SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL SCREENING OF SOME NOVEL INDOLE BASED 1,2,4-TRIAZOLO 1,3,4-THIADIAZINES

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ABSTRACTS:
A series of novel 3-(1H-indole-4-yl)-6-aryl-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazines (6a-f) were synthesized by involving 1H-indole-4-carboxylic acid (1) as raw material and 1H-indole-4-carboxylic acid ethyl ester (2), 1H-indole-4-carboxylic acid hydrazide (3), 5-(1H-indole-4-yl)-[1,3,4]oxadiazole-2-thiol (4) and 4-amino-5-(1H-indol-4-yl)-4H-[1,2,4]-triazole-3-thiol (5) as intermediates. The chemical structures of the all newly synthesized compounds were elucidated by their IR, 1H and 13C NMR, mass spectral data and elemental analysis. Further, the target compounds were used to find their antifungal and nematocidal activity.

Key-Words: Indole, 1,2,4-Triazole, 1,3,4-Thiadiazines, Antifungal activity, Nematicidal activity.

INTRODUCTION:
Recently, it was reported that the heterocyclic moiety such as triazole based thiadiazoles and thiadiazines possess variety of pharmacological activities like antimicrobial 1, antiviral 2, antibacterial, 3 antinflammatory, 4 herbical 5 and anti-HIV-1, 6. On the other hand, it has been reported that certain compounds bearing a triazole and 1,2,4-triazole nucleus possess significant anti-inflammatory activity. 7

These initial reports stimulated us to integrate thiadiazine moiety in triazole frame work, since these systems possess well documented antimicrobial and nematicidal activity. The target compounds, 3-(1H-indole-4-yl)-6-aryl-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazines (6a-f) have been prepared by using commercially available 1H-indole-4-carboxylic acid (1) as raw material and by involving 1H-indole-4-carboxylic acid ethyl ester (2), 1H-indole-4-carboxylic acid hydrazide (3), 5-(1H-indole-4-yl)-[1,3,4]oxadiazole-2-thiol (4) and 4-amino-5-(1H-indol-4-yl)-4H-[1,2,4]-triazole-3-thiol (5) as intermediates.

The initial intermediate, 1H-indole-4-carboxylic acid ethyl ester (2) has been prepared through esterification by boiling of a mixture of 1H-indole-4-carboxylic acid (1) and sulfuric acid in ethanol for 4 h. The compound 2 was reacted with hydrazine hydrate in absolute ethyl alcohol at reflux for 8 h to get 1H-indole-4-carboxylic acid hydrazide (3). The intermediate, 5-(1H-indole-4-yl)-[1,3,4]oxadiazole-2-thiol (4) for the synthesis of title compounds was prepared by the cyclization of compound 3 with carbon disulphide in the presence of potassium hydroxide in ethanol at reflux for 14 h followed by acidification. Further the compound 4 when reacted with hydrazine hydrate in ethanol at reflux for 6 h resulted 4-amino-5-(1H-indol-4-yl)-4H-[1,2,4]triazole-3-thiol (5). Finally, the compound 5 has been condensed successively with a variety of phenacylbromides in ethyl alcohol under reflux for 8-10 h to get the title compounds, 3-(1H-indole-4-yl)-6-aryl-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiadiazines (6a-f). The chemical structures of the all newly synthesized compounds were elucidated by their IR, 1H and 13C NMR, mass spectral data and elemental analysis. Further, the target compounds were used to find their antifungal and nematocidal activity.

Scheme 1: 6 Ar = (a) C6H5; (b) 4-OCH3C6H4; (c) 4-ClC6H4; (d) 4-BrC6H4; (e) 4-NO2C6H4; (f) 4-OHC6H4

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ISSN: 2250-1177
CODEN (USA): JDDTAO
ANTIFungal ACTIVITY

Compounds 6a-f were screened for their antifungal activity against four fungal organisms viz., Candida albicans, Aspergillus fumigatus, Trichophyton rubrum, and Trichophyton mentagrophytes in dimethyl sulfoxide by broth dilution method. The minimum inhibitory concentration (MIC, µg/mL) were measured and compared with the standard drug Amphotericin B (Table 1). Among the screened compounds, 6c is highly active against T. rubrum. T. mentagrophytes, 6e is also active against only C. albicans and 6f is highly active against C. albicans, T. mentagrophytes and the activity of these compounds is almost equal to the standard. All the compounds in this series exhibited either excellent or moderate activity towards different organisms. None of the compounds showed excellent antifungal activity towards D. myceliophagus.

NEMATICIDAL ACTIVITY

All the newly synthesized compounds 6a-f in this study were also assayed for their nematicidal activity against Ditylenchus myceliophagus and Caenorhabditis elegans by aqueous in vitro screening technique at various concentrations. The results have been expressed in terms of LD₅₀ i.e. median lethal dose at which 50% nematodes became immobile (dead), and compared with the standard drug levamisole. The screened data reveal that, 6a is the most effective against D. myceliophagus and C. elegans with LD₅₀ of 170 and 190 ppm, respectively. The compounds 6d and 6f are also most active against C. elegans with LD₅₀ of 200 ppm and D. myceliophagus with LD₅₀ of 190 ppm, respectively. The activity of 6a is almost equal to the activity of the standard Levamisole. The other tested compounds showed moderate activity. The LD₅₀ values of the compounds screened are presented in Table 2.

EXPERIMENTAL

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Table 1: In vitro antifungal activity of compounds 6a-f (MIC in µg/mL)

<table>
<thead>
<tr>
<th>Compound</th>
<th>C. albicans</th>
<th>A. Fumigatus</th>
<th>T. Rubrum</th>
<th>T. Mentagrophytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>6a</td>
<td>25.0</td>
<td>12.5</td>
<td>&gt;50.0</td>
<td>25.0</td>
</tr>
<tr>
<td>6b</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
<td>25.0</td>
</tr>
<tr>
<td>6c</td>
<td>12.5</td>
<td>6.25</td>
<td>3.12</td>
<td>3.12</td>
</tr>
<tr>
<td>6d</td>
<td>25.0</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
</tr>
<tr>
<td>6e</td>
<td>3.12</td>
<td>12.5</td>
<td>12.5</td>
<td>25.0</td>
</tr>
<tr>
<td>6f</td>
<td>50.0</td>
<td>25.0</td>
<td>12.5</td>
<td>12.5</td>
</tr>
<tr>
<td>Amphotericin B</td>
<td>6.25</td>
<td>3.12</td>
<td>3.12</td>
<td>3.12</td>
</tr>
</tbody>
</table>

Table 2: Median lethal dose (LD₅₀, ppm) of compounds 6a-f

<table>
<thead>
<tr>
<th>Nematicide</th>
<th>6a</th>
<th>6b</th>
<th>6c</th>
<th>6d</th>
<th>6e</th>
<th>6f</th>
<th>Levamisole</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. myceliophagus</td>
<td>170</td>
<td>270</td>
<td>950</td>
<td>430</td>
<td>570</td>
<td>190</td>
<td>170</td>
</tr>
<tr>
<td>C. elegans</td>
<td>190</td>
<td>220</td>
<td>870</td>
<td>200</td>
<td>610</td>
<td>780</td>
<td>180</td>
</tr>
</tbody>
</table>

1H-Indole-4-carboxylic acid ethyl ester (2) To the solution of 1H-indole-4-carboxylic acid (1) (0.01 mol) in absolute ethyl alcohol (15 ml), conc. H₂SO₄ (2 ml) was added. The mixture was refluxed for 4 h. After completion of the reaction (monitored by TLC), the solvent was removed under reduced pressure and the obtained residue was recrystallized with petroleum ether to get pure 1H-indole-4-carboxylic acid ethyl ester (2).

1H-Indole-4-carboxylic acid hydrazide (3) A mixture of 1H-indole-4-carboxylic acid ethyl ester (2) (0.01 mol) and hydrazine hydrate (0.025 mol) in ethanol (20 ml) was refluxed for 8 h. After completion of the reaction (monitored by TLC), the mixture was cooled to room temperature and filtered. The crude product was recrystallized from ethanol to give 1H-indole-4-carboxylic acid hydrazide (3) in pure form.

5-(1H-Indole-4-yl)1,3,4oxadiazole-2-thiol (4) A mixture of 1H-indole-4-carboxylic acid hydrazide (3) (0.01 mol), potassium hydroxide (0.02 mol) and carbon disulfide (0.03 mol) in ethanol (100 mL) was heated under reflux with stirring for 14 h. The solvent was distilled in vacuo, the residual mass was poured over crushed ice and neutralized the alkaline solution with 10% hydrochloric acid. The precipitated crude product was filtered, washed with water, dried and recrystallized from ethanol to get the pure compound 5-(1H-indole-4-yl)1,3,4oxadiazole-2-thiol (4).
4-Amino-5-(1H-indol-4-yl)-4H-[1,2,4]triazole-3-thiol (5) To a warm solution of 5-(1H-indole-4-yl)-[1,3,4]-oxadiazole-2-thiol (4) (0.01 mol) in ethanol (20 mL), 80% hydrazine hydrate (0.03 mol) was added drop wise and the reaction mixture was heated under reflux for 7 h. The reaction mixture was cooled in vacuo, cooled, and the solid separated were filtered, washed with cold ethanol and recrystallized from chloroform to give pure compound 4-amino-5-(1H-indol-4-yl)-4H-[1,2,4]-triazole-3-thiol (5).

3-(1H-indole-4-yl)-6-aryl-7H-[1,2,4]triazolo-[3,4-b][1,3,4]-thiazidiones (6a-f) A mixture of 4-amino-5-(1H-indol-4-yl)-4H-[1,2,4]-triazole-3-thiol (5) (0.01 mol) and corresponding phenacyl bromide (0.02 mol) in absolute ethanol (20 mL) was refluxed for 8–10 h. The reaction mixture was concentrated and cooled to room temperature, and the remaining solvent was removed under reduced pressure, then diethyl ether (25 mL) was added and the reaction mixture was left at 0 °C for overnight. The precipitated solid was filtered off. The crude product thus obtained was purified by column chromatography on silica gel with hexane-ethyl acetate as eluent to afford pure 3-(1H-indole-4-yl)-6-aryl-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiazidiones (6a-f).

PHYSICAL AND SPECTRAL DATA

1H-Indole-4-carboxylic acid ethyl ester (2) Yellow solid; yield 78%; bp 342-343 °C; IR (KBr) cm⁻¹: 3212 (N-H), 3024 (Ar-H), 2962 (C-H, CH₂), 1699(C=O), 1588 (C=C); ¹H-NMR (CDCl₃): δ = 1.24 (3H, t, J = 5.6 Hz, CH₃), 4.00 (2H, q, J = 5.6 Hz, CH₂), 7.42 (1H, d, J = 7.4 Hz, Ar-H), 7.39-7.62 (3H, m, Ar-H), 7.85 (1H, d, J = 7.4 Hz, Ar-H), 11.12 (1H, s, NH); ¹³C-NMR (CDCl₃): δ = 13.6, 9.21, 106.3, 112.5, 119.4, 121.7, 122.8, 126.3, 130.4, 132.5, 165.4; MS m/z: 189 (M⁺); Elemental analysis calculated for C₁₁H₁₂NO: C 76.59, H 5.42, N 7.06, O 15.98.

1H-Indole-4-carboxylic acid hydrazide (3) Brown solid; yield 81%; mp 187-189 °C; IR (KBr) cm⁻¹: 3318 (NH₂), 3218 (N-H), 3065 (Ar-H), 1645 (C=O), 1548 (C=C); ¹H-NMR (CDCl₃): δ = 5.30 (2H, s, NH₂), 7.38 (1H, d, J = 7.2 Hz, Ar-H), 7.42-7.64 (3H, m, Ar-H), 7.70 (1H, s, NH), 7.79 (1H, d, J = 7.2 Hz, Ar-H), 11.06 (1H, s, NH); ¹³C-NMR (CDCl₃): δ = 105.7, 114.2, 121.0, 123.7, 124.4, 127.3, 130.5, 162.3; MS m/z: 175 (M⁺); Elemental analysis calculated for C₁₀H₁₀N₂O: C 61.70, H 5.18, N 23.99, O 9.13. Found: C 60.12, H 4.89, N 22.17, O 8.89.

5-(1H-Indole-4-yl)-1,3,4oxadiazole-2-thiol (4) Pale yellow solid; yield 74%; mp 185-187 °C; IR (KBr) cm⁻¹: 3236 (N-H), 3028 (Ar-H), 2610 (S-H), 1648 (C=O), 1559 (C=C), 1155; ¹H-NMR (CDCl₃): δ = 3.81 (1H, s, SH), 7.35 (1H, d, J = 7.6 Hz, Ar-H), 7.41-7.58 (3H, m, Ar-H), 7.79 (1H, d, J = 7.6 Hz, Ar-H), 11.24 (1H, s, NH); ¹³C-NMR (CDCl₃): δ = 102.4, 116.3, 120.7, 123.4, 125.8, 128.4, 136.1, 139.8, 145.6, 158.9; MS m/z: 217 (M⁺); Elemental analysis calculated for C₁₀H₁₀N₂O: C 55.29, H 3.25, N 19.34, O 7.69, S 14.76. Found: C 53.69, H 3.12, N 18.45, O 7.02, S 13.38.

4-Amino-5-(1H-indol-4-yl)-4H-[1,2,4]triazole-3-thiol (5) White solid; yield 72%; mp 147-149 °C; IR (KBr) cm⁻¹: 3248 (NH), 3018 (Ar-H), 2648 (S-H), 1662 (C=N), 1552 (C=C); ¹H-NMR (CDCl₃): δ = 3.65 (1H, s, SH), 3.85 (2H, s, NH₂), 7.37 (1H, d, J = 7.3 Hz, Ar-H), 7.45-7.74 (3H, m, Ar-H), 7.80 (1H, d, J = 7.3 Hz, Ar-H), 11.21 (1H, s, NH); ¹³C-NMR (CDCl₃): δ = 105.6, 110.8, 116.7, 119.4, 127.6, 129.4, 132.4, 137.6, 142.3, 152.7; MS m/z: 231 (M⁺); Elemental analysis calculated for C₁₀H₁₃NO₂S: C 51.93, H 3.92, N 30.28, S 13.86. Found: C 50.12, H 3.45, N 29.65, S 12.98.

3-(1H-Indole-4-yl)-6-phenyl-7H-[1,2,4]triazolo-[3,4-b][1,3,4]-thiazidiones (6a-f) A mixture of 4-amino-5-(1H-indol-4-yl)-4H-[1,2,4]-triazole-3-thiol (5) (0.01 mol) and corresponding phenacyl bromide (0.02 mol) in absolute ethanol (20 mL) was refluxed for 8–10 h. The reaction mixture was concentrated and cooled to room temperature, and the remaining solvent was removed under reduced pressure, then diethyl ether (25 mL) was added and the reaction mixture was left at 0 °C for overnight. The precipitated solid was filtered off. The crude product thus obtained was purified by column chromatography on silica gel with hexane-ethyl acetate as eluent to afford pure 3-(1H-indole-4-yl)-6-phenyl-7H-[1,2,4]-triazolo-[3,4-b][1,3,4]-thiazidiones (6a-f).

**Journal of Drug Delivery & Therapeutics** 2014, 4(2), 43-46

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ISSN: 2250-1177  CODEN (USA): JDDTAO
3-(1H-Indole-4-yl)-6-(4-nitro-phenyl)-7H-[1,2,4]-triazolo[3,4-b][1,3,4]-thiadiazine (6e) White solid; yield 79%; mp 154-156 °C; IR (KBr) cm⁻¹: 3262 (N-H), 3027 (C=C); 1H-NMR (CDCl₃) δ: 1.58 (2H, s, CH₂), 7.26 (1H, d, J = 6.8 Hz, Ar-H), 7.31 (2H, d, J = 6.8 Hz, Ar-H), 7.38 (2H, d, J = 7.2 Hz, Ar-H), 7.45-7.72 (3H, m, Ar-H), 7.78 (1H, d, J = 7.2 Hz, Ar-H), 11.08 (1H, s, NH); 13C-NMR (CDCl₃) δ: 33.7, 108.9, 114.7, 117.4, 119.6, 122.3, 124.7 (2), 126.8, 133.7, 135.8 (2), 139.7, 142.4, 148.7, 149.6, 153.7, 164.9; MS m/z: 376 (M⁺); Elemental analysis calculated for C₁₉H₂₃N₅O₂S: C: 57.44, H: 3.21, N: 22.33, O: 8.50, S: 8.52. Found: C: 55.98, H: 3.12, N: 21.28, O: 7.87, S: 7.95.

REFERENCES: