INTRODUCTION

Author has synthesized the novel compounds of 2-chloro (N-aryl substituted)acetamide derivatives of 5-[2-(4-methoxyphenyl) pyridin-3-yl]-1, 3, 4-Oxadiazole-2-Thiol and screened these compounds for cytotoxicity on three different human leukemic cell lines. Synthetic chemistry was started with 2-chloro nicotinic acid which is converted into ethyl ester and subsequently synthesized the carbohydrazide. The carbohydrazide was cyclised using carbon disulphide and potassium hydroxide and obtained the key intermediate. This 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 anticancer, anti-tubercular, anti-inflammatory, anti-bacterial and kinase inhibition 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 Log-P values and thus increasing the potency. In order to validate this hypothesis the author has synthesized five novel 2-chloro(N-aryl substituted) acetamide derivatives to the 1, 3, 4-oxadiazole-2-thiol compounds and tested their invitro cytotoxicity against cancer cell lines. The study revealed that the different 1, 3, 4-oxadiazole derivatives possesses excellent anticancer activity. In this synthesis compounds have been reported for their potent activity towards anticancer, anti-tubercular, anti-inflammatory, anti-bacterial and kinase inhibition 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 Log-P values and thus increasing the potency. In order to validate this hypothesis the author has synthesized five novel 2-chloro(N-aryl substituted) acetamide derivatives to the 1, 3, 4-oxadiazole-2-thiol compounds and tested their invitro cytotoxicity against cancer cell lines. The study revealed that the different 1, 3, 4-oxadiazole derivatives possesses excellent anticancer activity. In this synthesis compounds...
6e has showed good antiproliferative\(^{(13)}\) activity on PANC-1 and HepG2 cell lines having IC\(_{50}\) of 4.6 µM and 2.2 µM respectively.

Scheme 1: Synthesis of 5-(2-(4-methoxyphenyl) 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-(4-methoxyphenyl) pyridin-3-yl)-1, 3, 4-oxadiazole-2-thiol 6a-6e:

**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-chloronicotinic acid 1 (10g, 0.0636mol) was taken in a 1L single necked round bottom flask, 200mL of ethanol and concentrated H\(_2\)SO\(_4\) (3-5 drops) were added, reaction mixture was refluxed at 80°C for 8 h. TLC (Thin layer chromatography) was monitored to check the completion of the reaction. Solvent was evaporated and the residue was neutralized with
10% NaHCO₃ solution. Aqueous was extracted with ethyl acetate (35mL x2), washed with brine (20mL) and dried over Na₂SO₄, evaporated. The obtained pale yellow oil was recrystallized form ethanol-water as yellow needles. Yield 8.5g, MS-(M+H)- 187; HPLC purity = 96.7%; TLC-ethyl acetate: hexane (1:9); IR(KBr), νmax/cm⁻¹: 980, 1089, 2845, 3006 ; ¹H NMR (CDCl₃, 400MHz) : δ 1.18(t, 3H), 3.89(q, 2H), 7.41(t, 1H, J 13.4Hz), 8.44(dd, 1H, J 8.5Hz), 8.85(d, 1H, J 7.8 Hz).

**Synthesis of ethyl 2-(4-methoxy phenyl) pyridine-3-carboxylate 3**
Ethyl2-chloropyridine-3-carboxylate(8.5g, 0.0457mol),Na₂CO₃ (19.37g, 0.182mol),4-methoxy phenylboronicacid (8.335g, 0.0448mol),tetrakis(triphenyl phosphine)palladium (0)(0.263g,304.8mol) 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 (25mL x3), washed with brine (15mL) and dried over Na₂SO₄. 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)-258; m.p-143-148°C; IR(KBr),vmax/cm⁻¹ : 1130, 2965,3126; ¹H NMR(CDCls, 400 MHz) : δ 0.9(t,2H), 2.6(s, 3H), 3.7(q,3H), 7.26(dd,J 7.8 Hz, 2H), 7.68(q,2H), 8.75(m,J 13.2Hz, 1H), 9.34(q,2H).

**Synthesis of 2-(4-methoxy phenyl)-nicotinic acid hydrazide:**
Ethyl 2-(4-methoxy phenyl) pyridine-3-carboxylate(4.6g) was taken in a 250mL single necked round bottom flask added with excess (15mL) of hydrazine hydrateand refluxed in100mL of 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-ethylacetate: Hexane (50:50);m.p-162-164°C;MS (ESI) m/z: (M+H)-244;IR (KBr),vmax/cm⁻¹:1100, 2975, 3176, 3385; ¹H NMR(CDCls, 400MHz) δ 2.64(bs,2H,NH₂), 7.39(dd,J 12.8Hz, 2H), 7.56(q,2H), 8.75(m,J 8.5Hz, 1H), 9.23(q,2H).

**General procedure for the synthesis of 2-chloro (N-Aryl substituted) acetamide derivatives a-e:**
The variousamines (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 (2.5-3.5equivalent) 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-(4-methoxyphenyl) pyridin-3-yl)-1, 3, 4-oxadiazole-2-thiol: 6a-6e.

5-(2-(4-methoxyphenyl) pyridin-3-yl)-1, 3, 4-oxadiazole-2-thiol was taken in a 100 mL single necked RB flask to this solvent 10-15 mL of acetone 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 3-4.5h. TLC was monitored to check the completion of the reaction. If the reaction was not completed RM was warmed to 50°C for 4-6h. 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. The entire final compounds 6a-6e were purified by column chromatography using silica gel 100-200mesh. Eluent started with 100% n-hexane and polarity was increased to 80% using ethyl acetate.

Analytical data of the final novel derivatives of 2-chloro-(N-aryl substituted) acetamide compounds of 5-(2-(4-Fluorophenyl) Pyridin-3-yl)-1, 3, 4-Oxadiazole-2-Thiol: 6a-6e

2-(5-(2-(4-Fluoro-phenyl)-pyridin-3-yl)-(1, 3, 4) oxadiazol-2-ylsulfanyl)-N-pyridin-2-yl-acetamide (6b): R = Pyridin-2yl

Pale yellow coloured solid; yield 67%; m.p -123-124°C; IR (KBr), $\nu_{max}/cm^{-1}$: 1235, 2985, 3356, 2865, 3310; $1^H$-NMR($CDCl_3$, 400MHz): 6.52 (s, 3H), 2.87 (s, 2H, CH$_2$), 7.34 (dd, J 8.2Hz, 2H), 7.75 (m, 3H), 7.89 (dd, J 13.4Hz, 2H), 9.05 (dd, J 8.5Hz, 2H), 10.03 (bs, 1H, NH); $13^C$ NMR ($CDCl_3$, 100MHz): 65, 113.5, 116, 123.2, 124, 129, 135, 136, 137, 144, 150, 155, 163, 173; molecular formula C$_{21}$H$_{17}$N$_5$O$_3$S; MS: (ESI) m/z(M+H)-420; HPLC 94.7% ; anal. Calculated for C$_{21}$H$_{17}$N$_5$O$_3$S: C, 60.13; H, 4.09; N, 16.70; O, 11.44; S, 7.64; Found C, 60.14; H, 4.10; N, 16.72; O, 11.45; S, 7.65.

N-(5-Bromo-pyridin-2-yl)-2-(5-(2-(4-Fluoro-phenyl)-pyridin-3-yl)-(1, 3, 4) oxadiazol-2-ylsulfanyl)-acetamide (6c): R = 5-Bromo-pyridin-2yl.

Off white coloured solid; yield 47%; IR (KBr), $\nu_{max}/cm^{-1}$: 1215, 2985, 3356,2786, 2815, 3350; $1^H$-NMR($CDCl_3$, 400MHz): 2.53 (s, 3H), 6.31 (s, 2H, CH$_2$), 7.23 (dd, J 13.2Hz, 2H), 7.34 (dd, J 8.3Hz, 2H), 7.87 (m, 3H), 8.01 (dd, 2H), 9.23 (dd, J 6.8Hz, 2H), 10.23 (bs, 1H, NH); $13^C$ NMR($CDCl_3$, 100MHz): 65, 115, 116, 118, 124, 129, 135, 137, 139, 148, 150, 153.
N-(5-(4-Fluoro-phenyl)-pyridin-2-yl)-2-(5-(2-(4-fluoro-phenyl)-pyridin-3-yl)-1, 3, 4 oxadiazol-2-ylsulfanyl)-acetamide(6d): R = 4-Fluoro-phenyl)-pyridin-2-yl

White coloured solid; yield 56%; m.p- 122-126°C ; IR (KBr), ν max/cm⁻¹: 1235, 2935, 3396, 2886, 2815, 3250; ¹H-NMR(CDCl₃, 400MHz): δ 2.53(s, 3H), 2.65(s, 2H, CH₂), 7.45(dd, 2H), 7.53(dd, J 8.5Hz, 2H), 7.78(m, 3H), 7.89(dd, J 7.8Hz, 2H), 8.43 (dd, J 12.4Hz, 2H), 9.13(dd, J 13.4, 2H), 9.34(dd, 1H), 10.23(bs, 1H,NH); ¹³C NMR( CDCl₃, 100MHz): 65, 114, 116, 124, 129, 135, 137, 144.5, 149, 151, 163, 173; molecular formula C₂₇H₂₀FN₇O₃S; MS: (ESI) m/z: [M+H]- 514; HPLC 96% ; anal. Calculated for C₂₇H₂₀FN₇O₃S; C, 63.15; H, 3.93; F, 3.70; N, 13.64; O, 9.35; S, 6.24; Found C, 63.16; H, 3.94; F, 3.71; N, 13.65; O, 9.36; S, 6.25.

2-(5-(2-(4-Methoxy-phenyl)-pyridin-3-yl)-1, 3, 4 oxadiazol-2-ylsulfanyl)-N-(5-(4-methoxy-phenyl)-pyridin-2-yl)-acetamide(6e): R = 4-Methoxy-phenyl)-pyridin-2-yl

Brown coloured solid; yield 67% ; m.p: 139-141°C ; IR (KBr), ν max/cm⁻¹: 1285, 2945, 3326, 2916, 2835, 3240; ¹H-NMR(CDCl₃, 400MHz): δ 2.05 (s, 3H, O-CH₃), 2.45(s, 3H), 2.65 (s,2H,CH₂),  7.32(dd, J 12.4, 2H),  7.56(m, 3H), 7.87(m, 3H), 7.82(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): 65, 67, 114, 116, 124, 128.5, 129, 134, 135, 137, 145, 149, 150.5, 155, 159, 162, 163, 173; molecular formula C₂₈H₂₃FN₅O₄S; MS: (ESI) m/z(M+H)- 526; HPLC 96% ; anal. Calculated for C₂₈H₂₃FN₅O₄S; C, 63.99; H, 4.41; N, 13.33; O, 12.18; S, 6.10; Found C, 63.99; H, 4.41; N, 13.33; O, 12.18; S, 6.10.

Table 1: Structures of amine and 2-chloro (N-aryl substituted) acetamide derivativesa-e.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Amines</th>
<th>2-chloro (N-aryl substituted) acetamides</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td><img src="image1.png" alt="A1" /></td>
<td><img src="image2.png" alt="a" /></td>
</tr>
<tr>
<td>2</td>
<td><img src="image3.png" alt="B1" /></td>
<td><img src="image4.png" alt="b" /></td>
</tr>
<tr>
<td>3</td>
<td><img src="image5.png" alt="C1" /></td>
<td><img src="image6.png" alt="c" /></td>
</tr>
<tr>
<td>4</td>
<td><img src="image7.png" alt="D1" /></td>
<td><img src="image8.png" alt="d" /></td>
</tr>
<tr>
<td>5</td>
<td><img src="image9.png" alt="E1" /></td>
<td><img src="image10.png" alt="e" /></td>
</tr>
</tbody>
</table>

Table 2: IC₅₀ and CC₅₀ values of the novel 2-chloro (N-aryl substituted) acetamiderervatives of 5-(2-(4-methoxyphenyl) pyridin-3-yl)-1, 3, 4-oxadiazole-2-thiol.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC₅₀ and CC₅₀ values of 1, 3, 4-oxadiazoles</th>
</tr>
</thead>
<tbody>
<tr>
<td>PANC-1</td>
<td>HepG2</td>
</tr>
<tr>
<td>6a</td>
<td>34.4(68.9)</td>
</tr>
<tr>
<td>6b</td>
<td>49.8(78.9)</td>
</tr>
<tr>
<td>6c</td>
<td>47.5(43.3)</td>
</tr>
<tr>
<td>6d</td>
<td>29.8(45.8)</td>
</tr>
<tr>
<td>6e</td>
<td>4.6(&gt;150)</td>
</tr>
<tr>
<td>5-FU</td>
<td>7.8(39.9)</td>
</tr>
</tbody>
</table>

IC₅₀ - Is the concentration that induces 50% of the growth inhibition as compared to untreated cells. CC₅₀ - Is the concentration of the 50% of the remaining cells after inhibition. 5-Fluoro uracil, standard used in the experiment.


**CYTOXIC EVALUATION**

**Cell Lines fixation and Culture Conditions:**

The *in vitro* anti-proliferative study was carried out on three human carcinoma cell lines namely PANC-1, HepG2 and MCF7. 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% CO₂. 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 acetamide derivatives of 1, 3, 4-oxadiazole have been determined using MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay.

**In vitro Cell Viability Assay (MTT Assay):**

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-6e. 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 h. The culture medium was then aspirated and 200µL dimethyl sulfoxide (DMSO; Sigma-Aldrich, India) was added. 5-fluorouracil 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 = \( \frac{A_{570} \text{ of treated cells}}{A_{570} \text{ of control cells}} \times 100\% \)).

**RESULTS AND DISCUSSIONS**

**Chemistry (Figure 1 & 2):** The synthetic chemistry of novel 1, 3, 4-oxadiazole compounds started with the synthesis of key intermediate 5-(2-(4-Fluorophenyl) Pyridin-3-yl)-1, 3, 4-Oxadiazole-2-Thiol. This intermediate was obtained by reacting compound 4 with carbon disulphide and potassium hydroxide, 2-chloro-(N-aryl substituted) acetamidea-e were synthesized by treating corresponding amines with chloroacetyl chloride. Final compounds 6a-6e were synthesized by reacting different acetamides with 5-(2-(4-methoxyphenyl) pyridin-3-yl)-1, 3, 4-oxadiazole-2-thiol. The final compounds 6a-6e were synthesized by fusing different 2-chloro-(N-aryl substituted) acetamides to the key intermediate 5-(2-(4-methoxyphenyl) pyridin-3-yl)-1, 3, 4-oxadiazole-2-thiol. The author envisaged that by introducing 4-methoxy phenyl boronic acid group at the second position of the pyridine ring may enhance the Log-P and TPSA values of 1, 3, 4-oxadiazoles and thus increasing the more bioavailability of the compounds.

**a) SAR: Structural Activity Relationship:** Studies related to SAR of these 1, 3, 4-oxadiazoles -2-thiol showed that the 2-chloro (N-aryl substituted) acetamide derivatives coupled with the cyclised key intermediate 5-(2-(4-methoxyphenyl) pyridin-3-yl)-1, 3, 4-oxadiazole-2-thiol ring enhances the water solubility and thereby more bio available molecules. By introducing the 4-methoxy phenyl group at the second position of the pyridine enhances further the Log-P values as well as increases the TPSA of the molecules. 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.

b) **Biology:** The obtained series of novel 1, 3, 4-oxadiazole derivatives 6a-6e have been screened for cytotoxicity\(^{14, 15}\) on three different human leukemic cell lines to obtain the IC\(_{50}\) and CC\(_{50}\) of the molecules. The cancer cell lines used was PANC-1, HepG2 and MCF7. The MTT assay of the novel 1, 3, 4-oxadiazoles\(^{14}\) have been screened for these cell lines and obtained the interesting data (Table 2).

**CONCLUSIONS**

In this research author has synthesized five novel derivatives of 1, 3, 4-oxadiazole and screened for MIT assay.

**ACKNOWLEDGEMENTS**

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