SYNTHESIS, CHARACTERIZATION, AND ANTIBACTERIAL EVALUATION OF SOME NOVEL SUBSTITUTED 2-AMINO BENZOTHIAZOLE DERIVATIVES

Mrudula Devi Kakara* and Govindarao Kamala.

Department of Pharmaceutical Chemistry, Aditya Institute of Pharmaceutical Sciences, Surampalem, Dist- East Godavari, Andhra Pradesh- 533437, India.

ABSTRACT

The present study deals with synthesis of some novel substituted 2-amino benzothiazole and their spectral characterization by means of UV, IR and ¹H NMR. The compounds were screened for antibacterial activity against standard strains of both Gram positive and Gram negative bacteria. Results obtained establish compounds MD1 and MD2 to be significantly responsive against different bacterial strains and as such these compounds can pave the way for development of potent antibacterial agents.

KEYWORDS: Benzothiazole, Aniline Derivatives, NH₄SCN, Antibacterial, Cup-plate method.

INTRODUCTION

Benzothiazole is revealed to have shown potential for application in a variety of pharmacological targets and is a privileged bicyclic ring system.¹ It contains a benzene ring fused to a thiazole ring.² The small and simple benzothiazole nucleus is present in compounds involved in research aimed at evaluating new products that possess interesting biological activities like- antimicrobial³⁻¹⁰, antitubercular¹¹, anticancer¹²⁻¹³, local anaesthetic activity¹⁴, anticonvulsant¹⁵⁻¹⁷, antifungal¹⁸, anthelmintic¹⁹, analgesic, anti-inflammatory activity²⁰,²¹, and antibacterial activity.²² Most alarming of all are diseases where resistance is developing for all currently available drugs; current trends suggest that some diseases will have no effective therapies within the next ten years. So, there is a
requirement to develop new replacement drug immediately which is effective against resistant bacteria having lesser toxicity as well as economical also \(^{[18]}\). In view of the biological importance of the benzothiazole nucleus containing compounds, in the present work, it is plan to synthesize substituted 2-amino benzothiazoles by developing novel methodology. Different synthetic methods are reported for the synthesis of benzothiazole and its derivatives which include processes like aniline was treated with KH\(_4\)SCN in presence of and glacial acetic acid in bromine.\(^{[23]}\) The present study utilizes the same reaction phenomenon of substituted aniline were treated with NH\(_4\)SCN in presence of chloroform and bromine to get substituted 2-amino benzothiazole derivatives followed by their antibacterial screening.\(^{[24-26]}\)

**MATERIALS AND METHODS**

**Synthetic Scheme**

![Synthetic scheme for 2-substituted benzothiazole](image)

All the reagents used for synthesis were of analytical grade commercial products and used without further purification. The melting points of the synthesized compounds were determined using an electric melting point apparatus by open capillary method. (Expressed in degree Celsius) and are uncorrected. The progress of reactions and purity of synthesized compounds were checked on silica gel-G TLC plates using various solvent combinations of different polarity. The spots were detected with iodine vapors as visualizing agent. The \(\lambda_{\text{max}}\) (in nm) of the synthesized compounds was recorded on *Elico SL 164* UV-visible spectrophotometer using alcohol as solvent. The FT-IR spectra of the synthesized compounds
were recorded on a FT-IR *Perkin Elmer Spectrum RX-I* spectrometer using KBr disc in the range of 4000-400 cm$^{-1}$. The Proton NMR ($^1$H NMR) spectra were recorded in *Bruker AC-F 400* FT-NMR spectrometer at a frequency of 400 MHz. Spectra were obtained in deuterated acetone (acetone-$d_6$) using TMS (δ 0.00 ppm) as an internal standard at room temperature. Chemical shift (δ) values are expressed in ppm relative to internal standard.

**Substituted aniline used for synthesis**

**Table.1: Substrates used for the reaction process to form target compounds**

<table>
<thead>
<tr>
<th>Sample Code</th>
<th>Substrate</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD1</td>
<td><img src="image1" alt="Substrates" /></td>
<td><img src="image2" alt="Compounds" /></td>
</tr>
<tr>
<td>MD2</td>
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</tr>
<tr>
<td>MD3</td>
<td><img src="image5" alt="Substrates" /></td>
<td><img src="image6" alt="Compounds" /></td>
</tr>
<tr>
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</tr>
<tr>
<td>MD5</td>
<td><img src="image9" alt="Substrates" /></td>
<td><img src="image10" alt="Compounds" /></td>
</tr>
</tbody>
</table>

**General method for synthesis of Substituted 2-amino benzothiazole**

**In 1st step,** the substituted aniline (0.01mol) was taken in a beaker with magnetic stirrer and it was placed into Hotplate. A mixture of 5ml of HCl & 20ml of water was added & then it was stirring at temperature 50°C for 15min. The solution of aniline HCl obtained & then ammonium thio cyanate (0.01mol) was slowly added. The reaction mixture was again stirring to continue 1 hr. The solid separated out on cooling was filtered, washed with water, dried and crystallized from absolute alcohol.

**In 2nd step,** above obtained compound [0.01mol] in 20 ml chloroform was taken a beaker & then it was stirring at temperature 40°C for 20min. To this reaction mixture bromine
[0.01mol] in 20ml chloroform was added with stirring over a period of 1hr, during the addition of bromine, a temp of reaction mixture was maintained below 10°C. It was refluxed until the evaluation of HBr ceased [about 30min] chloroform was removed by filtration. The filtrate was neutralized with aq. ammonia. The precipitate of substituted 2-amino benzothiazole was filtered washed with water & recrystallised from absolute alcohol.

**Antibacterial Activity**

The antibacterial activity of the synthesized compounds was evaluated systematically against different strains of Gram-positive bacteria like *Staphylococcus aureus* and *Bacillus subtilis* and Gram-negative bacteria like *E.Coli* and *Pseudomonas aeruginosa*. The inhibition zones (in mm) of synthesized compounds were determined by cup-plate method [27]. The sterilized medium (autoclaved at 121°C for 20 min) was inoculated using 18hr slant cultures of the test organisms and transferred into sterile Petri dishes and allowed to the media to solidify. Cups of 8 mm diameters were made on solidified media. Solutions of the synthesized compounds at a concentration of 50µg/ml and 100µg/ml were prepared in acetone. 50µl of each solution was placed in cups by means of sterile pipette. In each plate one cup was used for standard and other two for test solutions. The plates thus prepared were left for 90 min in a refrigerator for diffusion. The plates were incubated at 37°C for 24hrs and examined for inhibition zones. The experiment was performed in duplicate and the average diameter of the zones of inhibition was recorded. Amikacin (50µg/ml) was used as standard.

**RESULTS AND DISCUSSION**

**Physico-chemical properties and spectral data of the synthesized compounds**

The yields of all the synthesized compounds were found to be satisfactory within the range of 75 to 85%. The spectral data generated upon analysis were found in accordance with the anticipated structure of the synthesized compounds.

**MD1: 6-methyl-1, 3-benzothiazol-2-amine**

Yield: 75%; Melting point: 135-137 °C; Rf value: 0.65; λ<sub>max</sub>: 420; IR (KBr cm<sup>-1</sup>): 3375, 3280, 3076, 2935, 1638, 1537, 1457, 1374, 1305, 1101; ¹H NMR (400 MHz, acetone-d6), δ (ppm): δ7.50-7.20(m, 3H, Ar-H), 5.63-5.38 (S,2H,-C-NH2), 2.48 (S,3H, CH3).

**MD2: 6-methoxy-1, 3-benzothiazol-2-amine**

Yield: 79%; Melting point: 165-167 °C; Rf value: 0.70; λ<sub>max</sub>: 440; IR (KBr cm<sup>-1</sup>): 3380, 3281, 3096, 2937, 1636, 1544, 1459, 1339, 1273, 1050; ¹H NMR (400 MHz, acetone-d6), δ (ppm): δ7.49-7.10(m, 3H, Ar-H), 5.06-5.04 (bs,2H,-C-NH2), 3.78 (s,3H, -OCH3)
MD3: 4-nitro-1, 3-benzothiazol-2-amine

**Yield:** 81%; **Melting point:** 250-252 °C; **Rf value:** 0.93; **IR (KBr cm⁻¹):** 3324, 3088, 1612, 1585, 1509, 1352, 1253; **¹H NMR (400 MHz, acetone-d₆), δ (ppm):** δ8.22-7.35(m, 3H, Ar-H), 6.85-6.83 (S, 2H, C-NH2).

MD4: 4-bromo-1, 3-benzothiazol-2-amine

**Yield:** 80%; **Melting point:** 183-185 °C; **Rf value:** 0.95; **IR (KBr cm⁻¹):** 3451, 3260, 3083, 1636, 1541, 1450, 1310, 1270, 761; **¹H NMR (400 MHz, acetone-d₆), δ (ppm):** δ7.92-7.68(m, 3H, Ar-H), 7.20-6.78 (bs, 2H, C-NH2)

MD5: 4-methoxy-1, 3-benzothiazol-2-amine

**Yield:** 85%; **Melting point:** 153-155°C; **Rf value:** 0.90; **λ_max (KBr cm⁻¹):** 3373, 3281, 3096, 2937, 1636, 1546, 1459, 1339, 1273, 1049; **¹H NMR (400 MHz, acetone-d₆), δ (ppm):** δ7.45-7.15(m, 3H, Ar-H), 5.38-5.05 (bs, 2H, C-NH2), 3.78 (s, 3H, -OCH₃).

**Antibacterial activity data of the synthesized compounds**

Antibacterial screening of the synthesized compounds against different strains of Gram positive and Gram negative bacteria show compounds MD2 and MD1 exhibiting marked inhibition of both Gram positive and negative strains whereas compounds MD5 and MD3 too showed considerable amount of activity. Compound MD4 showed the least activity amongst the series. Stressing on the structural influence on the activity of the synthesized novel analogues, it can be observed that the P-methoxy group (-OCH₃) present in MD2 (from P-methoxy aniline as substituent) and P-methyl group (CH₃) present in MD1 (from P-methyl aniline as substituent) may have a vital role in the activity of the compounds. Whereas an O-bromde group (-Br) in MD4 (from O- bromo aniline as substituent) may possibly diminish the inhibitory activity to a considerable extent. It is evident from the research work that this series of synthesized and screened compounds along with further explored ones from the same series of Substituted 2-amino benzothiazole may pave the way for development of some very potent antibacterial agents.
Table 2: Antibacterial activity of synthesized compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gram positive bacteria</td>
</tr>
<tr>
<td></td>
<td>S. Aureus</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
</tr>
<tr>
<td>MD1</td>
<td>18</td>
</tr>
<tr>
<td>MD2</td>
<td>20</td>
</tr>
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<td>MD3</td>
<td>15</td>
</tr>
<tr>
<td>MD4</td>
<td>12</td>
</tr>
<tr>
<td>MD5</td>
<td>17</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
</tr>
<tr>
<td>Amikacin</td>
<td>18</td>
</tr>
</tbody>
</table>

*Amikacin (50 µg/ml) was used as positive control
**Acetone was used as negative control

CONCLUSION

In this study, we have synthesized five derivatives of substituted 2- amino benzothiazole by the scheme depicted in Figure 1. The test compounds were synthesized in good percentage of yield their physical and analytical determination was done by using melting point apparatus, purification of compounds by TLC, and the structural assignments of new compounds were made on the basis of UV-visible Spectrophotometer, IR and 1HNMR data. This scheme of reaction went to completion within $1\frac{1}{2}$ hr. After completion of reaction and work up the products were identified and characterized by using UV-visible Spectrophotometer, IR and 1HNMR techniques and their structures were elucidated as 6-methyl-1, 3-benzothiazol-2-amine, 6-methoxy-1, 3-benzothiazol-2-amine, 4-nitro-1, 3-benzothiazol-2-amine, 4-bromo-1, 3-benzothiazol-2-amine, 4-methoxy-1, 3-benzothiazol-2-amine. The isolated yield was 75%, 79%, 81%, 80%, 85%. From our present investigation, it can be concluded that substituted 2- amino benzothiazole are formed by fusion of aryl ring with thiazole and the condensing ring containing at 6th position are various substituent’s such as – CH₃, -OCH₃ of first two compounds (MD1 & MD2) where as at 4th position are various substituent’s such as –NO₂, -Br, -OCH₃ of next three compounds (MD3, MD4, & MD5) and the structural assignments of these compounds are made on the basis of UV-visible spectrophotometer, IR and 1HNMR data. Further exploration with this series can prove to be instrumental in the field of antibacterial drug development.
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