Original Research Article

Synthesis, Characterization and evaluation of Anti-inflammatory and Analgesic activity of Benzothiazole derivatives

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ABSTRACT

A parent benzothiazole nucleus was synthesized by para amino acetanilide, then it is subjected to treatment with various substituted aromatic aldehydes to get the corresponding Schiff’s bases followed by treatment with phthalic anhydride to form 2-(6-acetamidobenzo[d]thiazol-2-ylcarbamoyl)benzoic acid. The structures of synthesized compounds were confirmed by various spectroscopic methods such as IR, ¹H NMR and mass spectroscopy. The products were evaluated for their anti-inflammatory and analgesic activities. Some of the compounds exhibited potent activities when compared with the standards.

Introduction

Benzothiazole nucleus is one of the most important heterocyclic that has received much attention due to its diversified molecular design and remarkable optical and electronic properties. Among all the benzotheterocycles, benzothiazole has a considerable place in the area of research especially in synthetic as well as in pharmaceutical chemistry due to its potent and diversified pharmacological activities such as antimicrobial[1-2], antitubercular [3], anthelmintic [4], anti-inflammatory [5-6], analgesic [7], antidiabetic [8], anticancer [9-10], antioxidant [11] etc. Research in this area is still unexplored, therefore the present study is directed towards the synthesis of newer derivatives of benzothiazole with good yield and enhance anti-inflammatory and analgesic activities.

Material and methods

All the chemicals procured from CHEMCO Labs, NICE chemicals. The melting points were determined in open glass capillaries and were uncorrected. Thin Layer Chromatography using silica gel G (E. Merck) plates were used to access the reaction and purity of synthesized compounds. The IR spectra were recorded on Shimadzu FTIR system in KBr pellets and noted the absorption levels (cm⁻¹) were listed. ¹H NMR spectra were run on Bruker DPX 400 FTNMR in DMSO-d₆ as solvent and TMS as an internal standard. The Mass spectra were recorded on JEOL JMS600H mass spectrometer.

Step 1: Synthesis of N-(2-aminobenzo[d]thiazol-6-yl) acetamide

Glacial acetic acid (150ml) pre-cooled to 50 °C was added to potassium thiocyanate (5.82g, 0.06mole) and P-amino acetanilide (8.73g, 0.06mole) solution. The mixture was placed in freezing mixture of ice and salt and mechanically stirred while bromine (1.6ml, 0.02mol) in 10ml glacial acetic acid was added from the dropping funnel at such a rate that the temperature does not rise beyond 0-50 °C. After all the bromine was added (102 min.), the solution was stirred for an additional 2h at 0-100 °C. The residue was filtered off and then it was dissolved in hot water (150ml). The solution was filtered and the filtrate was neutralized with ammonia solution to pH 6. The precipitate was collected and recrystallized with ethanol.

Step 2: Synthesis of Schiff’s bases
N-(2-aminobenzod[2]thiazol-6-yl)acetamide (0.02mol) in toluene, substituted aromatic aldehydes (0.01mole) and 10 ml glacial acetic acid were added and the reaction mixture was refluxed on the steam bath for 10 hrs. The solvent was distilled off and the residue was filtered to get desired Schiff’s bases (BT<sub>2</sub>-BT<sub>5</sub>).


A solution of pure phthalic anhydride (0.05mol) in ether (20ml), N-(2-aminobenzod[2]thiazol-6-yl)acetamide (0.05mol) in ether (20ml) was added with swirling at room temperature. The warm reaction mixture was cooled. When a colorless product separated out, filtered off the product, Washed with ether and recrystallised from ethanol (BT<sub>1</sub>).

**Anti-inflammatory activity** [12]

The anti-inflammatory activity of the standard drug Acetaminophen and synthesized compounds was determined against carrageenan induced paw oedema method in albino rats (weighing 150-175g). The albino rats were divided into 4 groups containing 2 animals each. The animals were fasted for 12 hrs prior to the experiment. The 1% w/v solution of carrageenan for injection is prepared in normal saline and 0.1 ml is injected under subplanter region. The standard drug (200mg/Kg) and synthesized compound (BT<sub>1</sub>) (100mg/Kg, 200mg/Kg) were administered to the animals, by oral route. Volume of the injected paw after 3hr was measured by means of a plethysmometer. The differences in the paw volumes (i.e. oedema volumes) of each animal were calculated and compared with the changes in the oedema volumes of control and the drug treated animals. The results were expressed as percentage reduction in oedema volume, which can be calculated by using the formula:

Where X=P-Chloro, P-Methoxy, 3-Nitro, P-methyl
Percent Reduction = (Cvt − Tvt)/ Cvt × 100
Where,
Cvt = oedema volume of control animals at time,’t’
Tvt = oedema volume of drug treated animals at time,’t’

Analgesic activity [13]
Analgesic activity was determined by using 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 (100 mg/kg), which is considered as the standard group. Animals of Group – III and IV were treated with different concentration of test compound (BT1: 150, 75 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 = (T − C)/C × 100
C is the reaction time of the control and T is the reaction time of the test compound.

Result and Discussion
The melting points of all synthesized compounds were found in open capillary tubes and readings were uncorrected. The structures of the synthesized compounds were supported by physical data (Table 1) and following spectral analysis.

Table 1: Physical data of the compounds

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Percentage yield</th>
<th>m.p(°C)</th>
<th>Rf</th>
<th>Solvent system</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT1</td>
<td>C₇H₁₈N₃O₄S</td>
<td>355.37</td>
<td>65.5%</td>
<td>121-123°C</td>
<td>0.75</td>
<td>Chloroform ethanol (7:3)</td>
</tr>
<tr>
<td>BT2</td>
<td>C₇H₁₄N₃O₂S</td>
<td>325.39</td>
<td>68.75%</td>
<td>223-225°C</td>
<td>0.83</td>
<td>Chloroform ethanol (7:3)</td>
</tr>
<tr>
<td>BT3</td>
<td>C₇H₁₂N₂O₃S</td>
<td>340.36</td>
<td>65.52%</td>
<td>180-182°C</td>
<td>0.75</td>
<td>Chloroform ethanol (7:3)</td>
</tr>
<tr>
<td>BT4</td>
<td>C₁₀H₁₂CIN₃OS</td>
<td>329.81</td>
<td>63%</td>
<td>222-224°C</td>
<td>0.62</td>
<td>Chloroform ethanol (7:3)</td>
</tr>
<tr>
<td>BT5</td>
<td>C₁₂H₁₆N₃O₅S</td>
<td>309.39</td>
<td>62.65%</td>
<td>225-225°C</td>
<td>0.74</td>
<td>Chloroform ethanol (7:3)</td>
</tr>
</tbody>
</table>

Only three compounds i.e. BT1, BT2 and BT3 were taken for spectral studies. The results showed the presence of compounds which were predicted in the synthetic scheme.

2-(6-acetamidobenz[d][thiazol-2-ylcarbamoyl] benzoic acid: BT1
IR(υ cm⁻¹): 1649.14 (amide C=O, str), 2854.65 (aliphatic C-H, str), 3527.80 (N-H, str), 3115.04 (Ar C-H, str), 1556.55 (Ar C=C, str), 1400.32 (C-N, str), 1219.01 (C-S, str), 727.16 (C-S-C, str), 1602 (C=C, str), 2392.49 (carboxylic C=O, str), 1728.22 (carboxylic C=O, str), 1556.55 (Ar C=C, str), 1400.32 (C-N, str), 1556.55 (Ar C=C, str), 1400.32 (C-N, str), 1219.01 (C-S, str), 727.16 (C-S-C, str), 1602 (C=C, str), 2392.49 (carboxylic C=O, str), 1728.22 (carboxylic C=O, str), 1556.55 (Ar C=C, str), 1400.32 (C-N, str), 1219.01 (C-S, str), 727.16 (C-S-C, str)
1H NMR(DMSO-d6) δ: 2.024- Singlet, -CH₃ (3H), 7.245-7.693-Multiplet, Aromatic protons (7H), 8.007-Singlet, -NH-C=O (1H), 9.876-Singlet, -NH-C=O (1H), 13.245-Singlet, -OH (1H), LC-MS: m/z 355.24 (M⁺)

N-(E)-2-(4-methoxybenzylideneamino)benzo[d][thiazol-6-yl)acetamide: BT2
IR(υ cm⁻¹): 1681 (amide C=O, str), 2960.73 (aliphatic CH, str), 2377.36 (NH, str), 3080.32 (Ar C-H, str), 1404.18 (C-N, str), 1371.39 (C-S, str), 1631.78 (C=S, str), 1055(C-Cl, str), 1H NMR(DMSO-d6) δ: 2.024- Singlet, -CH₃ (3H), 6.894-8.002-Multiplet, Aromatic protons (7H), 9.875- Singlet, -NH-C=O (1H), 13.245-Singlet, -OH (1H), LC-MS: m/z 325.12 (M⁺)

N-(2-[(1Z)-(4-chlorophenyl)methylene]amino)-1,3-benzothiazol-6-yl)acetamide: BT4
IR(υ cm⁻¹): 1681 (amide C=O, str), 3077.36 (NH, str), 3080.32 (Ar C-H, str), 1404.18 (C-N, str), 1371.39 (C-S, str), 1631.78 (C=S, str), 1055(C-Cl, str), 1H NMR(DMSO-d6) δ: 2.024- Singlet, -CH₃ (3H), 6.894-8.002-Multiplet, Aromatic protons (7H), 9.875- Singlet, -NH-C=O (1H), 13.245-Singlet, -OH (1H), LC-MS: m/z 329.13 (M⁺)

Carrageenan induced rat paw edema method in Swiss albino rats was used for screening anti-inflammatory activity of compounds. The compound BT1 was selected for anti-inflammatory activity at 100 and 200 mg/kg body weight.
Acetaminophen was used as the standard drug at a dose 200 mg/kg. The control group was given 0.1% CMC. The results obtained were shown in Table 2 and Figure 1. The test compound BT$_1$ showed significant anti-inflammatory activity compared to acetaminophen (200 mg/kg). Thus the test compound can be developed into a drug or a lead molecule.

**Table 2: Anti-inflammatory activity screening of compound BT$_1$**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Difference in Paw Volume after 3 Hour</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>0.078</td>
<td>-</td>
</tr>
<tr>
<td>Test 1 (BT$_1$)</td>
<td>100 mg/kg</td>
<td>0.034</td>
<td>56%</td>
</tr>
<tr>
<td>Test 2 (BT$_1$)</td>
<td>200 mg/kg</td>
<td>0.023</td>
<td>70.5%</td>
</tr>
<tr>
<td>Standard</td>
<td>200 mg/kg</td>
<td>0.018</td>
<td>76.92%</td>
</tr>
</tbody>
</table>

**Figure 1: Percentage Anti-inflammatory activity of the compound BT$_1$**

Analgesic activity of the compound BT1 was determined using Eddy’s hot plate at a dose of 150, 75mg/kg. The standard drug aspirin was used at a dose of about 100mg/kg. The results observed for analgesic activity is given in Table 3 and Figure 2.

**Table 3: Analgesic activity screening of compound BT$_1$**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose</th>
<th>Reaction Time (in sec.) after 90 minutes of 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 1 (BT$_1$)</td>
<td>75 mg/kg</td>
<td>10.65</td>
<td>45.09%</td>
</tr>
<tr>
<td></td>
<td>150 mg/kg</td>
<td>12.87</td>
<td>75.34%</td>
</tr>
<tr>
<td>Standard (Aspirin)</td>
<td>100 mg/kg</td>
<td>13.53</td>
<td>84.33%</td>
</tr>
</tbody>
</table>
Conclusion

The research work was oriented towards the finding of newer derivatives of benzothiazole with enhance anti-inflammatory and analgesic activities. The different derivatives were synthesized. The synthesized derivatives showed very good anti-inflammatory and analgesic activities against previously reported derivatives of benzothiazole.

Acknowledgement

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Conflict of interest statement

We declare that we have no conflict of interest.

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