Research Article
Synthesis and Biological Activities of Some 1, 3, 4-Oxadiazole Based Schiff’s Bases
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Abstract
In the present study a series of 1, 3, 4-oxadiazole based Schiff’s bases (4a-4i) were synthesized and characterized by IR and NMR spectroscopy. The synthesized compounds were evaluated for their preliminary in-vitro anti-cancer activities. Compound 4h showed better anticancer activity (GIs value of 2.7 × 10⁻³M), which was closer to test standards doxorubicin (GIs value 1.3×10⁻³M).

Keywords:
1, 3, 4-oxadiazoles, Schiff’s base, Biological evaluation, Anti-Cancer study.

1. INTRODUCTION
The literature is flooded with numerous reports on biological, pharmacological and other important uses of various heterocycles comprising of azoles1 like oxadiazole2, Triazoles, indole3, benzotriazole, oxazoles, imidazoles4, pyridine and their derivatives. Among them 1,3,4-oxadiazoles have been associated with varieties of activities ranging from herbicidal, diuretic, monoamine oxidase inhibition, anti ulcer5, anti-inflammatory6 to anti cancer7, anti-tubercular and anti-human Immunodeficiency virus etc. Moreover schiffs bases of various heterocyclic scaffolds exhibits a broad spectrum of biological activities like anti human immunodeficiency virus8,9, anti cancer10, anti bacterial11, fungicidal and anti inflammatory12. The importance of benzotriazole as chemotherapeutic agent is also well known13, 14, 15, 16. With this background and our continuous interest17,18,19 in 1,3,4-oxadiazole chemistry, we herein report the synthesis of few 1,3,4 oxadiazole derivatives comprising 1,2,3-benzotriazole ar one end and schiff’s base structure at other end with biological activities of interest.

2. MATERIALS AND METHODS
The synthetic pathways adopted for the preparation of the parent compounds 3, 4a-4i are outlined in Scheme. (Fig. 1).

2.1 Synthesis of Ethyl-2-(1H-benzo[d] [1,2,3] triazole-1-yl) acetate (1)
To a solution of 1,2,3-(1H)-benzotriazole, (10g, 0.1 mole) in absolute ethanol (20 mL), ethyl bromoacetate (10 mL, 0.1 mole) and anhydrous potassium carbonate (5 g) were added and the reaction mixture was refluxed for 20-22 h. It was filtered off and the excess of ethanol was distilled under vacuum to yield a light yellow color thick liquid (1)20,21.

2.2 Synthesis of 2-(1H-benzo[d] [1,2,3] triazole-1-yl) acethyldrazide (2)
To a solution of 1 (9 g, 0.1 mole) in ethanol (10 mL), hydrazine hydrate (2.2 mL, 0.15 mole) was added and the reaction mixture was refluxed for 9-10 h. The excess of ethanol was distilled under vacuum and diluted with ice cold water. The white precipitate thus obtained was filtered, washed with ice cold water, dried and recrystallized from ethanol to yield 220,21,22.

2.3 Synthesis of 5-((1H-benzo[d][1,2,3]triazol-1-yl)methyl)-1,3,4- oxadiazole-2-amine (3)
A solution of 2 (5 g, 0.1 mole), cyanogen bromide (3.05 g, 0.1 mole,) and sodium bicarbonate (2.41g, 0.1 mole) in 15 mL dioxane was stirred for 7-8h at room temperature. Excess of solvent was distilled and residue thus obtained was poured into ice cold water. The brown precipitate was filtered, washed with ice cold water and dried.23

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2.4 Synthesis of Schiff’s bases (4a-4i)
In a mixture of 3 (4 g, 0.1 mole) in 20 mL ethanol, was added an appropriate aldehyde (0.1 mole). The mixture was refluxed for about 12 h in presence of 3 mL of glacial acetic acid. The solvent was distilled off and residue was poured into ice cold water. The precipitate thus obtained was filtered, washed with ice cold water and dried. All these compounds were subjected to purification through column chromatography using Ethyl acetate: Hexane (1:4).

2.5 Anti-cancer Activity
All the compounds were screened for in vitro anti-cancer activity against human Leukemia Cell line (K 562), human breast cancer cell line (MCF 7) and human colon cancer cell line (HCT 15). The cell lines were grown on 96 well micro-titer plates containing RPMI 1640 inoculated, incubated at 37°C, 5% CO₂, 95% air and 100% RH for 24 h prior to addition of experimental drugs. Aliquot of 10 µL of these different dilutions were added to micro-titer plate containing 90 µL of medium, resulting in required final concentration. Doxorubicin was used as a standard. Plates were incubated for 48 h and assay was terminated by addition of cold trichloro acetic acid (TCA). Supernatant was discarded, plates were washed with water. Sulforhodamine B in acetic acid was added to each well and incubated for 20 min at R.T. Unbound dye was removed by washing with 1 % acetic acid. Bound stain was eluted with 10 mM tris base and absorbance was read on Elisa plate reader at 540 nm. SRB protein assay was used to estimate cell viability or growth. This allows detection of both growth inhibition and lethality (Table 1). A value of 100 meant no growth inhibition, a value of 40 meant 60% growth inhibition, a value of 0 meant no net growth over the course of the experiment, value of -40 meant 40% lethality. Dose response parameter was calculated for each test compound. Growth inhibition of 50 % (GI₅₀) was calculated from \( \frac{([T_i-T_z]/(C-T_z)) \times 100 = 50^\circ} \), which is the drug concentration resulting in a 50% reduction in the net protein increase (as measured by SRB staining) in control cells during the drug incubation. Tz is the measurement of the cell population for each cell line at the time of drug addition and Ti is the measurement of the cell population for each cell line after interaction with drug for four different concentrations. Compound 4h showed GI₅₀ value of 2.7 × 10⁻⁵, which was closer to the doxorubicin a test standard (GI₅₀ value of 1.3 × 10⁻⁵). All other compounds showed GI₅₀ values more than 10⁻⁴.
Table 1: Anti-cancer activity of compound 3, 4a-i

<table>
<thead>
<tr>
<th>Cell lines</th>
<th>Compd./ Conc.</th>
<th>Growth percentage of treated cells (%)</th>
<th>Doxorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Leukemia Cell line K562</td>
<td>Compd. 4d</td>
<td>100.0</td>
<td>98.9</td>
</tr>
<tr>
<td>Human breast cancer cell line MCF 7</td>
<td>Compd. 4b</td>
<td>100.0</td>
<td>98.9</td>
</tr>
<tr>
<td>Human colon cancer cell line HCT 15</td>
<td>Compd. 4d</td>
<td>100.0</td>
<td>98.9</td>
</tr>
</tbody>
</table>

3. RESULTS AND DISCUSSION

All the Schiff's bases were obtained in quantitative yield and they were characterized by spectroscopic technique like NMR and IR (Table 2) after purification through column chromatography. These compounds were screened against cancer cell lines like K-562, MCF-7 and HCT-15 (Table 1). Compound 4h was found to be the active compound as it showed GI50 value of 2.7 × 10^-5 M. This value was found to be lower than the GI50 value of standard drug doxorubicin (1.3 × 10^-5 M).

Table 2: Physicochemical and spectral data of titled compounds 3, 4a-i

<table>
<thead>
<tr>
<th>Compd.</th>
<th>Mol. form.</th>
<th>m.p. (°C)</th>
<th>IR (cm^-1)</th>
<th>¹H NMR (δ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>C₂H₄N₂O₂</td>
<td>192-194</td>
<td>3300 (NH str); 3080 (Ar C-H str); 1500 (NH bend)</td>
<td>8.15-7.40 (m, 6H, Ar), 7.18 (s, 2H, Nh)</td>
</tr>
<tr>
<td>4a</td>
<td>C₂H₄N₂OC₆</td>
<td>232-236</td>
<td>3064, 2937 (Ar C-H str); 1676 (N=CH); 1558 (C=N); 1226</td>
<td>8.37 (s, 1H, N=CH), 8.00-7.20 (m, 8H, Ar)</td>
</tr>
<tr>
<td>4b</td>
<td>C₂H₄N₂OCl</td>
<td>241-245</td>
<td>3182, 2937 (Ar C-H str); 1676 (N=CH); 1276,1010</td>
<td>8.37 (s, 1H, N=CH), 8.00-7.20 (m, 8H, Ar)</td>
</tr>
<tr>
<td>4c</td>
<td>C₂H₄N₂O₂Cl₂</td>
<td>224-226</td>
<td>3463 (OH str); 3085, 2833 (Ar C-H str); 1693</td>
<td>10.06 (s, 1H, OH), 8.60 (s, 1H, N=CH)</td>
</tr>
<tr>
<td>4d</td>
<td>C₂H₄N₂O₂Cl₃</td>
<td>229-232</td>
<td>3461 (OH str); 3010, 2995 (Ar C-H str); 1681</td>
<td>8.23-7.42 (m, 8H, Ar), 6.00 (s, 2H, Nh)</td>
</tr>
<tr>
<td>4e</td>
<td>C₂H₄N₂O₂</td>
<td>204-208</td>
<td>3058, 2985 (Ar C-H str); 1666 (N=CH); 1211,1022</td>
<td>8.71 (s, 1H, N=CH), 7.80-7.00 (m, 8H, Ar)</td>
</tr>
<tr>
<td>4f</td>
<td>C₂H₄N₂O₂</td>
<td>198-200</td>
<td>3170, 3070 (Ar C-H str); 1641 (N=CH); 1190,1029</td>
<td>8.70 (s, 1H, N=CH), 7.85-6.95 (m, 8H, Ar)</td>
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<tr>
<td>4g</td>
<td>C₂H₄N₂O₂</td>
<td>222-225</td>
<td>2972 (Ar C-H str); 1639 (N=CH); 1598 (NO₂ str)</td>
<td>7.85 (s, 1H, N=CH), 8.15-8.85 (m, 8H, Ar)</td>
</tr>
<tr>
<td>4h</td>
<td>C₂H₄N₂O₂</td>
<td>218-220</td>
<td>3191, 3072 (Ar C-H str); 1664 (N=CH); 1139,1080</td>
<td>6.68 (s, 1H, N=CH), 8.52-7.4 (m, 13H, Ar)</td>
</tr>
<tr>
<td>4i</td>
<td>C₂H₄N₂O₂</td>
<td>212-215</td>
<td>3492 (OH str); 3147 (Ar C-H str); 1691 (N=CH); 1375</td>
<td>10.00 (s, 1H, OH), 8.46 (s, 1H, N=CH)</td>
</tr>
</tbody>
</table>

4. CONCLUSION

All the Schiff's bases were synthesized in good yields and they were characterized by different technique like NMR and IR as given in Table 2. These compounds were screened against cancer cell lines like K-562, MCF-7 and HCT-15. Hence it can be concluded that, the anticancer activity exhibited by Compound 4h was found to be the active compound as it showed GI50 value of 2.7 × 10^-5 M. This type of synthetic study needs to be thoroughly investigated and analyzed for getting new leads molecules and further it can be investigated by computer aided drug designing.

5. ACKNOWLEDGMENTS

The authors are thankful to Mr. N. C. Das Research Associate, Poona College of Pharmacy, Pune for his suggestion and ACTREC center, Kharghar for helping in anti-cancer studies.

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