2-Aminobenzothiazole containing novel Schiff bases derivatives: Search for new Antibacterial agents

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ABSTRACT. The benzothiazole and Schiff base moieties are crucial functionalities due to their wide variety of biological activities and have a wide range of therapeutic properties. Keeping in view the importance of these organic moieties, a new series of 2-Aminobenzothiazole containing novel Schiff bases derivatives were synthesized by sequential reaction. The structures of the synthesized compounds were confirmed by their analytical and spectral data. The synthesized compounds were evaluated for their in vitro antibacterial activity against gram positive and gram negative bacteria. Synthesized compounds showed significant activity against microorganisms, which can be correlated with the privileged heterocyclic structural scaffolds.

1. INTRODUCTION

Today, we are facing one of the major problems in the context of infectious diseases is the persistent increase and spread of antimicrobial resistance. Thus, studies for the identification of new targets and drugs for the action of infectious diseases are at the forefront. Many heterocyclic nuclei, benzimidazole, triazine, benzothiazole have been lately reviewed as antimicrobial agents. [1, 2] Our attention was focused to the benzothiazole nucleus.[3] Benzothiazole can serve as unique and versatile scaffolds in research area, especially in synthetic as well as in pharmaceutical chemistry because of its potent and significant pharmacological activities. In fact, benzothiazole derivatives possess a wide spectrum of biological applications such as antimicrobial,[4] anticancer,[5] Anthelmintic,[6] Anti-diabetic,[7] Anti-tuberculosis,[8] antitumor,[9] Antitrypanosomal,[10] antiviral, Antibacterial, antioxidant, Anti-glutamate and Anti-parkinsonism, analgesic, Anti-inflammatory, Antifungal, Anti-leishmanial, Anticonvulsant, [11] etc.

Schiff bases, named after Hugo Schiff,[12] are designed when any primary amine treated with an aldehyde or a ketone under particular conditions. A Schiff base is known as imine or azomethine, is a nitrogen analogue of an aldehyde or ketone in which the carbonyl group has been replaced by an imine or azomethine group. They are some of the most widely used organic compounds. They are used as catalysts, pigments and dyes, intermediates in organic synthesis, and as polymer stabilizers.[13] Schiff bases have also been shown to exhibit a broad range of biological activities such as antiviral, antibacterial, anti-inflammatory, antimalarial, antifungal, anti-proliferative and antipyretic properties.[14, 15] Imine or azomethine groups are present in various natural, naturally-derived, non-natural compounds and some compound which shows various biological activities. [16, 17]. There are very scarce recent literature data on antimicrobial potentials of benzothiazole containing Schiff base moiety that should combine favorable structural properties of both Schiff bases moiety and benzothiazole moiety. Therefore, in the present paper, we have prepared a set of new N-(4-(1-benzo[d]thiazol-2-ylimino)ethyl)phenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide derivatives and evaluated for their in-vitro antibacterial activities against Gram-positive and Gram-negative bacteria.
2. EXPERIMENT SECTION

All starting materials and other reagents were purchased from commercial suppliers. Thin layer chromatography (TLC) which was performed on silica gel G 60 F254 (Merck) plates and eluted with the appropriate solvent ratios (v/v). The melting points were recorded on optimel automated melting point system and were uncorrected. For column chromatography 60-120 mesh LR (Merck) silica gel was used. IR spectra were recorded on a Perkin-Elmer 377 spectrophotometer in KBr with absorption in cm\(^{-1}\). \(^1\)H NMR spectra were recorded on Bruker AV 400 MHz using DMSO-\(d_6\) as solvent and TMS as an internal standard. Chemical shifts are reported in parts per million (\(\delta\) in ppm). Mass spectra were recorded at Advion Expression CMS, USA. Elemental analysis was performed on the Vario MICRO cube, elementar CHN analyzer serial no.: 15084053.

**General procedure for the synthesis of N-(4-acetylphenyl)-2-chloroacetamide (2):**
A mixture of 4-amino acetophenone (0.01 mol) with chloroacetylchloride (0.015 mol) and Triethylamine (4-5 drops) in 25 ml RBF using DMF as solvent. The reaction mixture was stirred for 4 hours at Room temperature. The completion of the reaction was checked by TLC with Mobile phase Toluene: Acetone (30 %). The solution was poured into ice water. The product obtained was filtered by using a vacuum pump and crystalline it in Ethanol.[18]

**General procedure for the Synthesis of N-(4-Acetylphenyl)-2-(benzo[d]thiazole-2-ylthio) acetamide (4):**
Above synthesized compound N-(4-acetyl phenyl) -2-chloroacetamide (2) (0.01 mol) was further reacted with 2-mercaptobenzothiazole (0.01 mol). The reaction was stirred at room temperature for 4 hrs in the presence of K\(_2\)CO\(_3\) (0.02 mol) and acetone as a solvent. The completion of the reaction was monitored by TLC using Toluene: Acetone (20 %) as a mobile phase, the product was poured into water and stirred for 1 hour. The obtained precipitate was collected and dried. The product was crystallized into methanol.

**General procedure for the N-(4-(1-(benzo[d]thiazol-2-ylimino)ethyl)phenyl)-2-(benzo[d] thiazol-2-ylthio)acetamide derivatives (6a-6g):**
Compound N-(4-Acetylphenyl) -2-(benzo [d] thiazole-2-ylthio) acetamide (4) (0.01 mol) was further treated with different substituted 2-aminobenzothiazole (0.01 mol) in ethanol in the presence acetic acid as a catalyst and 4-5 drops of fused sodium acetate and refluxed for 8-12 hours. The completion of the reaction was monitored by the TLC using Toluene: Acetone (20 %) as mobile phase. Resulting solid was separated out, filtered, and washed with water, dried and crystallized by alcohol to get N-(4-(1-(benzo[d]thiazol-2-ylimino)ethyl)phenyl)-2-(benzo[d] thiazol-2-ylthio)acetamide derivatives (6a-6g) with 82-90 % good yield.

**\(N\)-(4-(1-(benzo[d]thiazol-2-ylthio)ethyl)phenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide (6a):**
IR (KBr) (\(v_{\text{max}}, \text{cm}^{-1}\)): 3056 (C-H Aromatic), 3016 (C-H alkene), 1635 (C=C alkene), 1538 (C=C aromatic), 1668 (C=O amide), 1388 (C-N Aromatic); ESI-MS: m/z Calculated 474.62, found [M+H]+ 475.6. \(^1\)H NMR (400 MHz, DMSO) \(\delta\) 11.37 (s, 1H), 8.55 \(\sim\) 7.97 (m, 4H), 7.65 (dd, 4H), 7.56 \(\sim\) 7.36 (m, 4H), 4.28 (s, 2H), 2.62 (s, 3H); Anal. Calcd for C\(_{24}\)H\(_{18}\)N\(_4\)OS\(_2\): C, 60.73; H, 3.82; N, 11.80; O, 3.37; S, 20.27 %; found C, 60.95; H, 3.96; N, 11.56; S, 20.49 %.

**2-(benzo[d]thiazol-2-ylthio)-N-(4-(1-((6-methylbenzo[d]thiazol-2-ylimino)ethyl) phenyl)acetamide (6b):**
IR (KBr) (\(v_{\text{max}}, \text{cm}^{-1}\)): 3045 (C-H Aromatic), 3078 (C-H alkene), 1635 (C=C alkene), 1536 (C=C aromatic), 1645 (C=O amide), 1375 (C-N Aromatic); ESI-MS: m/z Calculated 488.08, found [M+H]+ 489.0; \(^1\)H NMR (400 MHz, DMSO) \(\delta\) 11.38 (s, 1H), 8.55 \(\sim\) 7.98 (m, 4H), 7.65 (dd, 4H), 7.56 \(\sim\) 7.42 (m, 2H), 7.33 (dd, 1H), 4.28 (s, 2H), 2.64 (s, 3H), 2.37 (s, 3H); Anal. Calcd for
C₂₅H₂₀N₄OS₃: C, 61.45; H, 4.13; N, 11.47; O, 3.27; S, 19.69 %; found C, 61.67; H, 4.35; N, 11.68; S, 19.85 %.

2-(benzo[d]thiazol-2-ylthio)-N-(4-(1-((6-fluorobenzo[d]thiazol-2-yl)imino)ethyl)phenyl)acetamide (6c):
IR (KBr) (νmax, cm⁻¹): 3045 (C=H Aromatic), 3043 (C=H alkene), 1665 (C=O amide), 1662 (C=O amide), 1336 (C-N Aromatic); ESI-MS: m/z Calculated 504.65, found [M+H]^+ 505.6; ^1H NMR (400 MHz, DMSO) δ 9.40 (s, 1H), 9.11 – 7.82 (m, 4H), 7.63 (d, 1H), 7.55 (d, 2H), 7.49 – 7.28 (m, 3H), 4.24 (s, 2H), 2.50 (s, 3H); Anal. Calcd for C₂₅H₁₇FN₄OS₃: C, 58.52; H, 3.48; F, 3.86; N, 11.37; O, 3.25; S, 19.53 %; found C, 58.75; H, 3.71; N, 11.65; S, 19.49 %.

2-(benzo[d]thiazol-2-ylthio)-N-(4-(1-((6-methoxybenzo[d]thiazol-2-yl)imino)ethyl)phenyl)acetamide (6d):
IR (KBr) (νmax, cm⁻¹): 3086 (C=H Aromatic), 3056 (C=H alkene), 1652 (C=O amide), 1575 (C=O amide), 1462 (C=O amide), 1385 (C-N Aromatic); ESI-MS: m/z Calculated 519.62, found [M+H]^+ 520.6; ^1H NMR (400 MHz, DMSO) δ 9.57 (s, 1H), 8.59 – 7.90 (m, 3H), 7.74 (d, 1H), 7.63 (d, 2H), 7.53 (d, 2H), 7.42 (m, 2H), 7.13 (q, 1H), 4.29 (s, 2H), 3.81 (s, 3H), 2.48 (s, 3H); Anal. Calcd for C₂₅H₂₀N₄OS₃: C, 59.50; H, 3.99; N, 11.10; O, 6.34; S, 19.06 %; found C, 59.75; H, 3.69; N, 11.26; S, 19.29 %.

2-(benzo[d]thiazol-2-ylthio)-N-(4-(1-((6-nitrobenzo[d]thiazol-2-yl)imino)ethyl)phenyl)acetamide (6e):
IR (KBr) (νmax, cm⁻¹): 3025 (C=H Aromatic), 3015 (C=H alkene), 1673 (C=O amide), 1586 (C=O amide), 1674 (C=O amide), 1385 (C-N Aromatic); ESI-MS: m/z Calculated 519.07, found [M+H]^+ 519.62; ^1H NMR (400 MHz, DMSO) δ 8.21 (d, 1H), 8.18 – 7.79 (m, 2H), 7.66 (d, 2H), 7.51 (d, 2H), 7.46 – 7.32 (m, 2H), 4.27 (s, 2H), 2.61 (s, 3H); Anal. Calcd for C₂₅H₁₇N₄O₃S₃: C, 55.47; H, 3.30; N, 13.48; O, 9.24; S, 18.51 %; found C, 55.79; H, 3.49; N, 13.69; S, 18.75 %.

2-(benzo[d]thiazol-2-ylthio)-N-(4-(1-((6-chlorobenzo[d]thiazol-2-yl)imino)ethyl)phenyl)phenylacetamide (6f):
IR (KBr) (νmax, cm⁻¹): 3045 (C=H Aromatic), 3056 (C=H alkene), 1628 (C=O amide), 1578 (C=O amide), 1685 (C=O amide), 1378 (C-N Aromatic); ESI-MS: m/z Calculated 509.07, found [M+H]^+ 510.0; ^1H NMR (400 MHz, DMSO) δ 8.21 (d, 1H), 8.14 – 7.73 (m, 3H), 7.73 – 7.61 (m, 3H), 7.53 – 7.38 (m, 5H), 4.19 (s, 2H), 2.60 (s, 3H); Anal. Calcd for C₂₅H₁₇ClN₄O₃S₃: C, 56.62; H, 3.37; Cl, 6.96; N, 11.01; O, 3.14; S, 18.90 %; found C, 56.85; H, 3.49; N, 11.26; S, 19.12 %.

2-(benzo[d]thiazol-2-ylthio)-N-(4-(1-((6-ethoxybenzothiazol-2-yl)imino)ethyl)phenyl)phenylacetamide (6g):
IR (KBr) (νmax, cm⁻¹): 3058 (C=H Aromatic), 3026 (C=H alkene), 1686(C=O amide), 1578 (C=O amide), 1678 (C=O amide), 1328 (C-N Aromatic); ESI-MS: m/z Calculated 518.67, found [M+H]^+ 519.62; ^1H NMR (400 MHz, DMSO) δ 8.21 (d, 1H), 8.30 – 7.93 (m, 2H), 7.89 (d, 1H), 7.69 (dd, 3H), 7.60 (d, 2H), 7.50 – 7.36 (m, 2H), 7.05 (dd, 1H), 4.27 (s, 2H), 4.03 (q, 2H), 2.51 (s, 3H), 1.39 (t, 3H); Anal. Calcd for C₂₆H₂₂N₄O₃S₃: C, 60.21; H, 4.45; N, 10.80; O, 6.17; S, 18.55 %; found C, 60.49; H, 4.45; N, 10.98; S, 18.79 %.

Determination of Antibacterial Activity
Antibacterial activities of 6a-6g were carried out in Nutrient-agar plates by well diffusion assay. Cultures were activated in Nutrient broth. Isolates were inoculated in Nutrient broth and incubated at 37 °C for 24 hours for activation of cultures and then centrifuged at 3000 rpm for 15 min and the supernatant was collected to study antibacterial activity.
Using in-vitro agar well diffusion method, antimicrobial activity experiments were carried out. The activity of 6a-6g against test microorganisms (1000 µl volume with 1000 µg/ml concentration) of activated test cultures 2 Gram negative and 2 Gram positive; viz. Enterobacter aerogenes MTCC No. 8558, Escherichia coli MTCC No. 1610, Micrococcus luteus MTCC No. 11948 and Bacillus cereus MTCC No. 8557) was inoculated in molten agar and poured into sterile plates than allowed to solidify. Wells with 5mm diameter were prepared at equal distance in solidified agar plates using cup-borer. Various derivatives 6a-6g with 1000 µg/ml concentration were inoculated in the wells of nutrient agar whereas test microorganisms were inoculated by pour plate technique. The plates were incubated at 37 °C for 24 hours. The inhibition zones were measured at the end of the incubation period.

3. RESULT AND DISCUSSION

Chemistry

The synthetic pathway for preparation of different derivative of N-(4-(1-(benzo[d]thiazol-2-ylimino)ethyl)phenyl)-2-(benzo[d]thiazol-2-ylthio) acetamide (6a-6g) is shown in Scheme 1. To explore the scope and limitations of this reaction further, we extended our studies to the use of various substituted 2-aminobenzothiazole and chloroacetylchloride in the presence of diethyl amine. It was gratifying to observe that most of the tested substrates exhibited satisfactory reactivity profiles, in all cases leading to a link sequence such as hydrazine hydrate and 2-aminobenzothiazole that readily afforded the target product (Table 1).

![Scheme 1 Synthesis of N-(4-(1-(benzo[d]thiazol-2-ylimino)ethyl)phenyl)-2-(benzo[d] thiazol-2-ylthio)acetamide derivatives (6a-6g)]
Table 1 Synthesis of N-(4-(1-(benzo[d]thiazol-2-ylimino)ethyl)phenyl)-2-(benzo[d]thiazol-2-ylthio)acetamide derivatives (6a-6g)

<table>
<thead>
<tr>
<th>Entry</th>
<th>2-amino benzothiazole derivative</th>
<th>Product</th>
<th>yield</th>
<th>mp °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="5a.png" alt="Image" /></td>
<td><img src="6a.png" alt="Image" /></td>
<td>89.7</td>
<td>162-166</td>
</tr>
<tr>
<td>2</td>
<td><img src="5b.png" alt="Image" /></td>
<td><img src="6b.png" alt="Image" /></td>
<td>86.5</td>
<td>169-171</td>
</tr>
<tr>
<td>3</td>
<td><img src="5c.png" alt="Image" /></td>
<td><img src="6c.png" alt="Image" /></td>
<td>84.5</td>
<td>166-167</td>
</tr>
<tr>
<td>4</td>
<td><img src="5d.png" alt="Image" /></td>
<td><img src="6d.png" alt="Image" /></td>
<td>90.1</td>
<td>165-169</td>
</tr>
<tr>
<td>5</td>
<td><img src="5e.png" alt="Image" /></td>
<td><img src="6e.png" alt="Image" /></td>
<td>87.4</td>
<td>168-170</td>
</tr>
</tbody>
</table>
A series of compound 6a–6g has been synthesized by conventional method as illustrated in scheme 1. The structures of all the newly synthesized compounds were confirmed by elemental analysis and FT-IR, $^1$H NMR, and Mass analysis. The IR spectra of compounds 6a shows six main peaks at 33056 (C-H Aromatic), 3016 (C-H alkene), 1635 (C=C alkene), 1538 (C=C aromatic), 1668 (C=O amide), 1388 (C-N Aromatic) respectively. In $^1$H NMR spectrum of 6a shows characteristic peaks at 11.37 $\delta$ value resulting from -NH of amide group. Aromatic proton of targeted compound resonates at 8.55 – 7.36 $\delta$ ppm. The mass spectrum of selected compound 6a showed [M+H]$^+$ peak at m/z 475.6. The appearance of a molecular ion peak at 384.8 mass unit supports the structure of compound 6a.

**Antibacterial Activity**

The antibacterial activity of the synthesized compound (6a–6g) was carried out on Nutrient-agar plates by well–diffusion assay against test culture. Cultures were triggered in Nutrient broth. Isolates inhibits the above mentioned organisms or not were studied and zone of inhibition was measured in terms of zone diameter and with the help of that zone index was calculated where streptomycin was used as standard.

**Determination of activity index**

The activity index of the probiotic culture was calculated as:

$$
\text{Activity index (A.I.)} = \frac{\text{Mean of zone of inhibition of derivative}}{\text{Zone of inhibition obtained for standard antibiotic drug}}
$$

*Note: Standard drug used was Streptomycin with 1000 µg/ml concentration*

The synthesized compounds 5a–5g were tested in vitro for antibacterial activity against Gram-positive Micrococcus luteus (MTCC No. 11948), Bacillus cereus (MTCC No. 8558) and Gram-negative Enterobacter aerogens (MTCC No. 8558), Escherichia coli (MTCC No. 1610) by measuring the zone of inhibition in mm. Antibacterial screening results (the zone of inhibition), presented in Table 2, revealed that all compounds tested showed some degree of antibacterial activity. The antibacterial activity of all compounds except 6c showed more than 83.3 % inhibition than the standard against Enterobacter aerogens bacteria. When compound 6b, 6c, 6d, 6f, and 6g also shows good activity to standard drug against Micrococcus luteus gram positive bacteria. Compounds 6c-6g more pronounced activity compared to streptomycin against Escherichia coli bacteria. Compound 6b, 6c and 6g have showed very close activity to standard drug against Bacillus cereus gram positive bacteria.
Table 2 Antibacterial Activity of synthesized compounds 6a-6g

<table>
<thead>
<tr>
<th>Derivatives</th>
<th>Enterobacter aerogens MTCC No. 8558</th>
<th>Escherichia coli MTCC No. 1610</th>
<th>Micrococcus luteus MTCC No. 11948</th>
<th>Bacillus cereus MTCC No. 8558</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean value for Zone of Inhibition (mm)</td>
<td>Activity Index (A.I.)</td>
<td>Mean value for Zone of Inhibition (mm)</td>
<td>Activity Index (A.I.)</td>
</tr>
<tr>
<td>5a</td>
<td>21</td>
<td>0.875</td>
<td>19</td>
<td>0.791</td>
</tr>
<tr>
<td>5b</td>
<td>23</td>
<td>0.958</td>
<td>17</td>
<td>0.708</td>
</tr>
<tr>
<td>5c</td>
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<td>0.791</td>
<td>21</td>
<td>0.875</td>
</tr>
<tr>
<td>5d</td>
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<td>20</td>
<td>0.833</td>
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<tr>
<td>5e</td>
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<td>24</td>
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</tr>
<tr>
<td>5f</td>
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<td>26</td>
<td>1.08</td>
</tr>
<tr>
<td>5g</td>
<td>24</td>
<td>1.000</td>
<td>23</td>
<td>0.958</td>
</tr>
<tr>
<td>Std drug</td>
<td>24</td>
<td>---</td>
<td>24</td>
<td>---</td>
</tr>
</tbody>
</table>

4. CONCLUSION
We described in this context a methodology for the synthesis of 2-Aminobenzothiazole containing novel Schiff bases derivatives. The pharmacological study was undertaken to evaluate the effects of the substituent on the In Vitro antibacterial activity against gram positive and gram negative bacteria. All Compound has shown good activity with respect to the standard drug. Compound 6b, 6c and 6g have showed very close activity to standard drug against Bacillus cereus gram positive bacteria. When compound 6b, 6c, 6d, 6f, and 6g also shows good activity to standard drug against Micrococcus luteus gram positive bacteria. The antibacterial activity of all compounds except 6c showed more than 83.3 % inhibition than the standard against Enterobacter aerogens bacteria. Compounds 6c-6g more pronounced activity compared to streptomycin against Escherichia coli bacteria.

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