SYNTHESIS, CHARACTERIZATION AND EVALUATION OF ANTI-
MICROBIAL ACTIVITY OF SOME NOVEL 1,2,4-TRIAZOLES

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ABSTRACT

Some novel 4-(aryliidineamino)-5-(1-(4-isobutylphenyl) ethyl)-4-yl-4H-1,2,4-triazole-3-thiols were prepared from the reaction of carbon-di-sulphide and hydrazine hydrate in water to produce thiocarbohydrazides. The thiocarbohydrazides is then refluxed with ibuprofen for 2 hour, followed by cooling to room temperature and washing with sodium bicarbonate solution to produce 5-(1-(4-isobutylphenyl) ethyl)-4H-1,2,4- triazole-3-thiole. It is then refluxed with different aldehydes in the presence of ethanol and HCl to produce the title compounds. The synthesized compounds are recrystallized from ethanol and are analyzed for their physical and spectral data. The test compound T6 shows significant activity compared to Aspirin which was also employed both of which were employed at a dose of 200mg/kg. The fact that the test compound acted orally shows that there is better absorption and it is not degradable in GIT.

Keywords: 1,2,4-triazoles; spectral analysis; analgesic activity

INTRODUCTION

Nowadays research is concentrated towards the introduction of new and safe therapeutic agents of clinical importance. The heterocycles are enjoying their importance as being the centre of activity. The nitrogen containing heterocycles are found in abundance in most of the medicinal compounds. The success of the imidazole as an important moiety of number of medicinal agents led to the introduction of the triazoles.

The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isosterically replaced by nitrogen. Triazoles are 5 membered rings, which contain two carbon and three nitrogen atoms. According to the position of nitrogen atoms, the triazoles exist in isomeric forms. Two structural isomeric triazoles are known , the 1,2,3-(1,2,5) and the 1,2,4-(1,3,4), the former being known as osotriazole, and the latter as triazole. Each exists in two disimilar tautomeric forms. The different isomers are characterized by the position of the nascent hydrogen. Thus, 1,2,4-triazoles exist in two isomeric forms i.e. 1H and 4H.
Compounds containing triazole nucleus finds a unique place in medicinal chemistry and play a significant role as they are associated with immense biological activity.

Triazole derivatives have gained considerable attention owing to their effective biological activity and extensive use. A survey of literature reveals that 1,2,4-triazole derivatives are known for their biological activities like antibacterial, antifungal, anti-inflammatory, analgesic, anticonvulsant, diuretic, antith, anti tumor etc.

In the present work, our aim was to incorporate 1, 2, 4-triazole moiety in the side chain of Ibuprofen, so that the synergistic anti-inflammatory and analgesic activity was achieved with less adverse effects.

**EXPERIMENTAL WORK**

I. Synthesis of thiocarbohydrazide:

0.2 mole (12.6 ml) of carbon disulphide was added drop wise to vigorously stirred solution of hydrazine hydrate (95%) in water during 40-45 minutes. Then the temperature of the reaction was raised to 65°C. the reaction mixture was zapped inside a domestic microwave oven for 3 minutes at 210 watts, then cooled to 0°C. the precipitated thiocarbohydrazide was filtered, washed with ethanol followed by diethyl ether and then air dried. the product thus obtained was recrystallized from minimum amount of hot water containing a few drops of concentrated hydrochloric acid.
II. Synthesis of 5-(1-(4-isobutylphenyl) ethyl)-4H-1,2,4-triazole-3-thiole

A well triturated mixture of ibuprofen (0.01 mol, 2.06 g) and Thiocarbohydrazide (0.01 mol, 1.06 g) was fused in a RB flask for 1 hour. Then it was cooled to room temperature and washed with 5 % sodium bicarbonate solution to remove unreacted acid and again washed with water. The dried compound was recrystallized with ethanol. Yield: 76.55%

III. Synthesis of 4-(aryliidineamino)-5-(1-(4-isobutylphenyl) ethyl)-4-yl-4H-1,2,4-triazole-3-thiol (Schiff’s Bases)

Equal mole of triazole and corresponding aldehydes in 25ml ethanol was treated with 0.5ml concentrated HCl and refluxed for 2 hour. After cooling the reaction mixture was filtered, air dried and recrystallized from ethanol.

Analgesic Activity
Eddy’s Hot Plate Method

Male albino mice were selected and divided into four groups, containing one animal in each group. These animals were fasted for twenty four hours, prior to the experiment. Animal of Group – I considered as Control, was administered with 1 % Acacia suspension. Animal of Group – II was treated with standard drug, i.e., Aspirin (200 mg/kg), which is considered as standard group. Animals of Group – III and IV were treated with different concentrations of test compound (100, 200 mg/kg) respectively. The reaction time for each mouse was recorded at time interval of 30, 60 and 90 minutes after the administration of test substances by using Eddy’s hot plate method.

The % analgesic activity (PAA) was calculated by the following formula:

\[ PAA = \frac{(T-C)}{C} \times 100 \]

Where C is the reaction time of the control and T is the reaction time of the test compound. The results are shown in Table 2.

RESULTS AND DISCUSSIONS

Synthesized compounds were characterized by analytical and spectral analysis. The purity of the novel synthesized compounds was ascertained for consistency in melting point and Rf value TLC by Silica Gel G.

Table 1: Physical property data of oxadizole derivatives
Characterization

Formation of 1,2,4-triazole derivatives (T₁ to T₇) was confirmed by IR, ¹HNMR and Mass spectral data. The characterization data of the synthesized compounds has been given below:

**T₁:** 4-(benzylideneamino)-5-(1-(4-isobutyl phenyl)ethyl)-4h-1,2,4-triazole-3-thiol


IR (cm⁻¹): 3160.56 (C-H), 1538.58 (C-C, Ar-C), 2976.88 (C-H, Aliphatic), 1313.76 (C-N), 1612.52 (C=N), 1561.77 (N=N), 2578.52 (S-H), 615.06 (C-S).

¹HNMR (ppm): 7.385-8.004 (m, Ar-H), 13.412 (s, SH, 1H), 9.835 (s, N=CH, 1H), 3.692-3.717 (m, CH, 1H), 2.485-2.505 (d, CH₂, 2H), 2.401-2.085 (m, CH, 1H), 1.114-1.135 (d, CH₃, 9H). Mass spectra: (M⁺ peak) = 364.3590

**T₂:** 4-{(e)-(4-methoxyphenyl)methylidene]amino]-5-(1-(4-isobutyl phenyl)ethyl)-4h-1,2,4-triazole-3-thiol

MF: C₂₂H₂₆N₄OS, MW: 394.544, M.P.: 192-195°C, Rₚ: 0.48 (Benzene:Methanol = 8:2).

IR (cm⁻¹): 3155.68 (C-H, Ar-H), 1538.93 (C-C, Ar-C), 2993.32 (C-H, Aliphatic), 1309.77 (C-N), 1605.80 (C=N), 1571.44 (N=N), 2573.15 (S-H), 615.06 (C-S), 1243.69 (C-O-C).

**T₃:** 4-{(e)-(3,4,5-trimethoxyphenyl)methylidene]amino]-5-(1-(4-isobutyl phenyl)ethyl)-4h-1,2,4-triazole-3-thiol

MF: C₂₄H₃₀N₄O₃S, MW: 454.596, M.P.: 210-213°C, Rₚ: 0.54 (Benzene: Methanol = 8:2).

IR (cm⁻¹): 3158.19 (C-H, Ar-H), 1538.70 (C-C, Ar-C), 2996.56 (C-H, Aliphatic), 1307.34 (C-N), 1573.99 (C=N), 1563.99 (N=N), 2573.15 (S-H), 595.57 (C-S), 1236.00 (C-O-C).

**T₄:** 4-{(e)-(4-chlorophenyl)methylidene]amino]-5-(1-(4-isobutyl phenyl)ethyl)-4h-1,2,4-triazole-3-thiol

MF: C₂₁H₂₃ClN₄S, MW: 398.962, M.P.: 180-182°C, Rₚ: 0.42 (Benzene : Methanol = 8:2)

IR (cm⁻¹): 3192.49 (C-H, Ar-H), 1508.17 (C-C, Ar-C), 2957.56 (C-H, Aliphatic), 1327.30 (C-N), 1595.36 (C=N), 2559.55 (S-H), 596.22 (C-S), 1083.73 (Chlorobenzene)

**T₅:** 4-{(e)-(4-hydroxyphenyl)methylidene]amino]-5-(1-(4-isobutyl phenyl)ethyl)-4h-1,2,4-triazole-3-thiol

MF: C₂₁H₂₄N₄OS, MW: 380.517, M.P.: 196-198°C, Rₚ: 0.52 (Benzene : Methanol = 8:2)

IR (cm⁻¹): 3148 (C-H, Ar-H), 1551.86 (C-C, Ar-C), 2835.98 (C-H, Aliphatic), 1340.38 (C-N), 1605.62 (C=N), 1551.86 (N=N), 2572.14 (S-H), 618.11 (C-S), 3351.72 (O-H), 1219.46 (C-O)

**T₆:** 4-{(e)-(4-hydroxy-3-methoxyphenyl)methylidene]amino]-5-(1-(4-isobutyl phenyl)ethyl)-4h-1,2,4-triazole-3-thiol

MF: C₂₂H₂₆N₄O₂S, MW: 410.543, M.P.: 194-196°C, Rₚ: 0.51 (Benzene : Methanol = 8:2)

IR (cm⁻¹): 3109.62 (C-H, Ar-H), 1544.75 (C-C, Ar-C), 2835.98 (C-H, Aliphatic), 1315.23 (C-N), 1602.97 (C=N), 1585.73 (N=N), 2561.84 (S-H), 618.58 (C-S), 1260.88 (C-O-C), 3373.05 (O-H), 1221.85 (C-O)

**T₇:** 4-{(e)-(3,4,5-trimethoxyphenyl)methylidene]amino]-5-(1-(4-isobutyl phenyl)ethyl)-4h-1,2,4-triazole-3-thiol

MF: C₂₃H₃₀N₄O₃S, MW: 454.596, M.P.: 210-213°C, Rₚ: 0.54 (Benzene: Methanol = 8:2)

IR (cm⁻¹): 3158.19 (C-H, Ar-H), 1538.70 (C-C, Ar-C), 2996.56 (C-H, Aliphatic), 1307.34 (C-N), 1573.99 (C=N), 1563.99 (N=N), 2573.15 (S-H), 595.57 (C-S), 1236.00 (C-O-C)

¹HNMR (ppm): 6.777-8.540 (m, Ar-H, 7H), 13.499 (s, Ar-C), 2996.56 (C-H, Aliphatic), 1307.34 (C-N), 1573.99 (C=N), 1563.99 (N=N), 2573.15 (S-H), 595.57 (C-S), 1236.00 (C-O-C)
SH, 1H), 10.359 (s, N=CH, 1H), 11.618 (s, OH, 1H), 5.531 (s, OCH₃, 3H), 3.831-3.871 (m, CH, 1H), 2.442-2.462 (d, CH₂, 2H), 1.816-1.897 (m, CH, 1H), 1.091-1.112 (d, CH₃, 9H). Mass Spectra (M⁺ peak): 410.3684

T₆: 4-{(e)-[(4-(dimethylamino)phenyl)methylidene]amino}-5-(1-(4-isobutyl phenyl)ethyl)-4h-1,2,4-triazole-3-thiol

IR(cm⁻¹): 3292.49 (C-H, Ar-H), 1515.28 (C-C, Ar-C), 2969.06 (C-H, Aliphatic), 1300.65 (C-N), 1593.85 (C=N), 1553.66 (N=N), 2568.07 (S-H), 594.56 (C-S).

Analgesic Activity

Eddy’s Hot Plate Method
Eddy’s hot plate method was used for screening analgesic activity. T₆ was selected and evaluated for the activity at a dose of 100 mg/Kg and 200 mg/Kg. Aspirin was used as standard drug at a dose of 200 mg/Kg body weight. The results observed for analgesic activity by Eddy’s hot plate method in albino mice is given in table 2.

Table 2: Analgesic activity Screening

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Reaction time (sec) After 90 minutes of drug administration</th>
<th>% Analgesic activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>7.34</td>
<td>-</td>
</tr>
<tr>
<td>Test</td>
<td>100</td>
<td>11.17</td>
<td>52.17</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>13.84</td>
<td>88.55</td>
</tr>
<tr>
<td>+ve control (Aspirin)</td>
<td>200</td>
<td>13.53</td>
<td>84.33</td>
</tr>
</tbody>
</table>

CONCLUSION

Seven different novel derivatives of 1, 2, 4-triazole were synthesized by reaction with seven different aromatic aldehydes. The yield of all the synthesized compounds was found to be in the range of 69-82 %. The titled compounds were characterized by physico-chemical parameters like melting point and Rf value. The structure of all the synthesized compounds was characterized by IR, NMR and Mass spectra. The spectral data also supported the assigned structure by showing the characteristic absorption peaks.

The test compound T₆ (at a dose of 200 mg/Kg) showed significant analgesic activity compared to Aspirin by Eddy’s hot plate method. This indicates that the test compound shows significant activity compared to Aspirin which was also employed in a dose of 200mg/Kg. The fact that the test compound acted orally shows that there is better absorption and it is not degradable in GIT.

ACKNOWLEDGMENT

The authors are thankful to Dr. Ramanpreet Walia, Principal, Dept. of Pharmaceutical Chemistry, Spectrum Institute of Pharmaceutical sciences and Research, Greater Noida, for providing Laboratory facilities, guidance and financial support.

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