SYNTHESIS OF SOME 1,3-BENZOTHIAZOL-2-YL HYDRAZONE DERIVATIVES AS ANTIMICROBIAL AGENTS

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ABSTRACT
A series of 1,3-benzothiazol-2-yl hydrazones were synthesized(IIIa-IIIe) and evaluated for antibacterial activity against two different bacterial species and antifungal activity against one fungal species by disk diffusion method displaying different degree of antimicrobial activity. All the synthesized compounds were in good agreement with spectral data (FTIR, 1H-NMR and mass spectroscopy). In-vitro antibacterial activity was evaluated against two pathogenic bacterial strains, Staphylococcus aureus and Escherichia coli and one fungal strain Aspergillus niger. Synthesized compound IIIa has shown significant antibacterial and antifungal activity compared with standard ciprofloxacin and miconazole at 200µg/ml and 500µg/ml respectively.

KEYWORDS: Antimicrobial, Antibacterial, Antifungal, 1,3-benzothiazol-2-yl hydrazine.

INTRODUCTION
Benzothiazole is a heterocyclic compound, weak base, having varied biological activities and still of great scientific interest now a days.[1] Benzothiazoles are fused membered rings, which contain the heterocycles bearing thiazole. Sulphur and nitrogen atoms constitute the core structure of thiazole and many pharmacologically and biologically active compounds.
Thiazole (1) is structurally related to thiophene and pyridine, but in most of its properties it resembles to the latter. Thiazole was first described by Hantzsch and Waber in 1887. Popp confirmed its structure in 1889. The numbering in thiazole starts from the sulphur atom. Structure (2) is benzothiazole. The basic structure of benzothiazole consist of benzene ring fused with 4, 5 position of thiazole. The two rings together constitute the basic nucleus 1, 3 benzothiazole.\(^2\)

Biologist’s attention was drawn to this series when pharmacological profile of Riluzole (6-trifluoormethoxy-2-benzothiazolamines, Rilutek), as a Glutamate neurotransmission inhibitor was discovered. After that benzothiazole derivatives have been extensively studied and found to have diverse chemical reactivity and broad spectrum of activity.\(^3\)

The benzothiazole ring is present in various marine or terrestrial natural compounds. They show potent and selective biological activities.\(^4\)

Benzothiazole moites are part of compounds showing numerous biological activities such as antimicrobial,\(^5\)\(^,\)\(^9\) anti-inflammatory,\(^10\)\(^,\)\(^13\) anticancer,\(^14\)\(^,\)\(^15\) anthelmintic,\(^16\) anti-diabetic,\(^17\) anticonvulsant,\(^18\) schistosomicidal,\(^19\) diuretic,\(^20\) activity.

**Experimental**

NMR spectra were recorded with AVANCE-300, (300MHz FT NMR). Mass spectral analysis was carried out with a Micromass Quatto II triple quadrapole Mass Spectrometer. Infrared spectra were obtained with a FT-IR – 84. Melting points were determined using a DBK Programmed Melting Point Apparatus and are uncorrected. UV-Vis spectral analysis was carried out with a U.V. 2401 PC.

**MATERIALS**

p-Chloro aniline and substituted benaldehyde were purchased from LOBA Chemie Pvt. Ltd., Mumbai, India.
Scheme for synthesis of compounds IIIa-e

General Procedure for Synthesis of Compound IIIa-e

**Step-1: Synthesis of 6-Chloro-1,3-benzothiazol-2-amine (I)**

p-Chloro aniline (0.01 mol) and potassium thiocyanate (0.08 mol) were dissolved in glacial acetic acid (20 ml), cooled and stirred for 15 min at 2-4 °C. Cold bromine solution (0.01 mol, 1.6 ml in 6 ml acetic acid) was added dropwise. Stirring was continued for 2 h and then at room temperature for 10 h. The separated product was filtered off, washed with acetic acid, dissolved in hot water and neutralized with aqueous ammonia solution (25%). The resulting precipitate was filtered off, washed with water, dried and recrystallized from ethanol.
Step II: Synthesis of 1-(6-chloro-1,3-benzothiazol-2-yl) hydrazine (II)\textsuperscript{[22]}

The compound 6-chloro-1,3-benzothiazol-2-amine (I) (0.075 mol) was dissolved in mixture of 21 ml of conc. HCl and an equal volume of water and cooled rapidly to 0\textdegree C. A solution of sodium nitrite in 12 ml water was added gradually. Stirring was continued for few min, the solution was filtered rapidly and added dropwise with stirring to an ice cold solution of sodium sulphite (0.156 mol) in 100 ml of water containing 4g of sodium hydroxide. The solution was allowed to stand for 5 min, acidified with conc. HCl and heated on water bath for 3 min, then yellow crystals was separated. It was allowed to stand overnight, filtered, heated with conc. HCl on water bath for 7 min and allowed to cool. The precipitate was filtered off, dissolved in water and treated with concentrated solution of sodium acetate. The resulting precipitate was filtered off, dried and recrystallized from ethanol.

Step III: Synthesis of 2-(4-substituted benzyl)-1-(6-chloro-1,3-benzothiazol-2-yl) hydrazone (III\textsubscript{a-e})\textsuperscript{[23]}

To solution of compound II (0.005 mol) and substituted benzaldehyde (0.01 mol) in ethanol, 2-3 drops of concentrated sulphuric acid were added. The mixture was refluxed on a water bath for 3-4 h, till the completion of the reaction (monitered by TLC). The reaction mixture was cooled and poured onto crushed ice to obtained a solid, which was dried and crystallised by ethanol.

Physicochemical data of synthesized compounds III\textsubscript{a-e} are shown in Table 1.

### Table 1: Physicochemical data of synthesized compounds (III\textsubscript{a-e})

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Colour</th>
<th>Melting point (\textdegree C)</th>
<th>Yield (%)</th>
<th>Molecular Formula (Mol. Wt.)</th>
<th>Rf value</th>
</tr>
</thead>
<tbody>
<tr>
<td>III\textsubscript{a}</td>
<td>Yellow</td>
<td>180-182</td>
<td>75.66</td>
<td>C\textsubscript{14}H\textsubscript{9}ClN\textsubscript{4}O\textsubscript{2}S (332.76)</td>
<td>0.66</td>
</tr>
<tr>
<td>III\textsubscript{b}</td>
<td>Brown</td>
<td>156-158</td>
<td>60.32</td>
<td>C\textsubscript{14}H\textsubscript{10}ClN\textsubscript{3}OS (303.18)</td>
<td>0.58</td>
</tr>
<tr>
<td>III\textsubscript{c}</td>
<td>Orange</td>
<td>172-174</td>
<td>90.57</td>
<td>C\textsubscript{16}H\textsubscript{15}ClN\textsubscript{4}S (330.84)</td>
<td>0.63</td>
</tr>
<tr>
<td>III\textsubscript{d}</td>
<td>Brown</td>
<td>276-278</td>
<td>71.46</td>
<td>C\textsubscript{14}H\textsubscript{9}Cl\textsubscript{2}N\textsubscript{3}S (322.21)</td>
<td>0.59</td>
</tr>
<tr>
<td>III\textsubscript{e}</td>
<td>Brown</td>
<td>138-140</td>
<td>83.72</td>
<td>C\textsubscript{14}H\textsubscript{9}ClBrN\textsubscript{3}S (366.66)</td>
<td>0.68</td>
</tr>
</tbody>
</table>

Antimicrobial Activity

The antibacterial activity of synthesized compounds was studied against E. coli and S. aureus and antifungal activity was studied against A. niger. Ciprofloxacin and Miconazole were used
as standard for antibacterial and antifungal activity respectively. The agar dilution method was performed using Muller-Hinton agar (Hi-Media) medium for antibacterial activity and Sabouraud’s dextrose agar (Hi-Media) medium for antifungal activity. This method depends on the diffusion of drug from bore through the solidified agar layer of petri dish to an extent such that growth of the inoculated microorganism is prevented entirely in a circular area “zone” around the cup containing the solution of a compound under test.

The medium was sterilized by autoclaving at 12-15 lb pressure for 30 min. One loopful of the stock culture was inoculated at 10 ml of agar slant previously in sterilized test tubes and incubated at 37 °C for 24 and 72 h respectively for bacteria and fungi. About 3 ml of distilled water was added to the test tube and a suspension of the culture was obtained by shaking for few minutes.

**Determination of the In Vitro Anti-Microbial Activity by the Disk Diffusion Method**

All the operations were carried out under aseptic conditions. Sterile medium was melted on water bath and kept at 45 °C in constant temperature water bath and subcultured organism under study was inoculated. In each sterile petri dish molten medium was added so that thickness was approximately 8-10 mm. The inoculated dishes were allowed to set for 30 min at room temperature. Cups of 6 mm diameter were then made with the help of sterile stainless steel bore, 1ml of sample solution was added to each cup. Petri dishes were kept in refrigerator for 30 minutes so as to allow diffusion of the solutions in the medium, and then incubated at 37 °C for 24 hrs for antibacterial activity and 72 h for antifungal activity. The results are shown in Tables 2 and 3.

**RESULTS AND DISCUSSION**

**Table 2: Antibacterial activity data for compounds IIIa-e and Ciprofloxacin against S. aureus and E.coli**

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Bacteria along with zone of inhibition (mm)</th>
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<tbody>
<tr>
<td></td>
<td>S. aureus</td>
</tr>
<tr>
<td></td>
<td>50 µg/ml</td>
</tr>
<tr>
<td>IIIa</td>
<td>-</td>
</tr>
<tr>
<td>IIIb</td>
<td>-</td>
</tr>
<tr>
<td>IIIc</td>
<td>-</td>
</tr>
<tr>
<td>IIIId</td>
<td>-</td>
</tr>
<tr>
<td>IIIe</td>
<td>-</td>
</tr>
<tr>
<td>Std. Ciprofloxacin</td>
<td>18</td>
</tr>
</tbody>
</table>
Table 3: Antifungal activity data for compounds IIIa-e and Miconazole against A. niger

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Fungi along with zone of inhibition (mm)</th>
<th>A. niger</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>50 μg/ml</td>
</tr>
<tr>
<td>IIIa</td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>IIIb</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIIc</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIId</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>IIIe</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Std. Miconazole</td>
<td></td>
<td>13</td>
</tr>
</tbody>
</table>

In present investigation, cyclisation of aromatic amine in presence of KSCN with acetic acid gave the compound 6-chloro-1,3-benzothiazol-2-amine (I). The compound I was treated with NaNO₂ and Na₂SO₃ in concentrated HCl to give 1-(6-chloro-1,3-benzothiazol-2-yl) hydrazine (II). The compound II was treated with aromatic aldehyde in presence of ethanol to get 2-(4-substituted benzyl)-1-(6-chloro-1,3-benzothiazol-2-yl) hydrazone (IIIa-e). The synthesis procedure is given in scheme. The compound I display characteristic absorption bands in IR spectrum at 1631.78 cm⁻¹ due to C=N and 3456.44-3269.34 cm⁻¹ due to -NH₂ group. The compound II display characteristic absorption bands at 3197.96 cm⁻¹ due to -NH group and 3319.49-3120.82 cm⁻¹ due to -NH₂ group. The compounds IIIa-e were confirmed by physicochemical and spectral analysis (UV, IR, mass, NMR).

(IIIa) 2-(4-Nitrobenzyl)-1-(6-chloro-1,3-benzothiazol-2-yl) hydrazone
Yield 75.66%, yellow, molecular weight 332.76 cm⁻¹, melting point 180-182 °C, IR (KBr) 3380.70 cm⁻¹ (N-H stretching), 3105.18 cm⁻¹ (Aromatic C-H stretching), 2916.17 cm⁻¹, 2848.67 cm⁻¹ (Aliphatic C-H stretching), 1606.59 cm⁻¹ (C=N stretching), 738.69 cm⁻¹ (C-Cl stretching), Rf value 0.66 (benzene:acetone, 8:2), m/z, 332.22 (M⁺), ¹H NMR (DMSO), δ ppm: 8.4151-8.3987 m(Ar-H), 8.0929 s(CH=N), 4.0635 s(NHN-), UV λ max-264.80 nm.

(IIIb) 2-(4-Hydroxybenzyl)-1-(6-chloro-1,3-benzothiazol-2-yl) hydrazone
Yield 60.32%, brown, molecular weight 303.77, melting point 156-158 °C, IR (KBr) 3355.91 cm⁻¹ (N-H stretching), 3527.56-3263.33 cm⁻¹ (O-H stretching), 3095.54 cm⁻¹ (Aromatic C-H stretching), 2918.10 cm⁻¹, 2850.59 cm⁻¹ (Aliphatic C-H stretching), 1589.23 cm⁻¹ (C=N stretching), 811.98 cm⁻¹ (C-Cl stretching), Rf value 0.58 (benzene:acetone, 9:1), m/z, 303.32
(M\(^+\)), \(^1\)H NMR (DMSO), δ ppm: 8.1726-8.1223, m(Ar-H), 8.1021 s(CH=N), 5.0391 s(OH), 4.0370 s(NHN-), UV λ max-262.00 nm.

(IIIc) 2-(4-Dimethylaminobenzyl)-1-(6-chloro-1,3-benzothiazol-2-yl) hydrazone
Yield 90.57%, orange, molecular weight 330.84, melting point 172-174 ºC, IR (KBr) 3436.91 cm\(^{-1}\), 3373.27 cm\(^{-1}\) (N-H stretching), 3078.18 cm\(^{-1}\) (Aromatic C-H stretching), 2916.17 cm\(^{-1}\), 2848.67 cm\(^{-1}\) (Aliphatic C-H stretching), 1595.02 cm\(^{-1}\) (C=N stretching), 765.69 cm\(^{-1}\) (C-Cl stretching). Rf value 0.63 (chloroform:methanol, 8.5:1.5), m/z, 330.22 (M\(^+\)), \(^1\)H NMR (DMSO), δ ppm: 8.1250 s(CH=N), 7.7481-6.7070 m(Ar-H), 4.0370 s(NHN-), 2.8594 s(CH\(_3\))

(IIId) 2-(4-Chlorobenzyl)-1-(6-chloro-1,3-benzothiazol-2-yl) hydrazone
Yield 71.46 %, brown, molecular weight 322.21, melting point 276-278 ºC, IR (KBr) 3309.62 cm\(^{-1}\) (N-H stretching), 3063.68 cm\(^{-1}\) (Aromatic C-H stretching), 2904.60 cm\(^{-1}\), 2821.66 cm\(^{-1}\) (Aliphatic C-H stretching), 1591.16 cm\(^{-1}\) (C=N stretching), 727.11 cm\(^{-1}\) (C-Cl stretching), Rf value 0.59 (benzene:acetone, 8:2); m/z, 322.22 (M\(^+\)), \(^1\)H NMR (DMSO), δ ppm: 8.1301 s(CH=N), 7.7526-7.3070 m(Ar-H), 4.0370 s(NHN-), UV λ max-339.00 nm.

(IIIe) 2-(4-Bromobenzyl)-1-(6-chloro-1,3-benzothiazol-2-yl) hydrazone
Yield 83.72 %, brown, molecular weight 366.66, melting point 138-140 ºC, IR (KBr) 3384.19 cm\(^{-1}\) (N-H stretching), 3083.96 cm\(^{-1}\) (Aromatic C-H stretching), 2925.81 cm\(^{-1}\), 2858.31 cm\(^{-1}\) (Aliphatic C-H stretching), 1587.31 cm\(^{-1}\) (C=N stretching), 799.19 cm\(^{-1}\) (C-Cl stretching), Rf value 0.68 (chloroform:methanol, 9.5:0.5), m/z, 366.70 (M\(^+\)); \(^1\)H NMR (DMSO), δ ppm: 8.1014 s(CH=N), 7.6059-7.4127 m(Ar-H), 4.0448 s(NHN-), UV λ max-246.60.

ANTIMICROBIAL ACTIVITY
In vitro antimicrobial activity of synthesized compounds was studied by cup plate method using Ciprofloxacin and Miconazole as standard for antibacterial and antifungal activity respectively. The synthesized compounds were evaluated for their anti-bacterial activity against S. aureus and E. coli and antifungal activity against A. niger. The activity of synthesized compounds is reported by measuring zone of inhibition (in mm). The results showed that synthesized compounds IIIb, IIId and IIIe exhibited poor antibacterial activity against all the tested strains at the concentration of 500µg/ml when compared with
ciprofloxacin. Compound IIIa and IIIc at the concentration of 200 µg/ml and 500 µg/ml has shown significant activity against all strains of bacteria as compared with standard.

The result of antifungal activity showed that compound IIIa is effective against A. niger at the concentration of 50 µg/ml. Other compounds were found to be ineffective at the concentration of 50µg/ml against tested strain of fungi.

CONCLUSION
The compounds were prepared as per reported procedure in literature with a good yield. The physicochemical characteristic like melting point, % yield, Rf value was noted and data is given in Table 1. The spectral analysis has been done to ensure the formation of the compound and confirmation of the compounds synthesised.

The in-vitro antimicrobial activity was also carried out by using ciprofloxacin and miconazole as standard for antibacterial and antifungal activity respectively. Compound IIIa and IIIc has shown significant activity against Gram- positive and Gram-negative bacteria. Compound IIIa has also shown significant antifungal activity against A. niger. Thus all the synthesized compounds has shown significant antifungal activity hence further work is required to modify the compounds to give better activity.

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