscript{50}: Is the concentration of the 50% of the remaining cells after inhibition.

5-FU: 5-Fluoro uracil, standard used in the experiment.

**CYTOTOXIC EVALUATION**

**Cell Lines fixation and Culture Conditions**

The *invitro* anti-proliferative study was carried out on three human carcinoma cell lines namely PANC-1, Caco-2 and SK-N-SH. 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 of 95% air, 5% CO\textsubscript{2}. Following 24-48 hr. of incubation period, the adherent cells were detached using Trypsin-EDTA solution (HI Media Labs, Mumbai, India). Cell count was done using the Luna automated cell counter (Logos Bio systems, India) based on trypan blue dye exclusion method. Cytotoxicity of the novel methyl amine derivatives of 1, 3, 4-oxadiazoles have been determined using MTT 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay.

\textsuperscript{1}Potent molecule
By introducing the Corning®, USA derivatives (% MTT (5mg/ml; HI Media Labs, Mumbai, India) the Suzuki coupled products of 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol. It was prepared by the cyclization reaction using carbon disulphide and potassium hydroxide refluxed till the completion of the reaction. Derivatives of 2-chloro-(N-aryl substituted) acetamides¹⁰ coupled products of 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol were synthesized using 2-chloro nicotinic ethyl benzoate obtained by the esterification of 2-chloronicotinic acid. The ethylester(2) was reacted with 4-fluoro phenyl boronic acid in presence of tetrakis (triphenyl phosphine) palladium (0) and obtained the Suzuki coupled product 3 which upon refluxing with hydrazine hydrate obtained the carbohydrazide 4. Interestingly the carbohydrazide was cyclised in presence of carbon disulphide and potassium hydroxide and obtained the intermediate 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol. Different 2-chloro-(N-aryl substituted) acetamides⁰¹ a-g were synthesized by the treating choroacetic acid and sodium hydroxide to the respective amines. The final compounds 6a-6f were synthesized by fusing 2-chloro-(N-aryl substituted) acetamides to the key intermediate 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol in presence of K₂CO₃ and solvent acetone.In this linear synthesis six novel derivatives have been synthesized and purified by column chromatography using silica gel 100-200mesh and characterized by ¹³CNMR, ¹H-NMR and IR spectroscopies. Author envisaged that by introducing phenyl boronic acid group at the C2 position of the pyridine ring may enhance the water insolubility problem of 1, 3, 4-oxadiazoles and thus increasing the more bioavailability of the compounds.

**SAR: Structural Activity Relationship**

Studies related to SAR of these 1, 3, 4-oxadiazoles -2-thiol showed that the 2-chloro(N-aryl substituted) acetamidederivatives coupled with the cyclised key intermediate 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol ring enhances the water solubility and thereby more bio available molecules. By introducing the phenyl group at the C2 position of the pyridine enhances further the log-p values as well as increases the TPSA of the molecules. ring. Author envisaged that by coupling different 2-Chloro-(N-aryl substituted) acetamide group to the 1,3,4-oxadiazole thiolmoiety may further enhance the bioavailability of these molecules and thus increasing its potency.

**Invitro Cell Viability Assay (MTT Assay)**

The MTT assay was carried out in Genelon Institute of Life Sciences Pvt. Ltd. 200µL cell suspension was seeded in 96-well micro plates (Corning®, USA) at a density of 25,000 cells/well and incubated for 24hrs, all cells were seeded in duplicates with novel compounds 6a-6f. Having range of concentrations from 50µM-500µM, incubated in a CO₂ incubator at 37°C. Treated cells were thereafter incubated with 10% MTT (5mg/ml; HI Media Labs, Mumbai, India) for 3 hrs. The culture medium was then aspirated and 200µL dimethyl sulfoxide (DMSO; Sigma-Aldrich, India) was added. Doxorubicin was used as control. Cell viability was determined by measuring the absorbance on a micro plate reader (SPECTRO STAR NANO, BMG LABTECH, Germany) at 570nm. Cell viability was calculated as a percentage of viable cells at different test concentrations relative to the control (5-FU) cells [% cell viability = (A₅₇₀ of treated cells / A₅₇₀ of control cells) ×100%].

**RESULTS AND DISCUSSIONS**

**Chemistry (Figure 2)**

The chemistry of the synthesis of novel 1,3,4-oxadiazole compounds started with the synthesis of key intermediate 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol. It was prepared by the cyclization reaction using carbon disulphide and potassium hydroxide refluxed till the completion of the reaction. Derivatives of 2-chloro-(N-aryl substituted) acetamides¹⁰ coupled products of 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol were synthesized using 2-chloro nicotinic ethyl benzoate obtained by the esterification of 2-chloronicotinic acid. The ethylester(2) was reacted with 4-fluoro phenyl boronic acid in presence of tetrakis (triphenyl phosphine) palladium (0) and obtained the Suzuki coupled product 3 which upon refluxing with hydrazine hydrate obtained the carbohydrazide 4. Interestingly the carbohydrazide was cyclised in presence of carbon disulphide and potassium hydroxide and obtained the intermediate 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol. Different 2-chloro-(N-aryl substituted) acetamides⁰¹ a-g were synthesized by the treating choroacetic acid and sodium hydroxide to the respective amines. The final compounds 6a-6f were synthesized by fusing 2-chloro-(N-aryl substituted) acetamides to the key intermediate 5-[2-phenyl pyridin-3-yl]-1, 3, 4-oxadiazole-2-thiol in presence of K₂CO₃ and solvent acetone. In this linear synthesis six novel derivatives have been synthesized and purified by column chromatography using silica gel 100-200mesh and characterized by ¹³CNMR, ¹H-NMR and IR spectroscopies. Author envisaged that by introducing phenyl boronic acid group at the C2 position of the pyridine ring may enhance the water insolubility problem of 1, 3, 4-oxadiazoles and thus increasing the more bioavailability of the compounds.
Biology

The obtained series of novel 1,3,4-oxadiazole derivatives 6a-6f have been screened for cytotoxicity\[12\] on three different human leukemic cell lines to obtain the IC\(_{50}\) and CC\(_{50}\) of the molecules. The cancer cell lines used was SK-N-SH, Caco-2 and PANC-1. The MTT assay of the novel 1, 3, 4-oxadaizoles\[13\] have been screened for these cell lines and obtained the interesting data.

CONCLUSIONS

In this series six novel acetamide derivatives were synthesized and studied for their in vitro cytotoxic resistance against three human cancer cell lines. Most of the compounds in this series showed greater resistance. But, one compound 6(b) showed cytotoxicity of 24.3µM against PANC-1 cell line. Rest all the compounds showed moderate cytotoxicity against all the three cell lines.

ACKNOWLEDGEMENTS

First authors thankful to Mount Carmel College, Autonomous, Bangalore and Indian Institute of Science, IISc, Bangalore, India.

REFERENCES


