ANTI-OXIDANT AND ANTI-MICROBIAL ACTIVITIES OF PYRAZOLYL-BENZOTHIAZOLE DERIVATIVES USING VILSMEIER-HAACK REACTION

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ABSTRACT
Vilsmeier –Haack reaction provide a route involve formylation of activated aromatic and hetero-aromatic systems by ring formation. Recently pyrazole have been synthesized from various substituted hydrazones using Vilsmeier-Haack reaction. Our interest in synthesizing pyrazolyl benzothiazole from 2-amino benzothiazole which was reflux with hydrazine hydrate followed by condensation with substituted acetophenone to form hydrazones derivatives. Their structures were confirmed by IR, 1H NMR spectral data. The synthesized compounds were investigated for anti-oxidant and antimicrobial activities.

Keywords: Antibacterial activity, Anti-Oxidant, Benzothiazole, Pyrazole.

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INTRODUCTION
The development of simple synthetic routes to widely used organic compounds using readily available reagents is one of the main objectives of organic synthesis. Nitrogen heterocycles are of a special interest because they constitute an important class of natural and non-natural products, many of which exhibit useful biological activities.

Benzothiazole are an extremely important class of compounds that occur widely as biologically active natural products, as well as marketed drugs or drug candidates1. Accordingly, the development of efficient and general synthetic methodology for Benzothiazole is a meaningful research challenge having great potential for practical applications in the pharmaceutical industry. Benzothiazole have two heteroatom attached at the ortho position on the benzene ring.

The utility of Vilsmeier Haack Reaction2 as key intermediates in the synthesis of several series of heterocyclic compounds i.e. pyrazole and the broad spectrum of biological activities that have been reported for their cyclized products.

Pyrazole derivatives represent important building blocks in organic and medicinal chemistry. Pyrazole have remarkable pharmacological activities as antibacterial, antifungal, and hypoglycemic3.

EXPERIMENTAL
All the solvents used in the experimental work were redistilled and dried before use. Melting point of the synthesized compounds was recorded on Thermonik melting point apparatus (Campbell electronics) and is uncorrected. Thin Layer Chromatography (TLC) was performed on silica gel coated plates using Iodine
Scheme-1

\[
\begin{align*}
&\text{R} &\text{S} &\text{N} &\text{NHN} &\text{H}_2 &\text{2} \\
&\downarrow &\text{NH}_2 \text{NH}_2 \text{H}_2 \text{O} &\downarrow &\text{Gly acetic acid} &\downarrow &\text{Vilsmeier Haack Reaction} &\downarrow &\text{DMF/POCl}_3 \\
&\text{R} &\text{S} &\text{NHN} &\text{H}_2 &\text{NHN=C} &\text{CH}_3 &\text{R'} &\text{CHO} &\text{R'} \\
&\downarrow &\downarrow &\downarrow &\downarrow &\downarrow &\downarrow &\downarrow &\downarrow &\downarrow \\
&\text{R} &\text{S} &\text{NHN=C} &\text{CH}_3 &\text{R'} &\text{CHO} &\text{R'} &\text{R'} \\
\end{align*}
\]
vapor as visualizing agents. Infra-red spectra were recorded on Hartmann-Braun, MB series (Bomem, Quebec, Canada). ¹H NMR spectra were recorded on 300 MHz instrument (Jeol Ltd, Tokyo, Japan), using DMSO solvent.

**Synthesis of 2-hydrazino benzothiazole**

Conc. HCl (6 mL) was added drop wise with stirring to hydrazine hydrate (6 mL) at 5-10°C temp. To it ethylene glycol (24 mL) and 2-Amino Benzothiazole 4.5 gm, (0.03 M) .The mixture was refluxed for 3 hrs. Till the solid crystals appears. Cool, washed with water and recrystallized from ethanol.

IR (KBr): 3321, 3202, 1651, 1460 (C=N), 1195, 1159, 816.

¹H NMR (DMSO): δ 5.04, (2H, s, NH₂), δ 7.43 (1H, t, Ar-H), δ 7.60 (1H, q, Ar-H), δ 7.72 (1H, d, Ar-H), δ 9.34 (1H, s, NH);

**Elemental Analysis**: C₁₉H₁₄N₄O₃S₂; *Found*: C (%)-54.11, H (%)-3.99, N (%)-12.71

**Synthesis of Benzothiazol-2-yl hydrazone**

2-hydrazino benzothiazole 1.65 gm (1.5 m mol), appropriate substituted acetophenone (2.2 m mol) and glacial acetic acid (2-3 drops)were taken in absolute ethanol (20 mL).This mixture was refluxed on water bath for 5-10 hrs (till different spot on TLC may appear).On cooling solid separates out which was filtered, washed with water and recrystallized with ethanol.

IR (KBr): 3148, 3049, 2908, 1568, 1459 (NHNH=C), 1341, 1126, 918, 937.

¹H NMR (DMSO): δ 2.45 (3H, s, CH₃), δ 7.22 (1H, m, Ar-H), δ 7.47 (1H, d, Ar-H), δ 7.76 (1H, m, Ar-H), δ 11.63 (1H, s, NH);

**Elemental Analysis**:

C₁₅H₁₁Cl₂N₃S; *Found*: C (%)-51.23, H (%)-4.10, N (%)-13.01

**Table-1: Anti-bacterial and anti-oxidant assay of compounds**

<table>
<thead>
<tr>
<th>Comp</th>
<th>Substituent</th>
<th>S.aureus (µg/mL)</th>
<th>E.coli (µg/mL)</th>
<th>Antioxidant assay (IC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>4-fluoro</td>
<td>200</td>
<td>50</td>
<td>18 µg/mL</td>
</tr>
<tr>
<td>2b</td>
<td>4-Nitro</td>
<td>100</td>
<td>50</td>
<td>95 µg/mL</td>
</tr>
<tr>
<td>3c</td>
<td>2-Methoxy</td>
<td>200</td>
<td>200</td>
<td>28 µg/mL</td>
</tr>
<tr>
<td>4d</td>
<td>4-amino</td>
<td>NA</td>
<td>NA</td>
<td>78 µg/mL</td>
</tr>
<tr>
<td>5e</td>
<td>2, 4, 6-Trimethoxy</td>
<td>200</td>
<td>200</td>
<td>17 µg/mL</td>
</tr>
<tr>
<td>6f</td>
<td>2-hydroxy</td>
<td>NA</td>
<td>NA</td>
<td>17 µg/mL</td>
</tr>
<tr>
<td>7g</td>
<td>3,4-Dichloro</td>
<td>200</td>
<td>100</td>
<td>92 µg/mL</td>
</tr>
<tr>
<td>8h</td>
<td>4-chloro</td>
<td>100</td>
<td>100</td>
<td>35 µg/mL</td>
</tr>
<tr>
<td>9i</td>
<td>2, 4, 6-Trihydroxy</td>
<td>200</td>
<td>100</td>
<td>09 µg/mL</td>
</tr>
<tr>
<td>10j</td>
<td>2, 6-dihydroxy</td>
<td>100</td>
<td>200</td>
<td>89µg/mL</td>
</tr>
</tbody>
</table>

Ampicillin (MIC-0.04 µg/mL) used as standard against *S. aureus*; Trimethoprim (MIC 0.01 µg/mL) used as standard against *S. typhi*; Miconazole (MIC 6.25 µg/mL) as standard against *C. albicans* and *A. niger*; N.A.: Not active at 200 µg/mL.

**Synthesis of Formylated Pyrazolyl Benzothiazole**

Conventional Method

Substituted Benzothiazol-2-yl hydrazone 1.68 mL, (0 .005 M) was dissolved in 6 mL of DMF and this kept in ice cold condition. To this Vilsmeier Haack reagent [(1.5 mL) of POCl₃ was added drop by drop with stirring in (6 mL ) of DMF is added with stirring at room temp. for 4 hrs. then content was poured into crushed ice (previously neutralized with NaHCO₃ or liq.NH₃) solid separates out which was filter washed with water, dried and recrystallized from ethanol.

IR (KBr): 3131, 3062, 2923 (CHO), 2360, 2342, 1695, 1610, 1548, 1463(C=N),1356, 1207, 898.

¹H NMR (DMSO): δ7.49, (1H, m, Ar-H), δ7.63 (3H, s, Ar-H), δ7.63 (2H, s, Ar-H), δ9.75 (1H, s, CH=CH), 10.14 (1H, s, CHO); **Elemental Analysis**: C₁₅H₁₁Cl₂N₃S; *Found* C (%)=52.69, H (%)=3.85, N (%)=11.89

**Biological Activity of Tested Compounds**

**Antimicrobial activity**

All the synthesized compounds were screened for their *in vitro* anti-bacterial activity by tube dilution technique against *Staphylococcus aureus* (NCTC 6538) and *Escherichia coli* (ATCC10148). The
culture medium used in the screening was Muller-Hinton broth. Ampicillin and Trimethoprim were used as a standard for comparison of antibacterial activity. All the compounds were screened for antimicrobial activity at range of 10-200 µg/mL concentration against the following bacterial strains: *Staphylococcus aureus* and *Escherichia coli*. DMSO used as solvent for anti-bacterial activities. The results are presented in table 1.

**The antioxidant activity**

The antioxidant activity of the compounds and the standard was assessed on the basis of the radical Scavenging effect of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical activity. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 10-100 µg/mL solution. 0.002% of DPPH was prepared in methanol and 1 mL of this solution was mixed with 1 mL of sample solution and standard solution separately. This solution mixture were kept in dark for 30 min and optical density was measured at 517 nm using Spectrophotometer. Methanol (1 mL) with DPPH solution (0.002%, 1 mL) was used as blank. The optical density was recorded and % inhibition was Calculated using the formula given below -

\[
\text{Percent (\% inhibition of DPPH activity) = } \frac{(\text{Absorbance of control} - \text{Absorbance of test Sample})}{(\text{Absorbance Of control})} \times 100
\]

**RESULTS AND DISCUSSION**

The results of the *in vitro* anti-microbial activity are shown in Table 1. Most of the compounds showed activity against *S. aureus* and *E. coli* at conc. greater than 200 µg/mL. The active compounds were found to be Compound 1a and 2b against *E. coli*. This compound compared to the tested reference Gentamycine. The rest of the compounds showed varying degree of activity against test organisms.

Table 1 represents the radical scavenging activity of the compound. The antioxidant activities of compounds were tested by measuring their capacity to scavenge DPPH radical. The most active compounds were proved to be 9i shows good anti oxidant activity. Compounds 1a, 5e, 6f and 9i shows IC\(_{50}\) value 18% µg/mL, 17% µg/mL and 9% µg/mL respectively. Among them 9i was most active compound shows IC\(_{50}\) value 9% µg/mL. The rest of the compounds possessed moderate antioxidant activity.

**CONCLUSION**

The tested samples reduced the stable radical DPPH to the yellow-colored phenylpicryl hydrazine. In this work, we found that all tested materials were able to quench this radical in a concentration-dependent manner.

- Compounds (1a, 5e, 6f & 9i) showed strong radical scavenging activity in DPPH assay. (DPPH IC\(_{50}\) ≤ 20 µg/mL)
- Compounds (3c, 8h) showed moderate radical scavenging activity in DPPH assay. (DPPH IC\(_{50}\) ≤ 50 µg/mL)
- Compounds (2b, 7g & 10j) showed strong mild scavenging activity in DPPH assay. (DPPH IC\(_{50}\) ≤ 100 µg/mL)

In the DPPH assay compound with Trihydroxy substituent was found to be most potent with IC\(_{50}\) 09% µg/mL.

**REFERENCES**


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