Research Article

Synthesis, Characterization and Cytotoxic evaluation of Novel derivatives of 1-[2-(Aryl substituted)-5-(4'-Fluoro-3-methyl biphenyl-4-yl)-[1, 3, 4] oxadiazole-3-yl]-Ethanone

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Abstract

A series of novel 1,3,4-oxadiazole derivatives 6a-6f have been synthesized and characterized by 1H-NMR, 13CNMR, LCMS and elemental analysis. The key intermediate compound 4'-fluoro-3-methylbiphenyl-4-carboxylic acid was reacted with various aldehydes in presence of a catalytic amount of acetic acid and obtained the novel Schiff base compounds 5a-5f. The Schiff base compounds 5a-5f were acetylated by refluxing with acetic anhydride and obtained the final derivatives 6a-6f. All these compounds were evaluated for their MTT assay on three human cancer cell lines namely, HeLa, HepG2 and Caco-2. The antiproliferative activity of 1, 3, 4-oxadiazole compounds showed good cytotoxicity on Caco-2 cell line. Among the synthesized compounds, 6a and 6c showed good cytotoxicity on Caco-2 cell line having IC50 of 6.3µM and 4.4µM respectively. Compounds 6a, 6b, 6c and 6f showed mild cytotoxicity on all the three cell lines.

Keywords: Caco-2, 1, 3, 4-oxadiazole, MTT assay, Anticancer, Acetic anhydride

1. Introduction

The synthesis of novel derivatives of 1,3,4-oxadiazoles has been synthesized on the basis of the fact that 1,3,4-oxadiazoles known from decades as a potential molecule possessing various biological properties such as anti-inflammatory1, antibacterial14, antitubercular4 and anticancer. In this research work author has synthesized the novel ethanone derivatives of 1, 3, 4-oxadiazole compounds (Figure 1, A). The 1, 3, 4-oxadiazole compounds have been synthesized by linear synthetic method the starting material for this synthesis was 4-bromo-2-methyl benzoic acid which is converted into corresponding ester. The ester 2 was reacted with 4-fluoro phenyl boronic acid in presence of tetrakis (triphenyl phosphine) palladium (0) and obtain the compound 3. The compound 3 was converted into reactive intermediate carbodihydrazide 4 with the help of hydrazine hydrate by refluxing in ethanol. Thus obtained carbodihydrazide reacted with various aldehyde in presence of acetic acid as catalyst to get the novel Schiff base derivatives 5. The Schiff base compounds were refluxed in acetic anhydride to yield the novel ethanone derivatives (Figure 1, B) of 1, 3, 4-oxadiazoles 6a-6f. In total six derivatives have been synthesized and evaluated their antiproliferative activity on HeLa, HepG2 and Caco-2 cell line. Most of the compounds in this series showed mild cytotoxicity on all the three cell line but, two compound 6a and 6c showed good cytotoxicity on Caco-2 cell line having IC50 of 6.4µM and 4.4µM respectively.

2. Materials and Methods

All reagents, chemicals and solvents were purchased from S-d fine and Spectrum Ltd, Bengaluru. India. 1H NMR and 13C NMR were recorded by Brucker 400 MHz spectrophotometer. Melting points are determined using Buchi melting point 545.Mass spectra were recorded by Brucker 400 MHz spectrophotometer. Melting points were determined using Buchi melting point 545. Mass spectra were recorded by Brucker 400 MHz spectrophotometer. The antiproliferative activity of 1, 3, 4-oxadiazole derivatives (Figure 1, B) of 1, 3, 4-oxadiazoles 6a-6f. In total six derivatives have been synthesized and evaluated their antiproliferative activity on HeLa, HepG2 and Caco-2 cell line. Most of the compounds in this series showed mild cytotoxicity on all the three cell line but, two compounds 6a and 6c showed good cytotoxicity on Caco-2 cell line having IC50 of 6.4µM and 4.4µM respectively.

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2.3 Analytical data of the final novel derivatives of 1-[2-(Aryl substituted)-5-(4'-fluoro-3-methyl biphenyl-4-yl)-[1, 3, 4] oxadiazole-3-yl]-ethanone: 6a-f

2.3.1.1 -f[5-(4'-Fluoro-3-methyl-biphenyl-4-yl)-2-(2-fluoro-phenyl)-[1,3,4]oxadiazol-3-yl]-ethanone (6a): R = 2-Fluoro benzaldehyde ,
white coloured solid; yield 54.8%; m.p -165-168°C; IR (KBr), v max/cm-1: 1123, 1642, 2764,2936, 3252, 3347; 1H-NMR(CDCl3, 400MHz );
d1.05(s, CH3, 3H), 2.2(s, 3H), 6.7(s, 1H), 7.13(dd, J = 8.5Hz, 2H), 7.23(m, 3H), 7.56(m, J = 7.2Hz, 2H), 7.7(d, J = 12.4Hz, 2H), 8.9(d,J = 12.5Hz, 1H); 13C NMR (CDCl3, 100MHz): 114.5, 115.5, 123, 124, 128.5, 130, 134, 136.5, 150, 159, 163, 177; molecular formula C18H12F3NO3; ms: (ESI) m/z([M-H]-393; HPLC 94.4% ; anal. Calculated for C18H12F3NO3: C, 70.40; H, 4.62; F, 8.06; N, 7.14; O, 12.6; IR (KBr), ν max/cm-1: 3252, 3347, 1642, 2764,2936, 3252, 3347; 1H-NMR(CDCl3, 400MHz );
d0.8(s, CH3, 3H), 2.32(s, CH3, 3H), 6.45(s, H), 7.25(dd, J = 12.5Hz, 2H), 7.35(dd, J = 2H), 7.7(m, J = 7.2Hz, 3H), 7.8(m, J = 12.4Hz, 3H), 8.05(dd, J = 7.5Hz, 2H), 8.1(dd, J = 7.6, 2H); 13C NMR (CDCl3, 100MHz): 65, 114.5, 116, 123, 125, 127, 129, 131, 132, 134, 136.5, 150.5, 159, 162, 163, 177; molecular formula C18H12F3NO3; ms: (ESI) m/z([M-H]-451; HPLC 95.2%; anal. Calculated for C18H12F3NO3: C, 77.32, H, 5.15, F, 4.22; N, 6.22, O, 7.10; Found C, 77.33, H, 5.16; F, 4.23; N, 6.23; O, 7.11. 3a.

2.3.1.1 -f[2-(2,5-Dimethoxy-phenyl)-5-(4'-fluoro-3-methyl biphenyl-4-yl)-[1,3,4]oxadiazol-3-yl]-ethanone (6d): R = 2,5-Dimethoxy benzaldehyde ,
white coloured solid; yield 66%; m.p -124-126°C; IR (KBr), v max/cm-1: 812, 1235,1742,1890, 2287, 2815, 2935, 3255, 3396; 1H-NMR(CDCl3, 400MHz );
d0.8(s, CH3, 3H), 2.32(s, CH3, 3H), 6.45(s, H), 7.2(d, J = 12.5Hz, 2H), 7.6(m, 3H), 7.8(m, J = 12.4Hz, 3H), 8.15(dd, J = 7.5Hz, 2H), 8.8 (dd, J = 7.6, 2H), 9.1(dd, J = 1H); 13C NMR (CDCl3, 100MHz): 65, 90, 114.5, 122.5, 127.5, 128.5, 129, 131, 134, 136.5, 150.5, 159, 162, 177; molecular formula C18H12F3NO3; ms: (ESI) m/z([M-H]-451; HPLC 95.2%; anal. Calculated for C18H12F3NO3: C, 77.32, H, 5.15, F, 4.22; N, 6.22, O, 7.10; Found C, 77.33, H, 5.16; F, 4.23; N, 6.23; O, 7.11. 3a.

2.3.1.1 -f[5-(4'-Fluoro-3-methyl-biphenyl-4-yl)-2-(5-fluoro-phenyl)-[1,3,4]oxadiazol-3-yl]-ethanone (6e): R = 4-Fluoro-phenyl-thiophene-2-thiophen-2-aldehyde
Pale yellow coloured solid; yield 67.6%; m.p -124-126°C; IR (KBr), v max/cm-1: 776, 987, 1235, 1716, 1920,2824,2887, 2945, 3256, 3396; 1H-NMR(CDCl3, 400MHz );
d0.8(s, CH3, 3H), 2.32(s, CH3, 3H), 2.65(s, H), 6.7(s, 1H), 7.05(m, J = 12.5Hz, 3H), 7.11(dd, J = 7.5Hz, 2H), 7.65(dd, J = 12.4Hz, 2H), 8.5(dd, J = 7.5Hz, 1H); 13C NMR (CDCl3, 100MHz): 19, 65, 90, 114.5, 122.5, 128, 134, 136.5, 150, 159, 162, 163, 177; molecular formula C18H12F3NO3; ms: (ESI) m/z([M-H]-451; HPLC 95.2%; anal. Calculated for C18H12F3NO3: C, 69.11, H, 5.34; F, 4.37; N, 6.45; O, 14.73; Found C, 69.12, H; 5.35; F, 4.38; N, 6.46; O, 14.74. 3a.

2.3.1.1 -f[5-(4'-Fluoro-3-methyl-biphenyl-4-yl)-2-(5-fluoro-phenyl)-[1,3,4]oxadiazol-3-yl]-ethanone (6f): R = 5- Phenyl thiophene-2-carboxaldehyde
Yellow coloured solid; yield 76%; m.p -172-176°C; IR (KBr), v max/cm-1: 785, 1235, 1756, 2886, 2935, 3256, 3396; 1H-NMR(CDCl3, 400MHz );
d0.8(s, CH3, 3H), 2.32(s, CH3, 3H), 6.42(s, H), 7.25(dd, J = 12.5Hz, 2H), 7.45(dd, J = 7.12, 2H), 7.6(dd, J = 7.2Hz, 2H), 7.7(m, J = 12.4Hz, 4H), 8.05(dd, J = 12.6, 2H), 9.1 (dd, J = 11.8, 2H); 13C NMR (CDCl3, 100MHz): 90, 114.5, 122.5, 125, 126, 127.5, 128, 134, 136, 137, 141, 150, 159, 162, 163, 177; molecular formula C23H13F3N4O2S; ms: (ESI) m/z([M-H]-457; HPLC 96.6%; anal. Calculated for C23H13F3N4O2S: C, 71.03; H, 4.64; F, 4.16; N, 6.14; O, 7.01; S, 0.72; Found C, 71.04; H, 4.65; F, 4.17; N, 6.15; O, 7.02; S, 0.73. 3a.
Table 1: IC_{50} and CC_{50} values of the novel derivatives of 1-[2-(Aryl substituted)-5-(4'-fluoro-biphenyl-4-yl)-[1, 3, 4] oxadiazole-3-yl]-ethanone.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC_{50} and CC_{50} values of 1, 3, 4-oxadiazoles in µM</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>HeLa</td>
</tr>
<tr>
<td>6a</td>
<td>34.4(68.9)</td>
</tr>
<tr>
<td>6b</td>
<td>49.6(68.9)</td>
</tr>
<tr>
<td>6c</td>
<td>47.5(53.3)</td>
</tr>
<tr>
<td>6d</td>
<td>29.8(55.8)</td>
</tr>
<tr>
<td>6e</td>
<td>43.6(100)</td>
</tr>
<tr>
<td>6f</td>
<td>44.5(65.6)</td>
</tr>
<tr>
<td>5-FU</td>
<td>7.8(48.9)</td>
</tr>
</tbody>
</table>

2.4 Cytotoxic Evaluation

2.4.1. Cell Lines fixation and Culture Conditions: The in vitro anti-proliferative study was carried out on three human cancer cell lines namely HeLa, HepG2 and Caco-2. All the cell lines were grown in DMEM-HG supplemented with 10% heat-inactivated FBS, 2% Penicillin-Streptomycin and 2.5 µg/mL Ampicillin-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 ethanone derivatives of 1, 3, 4-oxadiazoles have been determined using MTT 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay.

2.4.2. Invitro Cell Viability Assay (MTT Assay): 200µL of 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 h. The culture medium was then aspirated and 200µL dimethyl sulfoxide (DMSO; Sigma-Aldrich, India) was added. 5-flourocurcil 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 = (A_{treated cell} / A_{control cell}) ×100%].

3. Results and Discussions

3.1. Chemistry (Scheme 1): The synthetic chemistry of novel ethanone derivatives of 1, 3, 4-oxadiazole compounds started with the synthesis of ethyl 4-bromo-2-methylbenzoxazole 2 which is coupled with 4-fluoro phenyl boronic acid(Suzuki coupling). The introduction of the 4-fluoro phenyl boronic acid group increases the Log-P as well as TPSA of the 1,3,4-oxadiazole molecules. The intermediate 3 was reacted with hydrazine hydrate and ethyl alcohol in order to obtain the corresponding carbohydrazide 4. The key intermediate 4-fluoro-3-methylphenyl-4-carboxyhydrazide was reacted with various substituted aldehydes a-fin presence of a catalytic amount of acetic acid yielded a series of novel Schiff base derivatives 5a-5f. The reactive novel Schiff base derivative 5a-5f were refluxed in acetic anhydride and obtained a series of novel derivatives of 1-[2-(Aryl substituted)-5-(4'-fluoro-3-methyl biphenyl-4-yl)-[1, 3, 4] oxadiazole-3-yl]-ethanone11,12. Author envisaged that by introducing 4-fluoro phenyl boronic acid group at the second position of the pyridine ring may enhance the Log-P and TPSA values of 1, 3, 4-oxadiazoles12,13 and thus increasing the more bioavailability of the novel ethanone derivatives of 1, 3, 4-oxadiazole compounds13.

3.1.2SAR: Structural Activity Relationship: Studies related to SAR of these 1, 3, 4-oxadiazole ethanone derivatives showed that the substitution of different aryl derivatives in the oxadiazole ring enhances the water solubility and thereby more bio available molecules. By introducing the 4-fluoro phenyl group at the forth position and constructing biphenyl ring enhances further the Log-P values as well as increases the TPSA of the molecules. Author envisaged that by reacting with various aldehydes and further cyclizing the intermediate obtained novel derivatives of 1, 3, 4-oxadiazole ethanone moiety13.

3.1.3. Biology: The obtained series of novel 1, 3, 4-oxadiazole derivatives 6a-6f have been screened for cytotoxicity13 on three different human cancer cell lines namely, HeLa, HepG2 and Caco-2 and obtain the IC_{50} and CC_{50} of the molecules. The MTT assay of the novel 1, 3, 4-oxadiazoles13 have been screened for these cell lines and obtained the interesting data (Table 1). Compound 6a and 6e showed good cytotoxicity on Caco-2 cell lines having IC_{50} of 6.4µM and 4.4µM respectively. Rest all the compounds showed moderate cytotoxicity as in the (Table 1).

3.1.4. Scheme 1: Synthesis of novel derivatives of 1-[2-(Aryl substituted)-5-(4'-fluoro-3-methyl biphenyl-4-yl)-[1, 3, 4] oxadiazole-3-yl]-ethanone.

Reagents and Conditions: (i) Ethyl alcohol, Conc. H2SO4; (ii) 4-Fluoro phenyl boronic acid; (iii) Hydrazine hydrate/reflux ; (iv) Acetic acid/ various aldehydes; (v) Acetic anhydride/ reflux
4. Conclusions

In this research author has synthesized six novel derivatives of 1, 3, 4-oxadiazole and screened for MTT assay. Compound 6a and 6e showed good antiproliferative activity on Caco-2 cell lines having IC_{50} 6.4µM and 4.4µM of respectively. The obtained IC_{50} values are better than the one obtained with the standard 5-FU. The compounds 6a and 6e can act as potent compound for the antiproliferative activity. The remaining compounds showed moderate to low cytotoxicity on all the three cell lines as compared with the standard 5-FU.

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References