Synthesis and Anti-Inflammatory Activity of a Novel Series of Diphenyl-1,2,4-triazoles and Related Derivatives

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Abstract: In the present investigation we have synthesized a series of new 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]urea and 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]thiourea derivatives (4Ia - 4IId). The newly synthesised derivatives were characterized by using the data of IR, ¹H NMR and Mass Spectral analysis. Thus synthesised and characterized targetted compounds were further screened for their anti-inflammatory activity by using Carrageenan – induced paw edema rat model. Among all the newly synthesized derivatives, Compounds 4Ia-4Ic and Compounds 4IIa-4IId were reduced the inflammation very significantly (p<0.0001), thus these compounds showed promising anti-inflammatory activity and only one compound (4Id) showed moderate anti-inflammatory activity (p<0.05).

Keywords: 1,2,4-triazoles, IR, ¹H NMR, Mass Spectroscopy and anti-inflammatory activity.

Introduction

Among all the widely used therapeutic agents Nonsteroidal anti-inflammatory drugs (NSAIDs) primarily important for the treatment of inflammation and pain in arthritis. 1,2,4-triazoles have long been known for their antiviral activity against the influenza ¹ and HIV viruses ²-³. Triazoles were also associated with central nervous⁴-⁶, antimicrobial⁷-⁹ activities. Various derivatives of 1,2,4-triazole have been reported to possess interesting biological activities such as hypoglycemic¹⁰ anti-inflammatory¹¹-¹³, antibacterial¹⁴, antifungal¹⁵, anticancer¹⁶ analgesic¹⁷ and antidepressant activities¹⁸.

In recent years, there has been a growing interest in the pharmacology of s-triazole and o-triazole derivatives, we have synthesized and reported 1-[3-(4-substitutedphenyl)-5-
phenyl-4H-1,2,4-triazol-4-yl]urea and 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]thiourea derivatives as the targeted compounds as per the scheme-1 and evaluated for their anti-inflammatory activity.

![Scheme 1](image)

**Experimental**

Melting points of all synthesized compounds were determined by open capillary tubes and are uncorrected. The IR spectra (KBr pellets) were recorded on Spectrum BX series model spectrometer for Compounds 4Ib and 4IIa. ¹H NMR spectra were recorded for compound 4Ib and 4IIa on AV 300 MHz NMR Spectrometer, using TMS as internal standard. The mass spectra were recorded on –LCQ ion Mass spectrometer. The purity of the compounds were checked by Thin Layer Chromatography (TLC) on Merck Silica gel 60 F254 pre coated sheet using Chloroform and Ethyl acetate in 1:1 v/v.

*General procedure for synthesis of Benzoic acid hydrazide*¹⁹ (1)

Methyl benzoate (0.01 mol) was dissolved in ethanol and added hydrazine hydrate (99%) (0.03 mol) and the reaction mixture was refluxed for 2 h, cooled in icebath. The obtained product was filtered, dried and recrystallised by using ethyl alcohol.
**General procedure for the synthesis of arylidenehydrazide**\(^{20}\) (2)

Benzoic acid hydrazide (0.01 mol) and appropriate aromatic aldehydes (0.015 mol) in alcohol (20 ml) with 2 to 3 drops of acetic acid, heated under reflux on a water bath for one hour. The solvent was removed to possible extent by distillation under reduced pressure. The product thus obtained was filtered, washed with water dried and purified by recrystallization from ethyl alcohol.

**General procedure for the synthesis of** \(2-(4\text{-substitutedphenyl})-5\text{-phenyl}-1,3,4\text{-oxadiazole}\)^{20} (3)

A mixture of compound (2) (0.01 mol) and sodium acetate (0.02 mol) were dissolved in 30 ml of glacial acetic acid and added with bromine (0.7 ml in 5 ml of glacial acetic acid) slowly while stirring until the colour of bromine exists. Then the reaction mixture was poured into crushed ice and collected the precipitate and washed with water, then dried. The resulting compound was purified by recrystallization from appropriate portion of DMF and water.

**General procedure for the synthesis of** \(1-[3\text{-}(4\text{-substitutedphenyl})-5\text{-phenyl}-4H-1,2,4\text{-triazol}-4\text{-yl}]\text{urea} \) (4I)

Compound (3) (0.001 mol) was dissolved in glacial acetic acid and added with semicarbazide hydrochloride (0.0015 mol) and pinch of sodium acetate. The contents of the flask were refluxed for 8 h, poured into crushed ice. The product obtained was filtered, washed with cold alcohol, dried and recrystallised from methyl alcohol.

**General procedure for the synthesis of** \(1-[3\text{-}(4\text{-substitutedphenyl})-5\text{-phenyl}-4H-1,2,4\text{-triazol}-4\text{-yl}]\text{thiourea} \) (4II)

Compound (3) (0.001 mol) was dissolved in glacial acetic acid and added with thiosemicarbazide (0.0015 mol) and pinch of sodium acetate. The contents of the flask were refluxed for 8 h, poured into crushed ice. The product obtained was filtered, washed with cold alcohol, dried and recrystallised from methyl alcohol.

Adopting this procedure totally we have synthesized eight di-phenyl-1,2,4-triazole derivatives. The yields, melting points and physical data of newly synthesized compounds were summarized in Table-1.

**Table 1.** Physical data of 1-(3,5-disubstituted-4H-1,2,4-triazol-4-yl)urea and 1-(3,5-disubstituted-4H-1,2,4-triazol-4-yl)thiourea derivatives (4Ia-4Id).

<table>
<thead>
<tr>
<th>S.No</th>
<th>compd</th>
<th>X</th>
<th>Ar</th>
<th>Mol.Form</th>
<th>Mol.Wt</th>
<th>M.P(°c)</th>
<th>%yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4Ia</td>
<td>O</td>
<td>C(_6)H(_5)</td>
<td>C(<em>{15})H(</em>{13})N(_2)O</td>
<td>279.29</td>
<td>230-235</td>
<td>75</td>
</tr>
<tr>
<td>2</td>
<td>4Ib</td>
<td>O</td>
<td>C(_6)H(_4)(p-Cl)</td>
<td>C(<em>{15})H(</em>{13})ClN(_2)O</td>
<td>313.74</td>
<td>190-210</td>
<td>80</td>
</tr>
<tr>
<td>3</td>
<td>4Ic</td>
<td>O</td>
<td>C(_6)H(_4)(p-OCH(_3))</td>
<td>C(<em>{16})H(</em>{15})N(_2)O(_2)</td>
<td>309.32</td>
<td>225-250</td>
<td>62</td>
</tr>
<tr>
<td>4</td>
<td>4Id</td>
<td>O</td>
<td>Furfuryl</td>
<td>C(<em>{13})H(</em>{11})N(_2)O(_2)</td>
<td>269.25</td>
<td>220-225</td>
<td>69</td>
</tr>
<tr>
<td>5</td>
<td>4IIa</td>
<td>S</td>
<td>C(_6)H(_5)</td>
<td>C(<em>{15})H(</em>{13})N(_2)S</td>
<td>295.36</td>
<td>180-185</td>
<td>81</td>
</tr>
<tr>
<td>6</td>
<td>4IIb</td>
<td>S</td>
<td>C(_6)H(_4)(p-Cl)</td>
<td>C(<em>{15})H(</em>{12})ClN(_2)S</td>
<td>329.80</td>
<td>250-255</td>
<td>78</td>
</tr>
<tr>
<td>7</td>
<td>4IIc</td>
<td>S</td>
<td>C(_6)H(_4)(p-OCH(_3))</td>
<td>C(<em>{16})H(</em>{15})N(_2)O(_2)</td>
<td>325.38</td>
<td>220-230</td>
<td>71</td>
</tr>
<tr>
<td>8</td>
<td>4IId</td>
<td>S</td>
<td>Furfuryl</td>
<td>C(<em>{13})H(</em>{11})N(_2)O(_2)</td>
<td>285.32</td>
<td>215-220</td>
<td>68</td>
</tr>
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</table>
Synthesis and Anti-Inflammatory Activity

1-(3-(4-chlorophenyl)-5-phenyl-4H-1,2,4-triazol-4-yl)urea (4Ib)

IR (cm⁻¹): 3402 (NH), 3258 (NH₂), 1605 (C=O), 1551 (C=C), 1478 (C-N), 1089 (C-Cl).

¹H NMR (δ, ppm): 8.2 (d, 2H, ArH), 7.8 (d, 2H, ArH), 7.7 (d, 2H, ArH), 7.6 (t, 2H, ArH), 7.4 (d, 1H, ArH), 6.4 (s, 2H, NH₂), 5.9 (s, 1H, NH).

M⁺ (theoretical): 313.07; M₂⁺ (experimental): 315.0.

1-(3,5-diphenyl-4H-1,2,4-triazol-4-yl)thiourea (4IIa)

IR (cm⁻¹): 3378 (NH), 3233 (NH₂), 1637 (C=O), 1511 (C-N), 1108 (C=S).

¹H NMR (δ, ppm): 9.6 (s, 2H, NH₂), 8.3 (d, 4H, ArH), 7.9 (t, 2H, ArH), 7.6 (t, 4H, ArH), 4.2 (s, 1H, NH).

M⁺ (theoretical): 295.09; M₂⁺ (experimental): 296.0.

Anti-inflammatory activity

Carrageenan-induced rat paw edema method was employed for evaluating the anti-inflammatory activity of the synthesized compounds (4Ia-4IId). Wistar Albino rats of either sex weighing approx 200-300 gm, were housed in clean polypropylene cages and kept under room temperature (25±2°C), and relative humidity 40-50% in a 12 h light-dark cycle. Food was withdrawn 12 h before and during experimental hours. In this study, the animals were divided into groups as shown in the Table 2. Acute inflammation was produced by subplantar injection of 0.1ml of 1% suspension of Carrageenan with 2% gum acacia in normal saline, in the right hind paw of the rats. After oral administration of the test compounds, the paw volume was measured Plethysmometrically at 1, 2, 3, and 4 h intervals. Diclofenac sodium 10mg/ml of 2% gum acacia in normal saline was used as standard drug.

Table 2. Anti-inflammatory activity of newly synthesized Compounds (4Ia-4IId).

<table>
<thead>
<tr>
<th>Time</th>
<th>1.0 h</th>
<th>% red</th>
<th>2.0 h</th>
<th>% red</th>
<th>3.0 h</th>
<th>% red</th>
<th>4.0 h</th>
<th>% red</th>
</tr>
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<tr>
<td>Crgn</td>
<td>2.82</td>
<td>NA</td>
<td>2.97</td>
<td>NA</td>
<td>3.24</td>
<td>NA</td>
<td>3.24</td>
<td>NA</td>
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<tr>
<td>DFS</td>
<td>1.04</td>
<td>62.88</td>
<td>1.02</td>
<td>65.65</td>
<td>0.86</td>
<td>73.25</td>
<td>0.53</td>
<td>83.53***</td>
</tr>
<tr>
<td>4Ia</td>
<td>1.43</td>
<td>42.89</td>
<td>1.13</td>
<td>60.23</td>
<td>0.87</td>
<td>74.12</td>
<td>0.50</td>
<td>85.02***</td>
</tr>
<tr>
<td>4Ib</td>
<td>1.4</td>
<td>50.35</td>
<td>0.96</td>
<td>67.45</td>
<td>0.76</td>
<td>76.33</td>
<td>0.43</td>
<td>86.62***</td>
</tr>
<tr>
<td>4Ic</td>
<td>1.77</td>
<td>29.61</td>
<td>1.47</td>
<td>48.53</td>
<td>1.03</td>
<td>69.19</td>
<td>0.63</td>
<td>81.03***</td>
</tr>
<tr>
<td>4Id</td>
<td>1.63</td>
<td>42.08</td>
<td>1.56</td>
<td>47.25</td>
<td>1.66</td>
<td>48.55</td>
<td>1.46</td>
<td>54.73*</td>
</tr>
<tr>
<td>4IIa</td>
<td>1.76</td>
<td>37.35</td>
<td>1.46</td>
<td>50.61</td>
<td>1.03</td>
<td>68.10</td>
<td>0.63</td>
<td>80.45***</td>
</tr>
<tr>
<td>4IIb</td>
<td>1.53</td>
<td>45.62</td>
<td>1.26</td>
<td>57.35</td>
<td>0.83</td>
<td>74.27</td>
<td>0.36</td>
<td>88.68***</td>
</tr>
<tr>
<td>4IIc</td>
<td>1.53</td>
<td>45.62</td>
<td>1.26</td>
<td>57.35</td>
<td>0.83</td>
<td>74.27</td>
<td>0.5</td>
<td>83.53***</td>
</tr>
<tr>
<td>4IId</td>
<td>1.9</td>
<td>32.62</td>
<td>1.23</td>
<td>58.47</td>
<td>0.83</td>
<td>74.27</td>
<td>0.53</td>
<td>83.5***</td>
</tr>
</tbody>
</table>

***statistically significant (p<0.0001) difference in comparison to control; *Statistically significant (p<0.05) difference in comparison to control; Values are in Mean ±Standard Deviation; NA = Not Applicable; DFC = Diclofenac Sodium; Crgn = Carrageenan; Each Group contains six animals.
Results and Discussion

The preliminary studies on anti-inflammatory activity of the new 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]urea and 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl] thiourea derivatives (4Ia-4Id) have generated some interesting data.

Acute toxicity studies

All the new 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]urea and 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl] thiourea derivatives (4Ia-4Id) employed in the investigation have been found to be free from toxicity as well as toxic symptoms up to a dose of 1000mg/kg (b.w).

Anti-inflammatory activity

The anti-inflammatory activity of all 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]urea and 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl] thiourea derivatives (4Ia - 4Id) of test compounds has been evaluated and data presented in Tables using Diclofenac sodium (10mg/kg) b.w., as the standard for in vivo method by using Carrageenan – induced paw edema rat model.

The data revealed that all the eight compounds 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]urea and 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl] thiourea derivatives (4Ia - 4Id) showed a significant anti-inflammatory activity in vivo. The data is presented in table 2. The maximum activity was observed at 3rd and 4th hour. The highest anti-inflammatory activity was observed with the compound 4Id (X= S; Ar= p-chlorophenyl) with 88.68 percentage of inhibition at 4th hour.

Compound 4Ib (X= O; Ar= p-chlorophenyl), 4Ia (X= O; Ar= phenyl), 4Ic (X= S; Ar= Anisyl) and 4Id (X= S; Ar= furfuryl) with percentage inhibition of 86.62, 85.02, 83.53 and 83.50 respectively. This was followed by compounds 4Ic (X= O; Ar= Anisyl) and 4Ia (X= S; Ar= phenyl) with percentage inhibition of 81.03 and 80.45 respectively.

Only one derivative i.e, compound 4Id (X= O; Ar= furfuryl) showed mild anti-inflammatory activity with the percentage of inhibition of 54.73.

Hence, present studies showed that all the compounds 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]urea and 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl] thiourea derivatives (4Ia-4Id) possess significant anti-inflammatory activity.

![Graphical representation of percentage inhibition of paw volume of 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl]urea and 1-[3-(4-substitutedphenyl)-5-phenyl-4H-1,2,4-triazol-4-yl] thiourea derivatives (4Ia-4Id) by carrageenan induced rat paw edema method.](Image 1)
Conclusion

This study reports the successful synthesis of the title compounds in good yields and moderate to potent anti-inflammatory activity of these derivatives containing 1,2,4-triazole moiety which is comparable with standard drug. It has been observed that the increased anti-inflammatory activity is attributed to the presence of pharmacologically active substituents like urea and thiourea groups.

References

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