Synthesis, Characterization and Antibacterial Activity of some 1,3- Benzthiazol-2-amine derivatives

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Abstract
Benzothiazole is an aromatic heterocyclic compounds with the general formula C\textsubscript{7}H\textsubscript{5}NS which consists of 5 membered heterocyclic 1, 3 thiazole ring fused to benzene. Benzothiazole is a simple analogues which play a major role in research which has wide number of biological activities like antimicrobial, antitubercular, antimalarial, anticonvulsant, antihelimenthic, analgesic and anti-inflammatory activity on the account of the reported anticancer activity of benzothiazoles containing pthalmide, a new series of benzothiazole derivatives were synthesized and tested for in-vitro anticancer activity against human carcinoma cell line. The newly synthesized compounds were screened for antibacterial activity against Gram positive [Bacillus subtilis (NCIM-2545), Staphylococcus epidermidis (NCIM-2493), Staphylococcus aureus (NCIM-5021)] and Gram negative [(Escherichia coli (NCIM-2803))] bacteria by agar diffusion method. All the compounds were found to be active against all the four bacterial strains at 20 µg/ml concentration. The compound 1,3-benthiazol-2-amine exhibited activity against Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis, at a concentration of 20 µg/ml. All the activity is done with Amoxicillin as reference
Key words: Benzothiazole, analgesic activity, anti-inflammatory activity, Escherichia coli

Introduction
Benzothiazole is an aromatic heterocyclic compounds with the general formula C\textsubscript{7}H\textsubscript{5}NS which consists of 5 membered heterocyclic 1, 3 thiazole ring fused to benzene.\textsuperscript{1} Benzothiazole is a simple analogues which play a major role in research which has wide number of biological activities like antimicrobial, antitubercular, antimalarial, anticonvulsant, antihelimenthic, analgesic and anti-inflammatory activity\textsuperscript{2}. On the account of the reported anticancer activity of benzothiazoles containing pthalmide, a new series of benzothiazole derivatives were synthesized and tested for in-vitro anticancer activity against human carcinoma cell line.\textsuperscript{3} An array of biological
activities such as antibacterial, antifungal, antiphlogistic, antitumor, and herbicidal has been reported to be shown by various benzothiazoles. It has been proved that these heterocyclic compounds are effective as inhibitors of inflammatory mediators in intact cells, M. tuberculosis, and human enterovirus. They also show inhibitory activity towards both tubulin polymerization, cyclin-dependent kinase and enzymatic assays on Src and Abl tyrosine kinases. Prompted by these claims and in continuing our synthetic studies on bioactive heterocycles, we have now synthesized a new series of some benzothiazoles to test their ability as antibacterial agents.

Experimental

Synthesis of 6-nitro-1,3-benzthiazol-2-amine: (1)

Reaction:

![Chemical structure of 6-nitro-1,3-benzothiazol-2-amine](image)

Procedure: 4-nitroaniline (0.5mol 6.54gm), sodium thio cyanide (1.0gm) and sulphuric acid (3ml) as catalyst and DMSO(dimethyl sulphoxide) and water are taken in a ratio of 9:1 as solvent (10ml) in a round bottomed flask and stirred for about 1 hour and then refluxed for 30 mins at 50°C for 30 min. then the reaction mixture is cooled to room temperature and poured into ice cold water and compound was extracted into an organic solvent ethyl acetate. Then organic solvent was washed with 10% sodium dithionite and 10% sodium bicarbonate. The product was dried with magnesium sulphate. The product was concentrated under reduced pressure by removing ethyl acetate. The obtained product was stored.

TLC Studies of Compound (1):
Solvent System: Chloroform: Methanol (9:1)
1. 4-nitroaniline
2. Co spot of 4-nitroaniline and Compound-1
3. Compound-1

Physical Properties
Recrystallisation solvent: Ethanol
Yield: 64.75 %
M.P of pure product: 156°C
Molecular Weight: 195.1
Molecular Formula: C₇H₅N₃O₂S

Rf value of Compound (1) : 0.72

Synthesis of 6-chloro-1,3-benzothiazol-2-amine: (2)

Reaction:
Procedure:

\[
\begin{align*}
\text{4-chloroaniline} & \quad + \quad \text{NaSCN} \\
\rightarrow & \\
\text{6-chloro-1,3-benzothiazol-2-amine}
\end{align*}
\]

4-chloro aniline (0.5mol, 6.54gm), sodium thio cyanide (1.0gm) and sulphuric acid (3ml) as catalyst and DMSO (dimethyl sulphoxide) and water are taken in a ratio of 9:1 as solvent (10ml) in a round bottomed flak and stirred for about 1 hour and then refluxed for 30 mins at 50°C for 30 min. Then the reaction mixture is cooled to room temperature and poured in to ice cold water and compound was extracted in to an organic solvent ethyl acetate. Then organic solvent was washed with 10% sodium dithionite and 10% sodium bicarbonate. The product was dried with magnesium sulphate. The product was concentrated under reduced pressure by removing ethyl acetate. The obtained product was stored.

TLC Studies of Compound (2):

Solvent System: Chloroform: Methanol (9:1)
1. 4-chloroaniline
2. Co spot of 4-chloroaniline and Compound-2
3. Compound-2

Physical Properties
Recrystallisation solvent: Ethanol  
Yield: 74.75 %  
M.P of pure product: 145°C  
Molecular Weight: 186.1  
Molecular Formula: C7H5N3S,Cl  
Rf value of Compound (2): 0.54  

**Synthesis of 1,3-benzothiazol-2-amine (3)**  

**Reaction:**

![Reaction Diagram](image)

Aniline (0.5mol 9.2ml), sodium thio cyanide (5.0gm) and sulphuric acid (3ml) as catalyst and DMSO (dimethyl sulphoxide) and water are taken in a ratio of 9:1 as solvent (10ml) in a round bottomed flask and stirred for about 1 hour and then refluxed for 30 mins at 50°C for 30 min. Then the reaction mixture is cooled to room temperature and poured in to ice cold water and compound was extracted in to an organic solvent ethyl acetate. Then organic solvent was washed with 10% sodium dithionite and 10% sodium bicarbonate. The product was dried with magnesium sulphate. The product was concentrated under reduced pressure by removing ethyl acetate. The obtained product was stored.

**TLC Studies of Compound (3):**

![TLC Diagram](image)

Solvent System: Chloroform: Methanol (9:1)  
1. Aniline  
2. Co spot of Aniline and Compound-3  
3. Compound-3

**Physical Properties**  
Recrystallisation solvent: Ethanol  
Yield: 79.62 %  
M.P of pure product: 128°C  
Molecular Weight: 150.20
Molecular Formula: $C_7H_6N_2S$
$R_f$ value of Compound (2) : 0.66

**Synthesis of 6-bromo-1,3-benzthiazol-2-amine: (4)**

**Reaction:**

4-bromo aniline (0.5mol 17.2gms), sodium thio cyanide (5.0gm) and sulphuric acid (3ml) as catalyst and DMSO(dimethyl sulphoxide) and water are taken in a ratio of 9:1 as solvent (10ml) in a round bottomed flak and stirred for about 1 hour and then refluxed for 30 mins at 50°C for 30 min. then the reaction mixture is cooled to room temperature and poured in to ice cold water and compound was extracted in to an organic solvent ethyl acetate. Then organic solvent was washed with 10% sodium dithionite and 10%sodium bicarbonate. The product was dried with magnesium sulphate. The product was concentrated under reduced pressure by removing ethyl acetate. The obtained product was stored.

**TLC Studies of Compound (4):**

**Solvent System:** Chloroform: Methanol (9:1)
1. Aniline
2. Co spot of Aniline and Compound-3
3. Compound-3

**Physical Properties**
Recrystallisation solvent: Ethanol
Yield: 74.75%
M.P of pure product: 229°C
Molecular Weight: 186.1
Molecular Formula: $C_7H_5N_2S, Br$
$R_f$ value of Compound (2) : 0.46

**Antibacterial Activity**
The synthesized compounds were screened for antibacterial activity. For determination of bacterial susceptibility test, both gram positive and gram negative organisms are used. All the bacterial strains were obtained from National
Collection of Industrial Microorganisms: A stock solution of amoxicillin was prepared and the dilutions are prepared. All the strains were maintained by weekly sub culturing on nutrient agar slant, stored at 4 °C after previous 24 h incubation at 37 °C. Before each experiment, the organism was activated by successive sub culturing and incubation. The activity is studied by using agar diffusion method 12.

**Gram Positive Bacteria:**
- *Bacillus subtilis*: (NCIM-2545)
- *Staphylococcus epidermidis*: (NCIM-2493)
- *Staphylococcus aureus*: (NCIM-5021)

**Gram Negative bacteria:**
- *Escherichia coli*: (NCIM-2803)

**PROCEDURE:**

**Composition of nutrient agar medium:**
- Peptone: 5.0g
- Beef extract: 5.0g
- NaCl: 5.0 mg
- Agar: 2 %
- Distilled water: up to 1000 mL

**Standardization of test microorganisms:**
A 10 mL volume of sterile water was added to the agar slant containing a 24 h old culture of purified test microorganism and shaken carefully to harvest the organism. Subsequently, dilutions were carried out to get microbial population of $10^5$ cfu/ml by comparing with BaSO$_4$, equivalent to McFarland 0.5 standard.

**Preparation of BaSO$_4$ suspension equivalent to McFarland 0.5 Standard:**
To standardize the inoculums density for a susceptibility test, a BaSO$_4$, turbidity standard, equivalent to a 0.5 McFarland standard is used. The BaSO$_4$, McFarland 0.5 standard is prepared as follows. A 0.5 mL of 1.175 % w/v of BaCl$_2$.2H$_2$O is added to 99.5 mL of 1% w/v of H$_2$SO$_4$ with constant stirring to maintain suspension. The correct density of the turbidity standard is verified by using a UV-spectrophotometer by determining the absorbance. The absorbance at 625 nm is 0.08-0.10 for this standard. This suspension is used to standardize the inoculums density 7.

## Results and Discussion

### Table 1: Physical data 6-nitro-1,3-benzthiazol-2-amine: (Compound 1)

<table>
<thead>
<tr>
<th>Compd. No</th>
<th>Compound structure</th>
<th>Mol. Formula</th>
<th>Mol. Wt</th>
<th>Recrys. Solvent</th>
<th>M.P (°C)</th>
<th>Yield (%)</th>
<th>Rf value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image" alt="Compound 1" /></td>
<td>C$_7$H$_5$N$_3$O$_2$S</td>
<td>195.1</td>
<td>Ethanol</td>
<td>229°C</td>
<td>64.75</td>
<td>0.72</td>
</tr>
</tbody>
</table>

*Solvent System: Chloroform: Methanol (9:1)

### Table 2: Physical data of 6-chloro-1,3-benzthiazol-2-amine: (Compound 2)

<table>
<thead>
<tr>
<th>Compd. No</th>
<th>Compound structure</th>
<th>Mol. Formula</th>
<th>Mol. Wt</th>
<th>Recrys. Solvent</th>
<th>M.P (°C)</th>
<th>Yield (%)</th>
<th>Rf value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td><img src="image" alt="Compound 2" /></td>
<td>C$_7$H$_5$N$_3$S,Cl</td>
<td>186.1</td>
<td>Ethanol</td>
<td>145</td>
<td>74.75</td>
<td>0.54</td>
</tr>
</tbody>
</table>

*Solvent System: Chloroform: Methanol (9:1)
Table 3: Physical data of 1,3-benzthiazol-2-amine: (Compound 3)

<table>
<thead>
<tr>
<th>Compd. No</th>
<th>Compound structure</th>
<th>Mol. Formula</th>
<th>Mol. Wt</th>
<th>Recrys. Solvent</th>
<th>M.P (°C)</th>
<th>Yield (%)</th>
<th>Rf value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>![Compound 3 Image]</td>
<td>C₇H₅N₂S,</td>
<td>150.20</td>
<td>Ethanol</td>
<td>128</td>
<td>79.62</td>
<td>0.66</td>
</tr>
</tbody>
</table>

*Solvent System: Chloroform: Methanol (9:1)

Table 4: Physical data of 6-bromo-1,3-benzthiazol-2-amine: (Compound 4)

<table>
<thead>
<tr>
<th>Compd. No</th>
<th>Compound structure</th>
<th>Mol. Formula</th>
<th>Mol. Wt</th>
<th>Recrys. Solvent</th>
<th>M.P (°C)</th>
<th>Yield (%)</th>
<th>Rf value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>![Compound 4 Image]</td>
<td>C₇H₅N₂S,Br</td>
<td>260.67</td>
<td>Chloroform</td>
<td>186.1</td>
<td>74.75</td>
<td>0.46</td>
</tr>
</tbody>
</table>

*Solvent System: Chloroform: Methanol (9:1)

Spectral Data

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Compound structure</th>
<th>IR (Cm⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>![Compound 1 Image]</td>
<td>IR (Cm⁻¹) (KBr): 2951 (S-H), 3283 (NH₂), 1558 (NO₂), 1458 (C-NH).</td>
</tr>
<tr>
<td>2</td>
<td>![Compound 2 Image]</td>
<td>IR (Cm⁻¹) (KBr): 2954 (S-H), 1636 (NH₂), 1428 (C-NH), 618 (C-Cl)</td>
</tr>
<tr>
<td>3</td>
<td>![Compound 3 Image]</td>
<td>IR (Cm⁻¹) (KBr): 2956 (S-H), 2932 (NH₂), 1474 (C-NH).</td>
</tr>
</tbody>
</table>
IR (KBr): 2928 (S-H), 3283 (NH$_2$), 1632 (C-NH), 885 (C-Br) \text{M+H} : 261

**Antibacterial Results**

**Antibacterial activity profile of 1,3-benzthiazol-2-amine for *Escherichia coli***

**Category:** Gram Negative

**Control:** Amoxicillin

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R</th>
<th><em>Escherichia coli</em> ($\mu$g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>6a</td>
<td>Compound-1</td>
<td>--</td>
</tr>
<tr>
<td>6b</td>
<td>Compound-2</td>
<td>--</td>
</tr>
<tr>
<td>6c</td>
<td>Compound-3</td>
<td>--</td>
</tr>
<tr>
<td>6d</td>
<td>Compound-4</td>
<td>--</td>
</tr>
<tr>
<td>Amox</td>
<td>--</td>
<td>15.5 ± 0.07</td>
</tr>
</tbody>
</table>

Zone of inhibition in millimeters. (Average ± SEM) (n=9)

**Figure 1:** Antibacterial activity profile of 1,3-benzthiazol-2-amine for *Bacillus subtilis*

**Category:** Gram Positive

**Control:** Amoxicillin

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R</th>
<th><em>Bacillus subtilis</em> ($\mu$g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>6a</td>
<td>Compound-1</td>
<td>--</td>
</tr>
<tr>
<td>6b</td>
<td>Compound-2</td>
<td>--</td>
</tr>
<tr>
<td>6c</td>
<td>Compound-3</td>
<td>--</td>
</tr>
<tr>
<td>6d</td>
<td>Compound-4</td>
<td>--</td>
</tr>
<tr>
<td>Amox</td>
<td>--</td>
<td>16.3 ± 0.05</td>
</tr>
</tbody>
</table>

Zone of inhibition in millimeters. (Average ± SEM) (n=9)
Antibacterial activity profile of 1,3-benzthiazol-2-amine for *Staphylococcus aureus*

Category: Gram Positive  
Control: Amoxicillin

### Table 7

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R</th>
<th><em>Staphylococcus aureus</em> (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>6a</td>
<td>-</td>
<td>12.6 ± 0.05</td>
</tr>
<tr>
<td>6b</td>
<td>-</td>
<td>13.2 ± 0.04</td>
</tr>
<tr>
<td>6c</td>
<td>-</td>
<td>13.3 ± 0.05</td>
</tr>
<tr>
<td>6d</td>
<td>-</td>
<td>13.0 ± 0.05</td>
</tr>
<tr>
<td>Amox</td>
<td>--</td>
<td>16.6 ± 0.05</td>
</tr>
</tbody>
</table>

Zone of inhibition in millimeters (Average ± SEM) (n=9)

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**Discussion**  

Synthesis of 6-Nitro-1,3-Benzthiazol-2-Amine

\[
\begin{align*}
4\text{-nitroaniline} + \text{NaSCN} & \rightarrow 6\text{-nitro-1,3-benzothiazol-2-amine} \\
\end{align*}
\]
In the above reaction the 4-nitroaniline was treated with Sulphuric which readily oxidizes the aniline which is then reacted with sodium thiocyanate and then reaction mixture was stirred with at room temperature for 1 hour which upon heated up to 50°C and then cooled to room temperature and compound was quenched in to ice cold water and kept aside. Then the compound was extracted by ethyl acetate and then washed with sodium thionite and sodium bicarbonate solutions. Then the organic layer was washed with magnesium sulphate and concentrated under reduced pressure. 

![IR-SPECTRA COMPOUND - 1](image1)

![MASS-SPECTRA COMPOUND – 1](image2)

![IR-SPECTRA COMPOUND-2](image3)
MASS-SPECTRA COMPOUND – 2

IR-SPECTRA COMPOUND – 3

MASS-SPECTRA COMPOUND – 3
IR-SPECTRA COMPOUND – 4

MASS-SPECTRA COMPOUND – 4

NMR-SPECTRA COMPOUND – 4
Conclusion
All the compounds synthesized were characterized by physical (Rf values, Melting point, Molecular weight, Molecular formula) and spectral data by IR, MASS, NMR spectra. The newly synthesized compounds were screened for antibacterial activity against Gram positive [Bacillus subtilis (NCIM-2545), Staphylococcus epidermidis (NCIM-2493), Staphylococcus aureus (NCIM-5021)] and Gram negative [(Escherichia coli (NCIM-2803))] bacteria by agar diffusion method. All the compounds were found to be active against all the four bacterial strains at 20 µg/mL concentration. And the activity of these compounds varied with the kind of organism. All compounds (compound 1-4) showed similar zone of inhibitions. The compound 1,3-benthiazol-2-amine exhibited activity against Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis, at a concentration of 20 µg/mL. All the activity is done with Amoxicillin as reference.

References