SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF N-MANNICH BASES OF 5-AMINO-4-[2-(6-BROMO-1, 3-BENZOTHIAZOL-2-YL) HYDRAZINYLIDENE]-2, 4-DIHYDRO-3H-PYRAZOL-3-ONE

Dissertation Submitted to
The Tamil Nadu Dr. M.G.R Medical University, Chennai
In partial fulfillment for the requirement of the Degree of

MASTER OF PHARMACY
(Pharmaceutical Chemistry)

April - 2012

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
KMCH COLLEGE OF PHARMACY,
KOVAI ESTATE, KALAPATTI ROAD,
COIMBATORE 641-048.
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MASTER OF PHARMACY
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Submitted by
S.BUGGA REDDY

Under the guidance of
Mr. K.K. SIVAKUMAR, M. Pharm., (Ph.D),
Assistant Professor,
Department of Pharmaceutical Chemistry
April-2012

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY,
KMCH COLLEGE OF PHARMACY
KOVAI ESTATE, KALAPATTI ROAD,
COIMBATORE 641-048
SCREENING FOR ANTI-MICROBIAL ACTIVITY

PRILIMINARY ANTI-MICROBIAL STUDIES

Methods

Procedure employed in anti-microbial assay may be divided into broad classification

Disc diffusion method

Minimum inhibitory concentration method

DISC DIFFUSION METHOD

In this method, the drug activity is based on measurement of the diameter of zone of inhibition surrounding the discs which are placed on the surface of a nutrient medium previously inoculated with a culture of suitable microorganisms. Inhibition produced by the test compound is compared with that of known standard.

MINIMUM INHIBITORY CONCENTRATION METHOD

Minimum inhibitory concentration (MIC) is the lowest concentration of an anti-microbial that will inhibit the visible growth of a microorganism after overnight incubation. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an antimicrobial agent and also to monitor the activity of new antimicrobial agents. A lower MIC is an indication of a better anti-microbial agent.

ANTIBACTERIAL SCREENING BY DISC DIFFUSION METHOD

Preparation of inoculum

The inoculum for the experiment was prepared fresh in Muller Hinton broth. Muller Hinton broth was prepared by dissolving 0.21gms of muller hinton broth in 10ml of distilled water and kept for sterilisation in auto clave for 30 minutes and afterwards the broth and culture was transferred to Laminar Air Flow bench. The sterile inoculation loop was taken and one time dipped in the bacterial culture and then it was dipped in the Muller Hinton broth it was kept in the B.O.D Incubator at 37°C for 24hrs.
Preparation of sample disc

Sample solutions of 100 µg/ml concentration were prepared by dissolving the sample in DMSO solvent and the sterile discs were dipped into the sample solution for 20 minutes.

List of bacterial strains used

Six gram-positive bacterial strains, Micrococcus luteus NCIM (National Collection of Industrial Microorganisms) 2169, staphylococcus aureus NCIM 2079, Bacillus subtilis NCIM 2063, Corynbacterium NCIM 2640, Bacillus lintus NCIM 2018, Staphylococcus albus NCIM 2178, and six gram-negative bacteria strain, Escherichia Coli NCIM 2065, Pseudomonas aruginosa NCIM 2018, Rhodosporum ruberum NCIM 5128, Vibrio cholera NCIM 1738, Salmonella Paratyphii NCIM 2075, Klbsellia pneumonia NCIM 2957 were grown in Pharmaceutical Biotechnology Laboratory, KMCH College of pharmacy, Coimbatore. For the study, fresh 24hr broth cultures were used.

ANTI-BACTERIAL SCREENING BY

Kirby-Bauer Method

38 gms of Muller Hinton agar was dissolved in 1000ml distilled water and 2gms of agar was added and kept for sterilisation in auto clave. The Petri plates were cleaned, sterilised and marked. Both media and plates were transferred to Laminar Air Flow bench and the medium was poured uniformly into the plates while in hot condition and allowed to solidify. The bacteria were streaked onto the medium by dipping the sterile swab in the inoculum by removing the excess of inoculum by pressing and rotating the swab firmly against the sides of the culture tube above the level of liquid. The sterile disc containing the drugs, standard were placed on the previously inoculated surface of the media and kept in the B.O.D Incubator for 37°C for 24hrs.

Observations were made for zone inhibition around the tested drugs (100µg/disc) and compared with that of standard (Ciprofloxacin 5µg/disc). All the synthesised compounds were tested against six Gram-positive and six Gram-negative bacteria and their results were given in the table no: 6.1 & 6.2.
ANTIFUNGAL SCREENING BY DISC DIFFUSION METHOD

Preparation of inoculums

Preparation of inoculums of fungus was carried out by Sabourand’s Dextrose Broth and transferred to test tube and kept it for sterilization in autoclave at 120°C for 15 min. Then added culture of each fungal to each tube (this step was carried out in aseptic room near laminar air flow) then kept it for incubation in incubator for 24-48 h at 29°C.

Preparation of sample disc

Sample solutions of 100 µg/ml concentration were prepared by dissolving the sample in DMSO solvent and the sterile discs were dipped into the sample solution for 20 minutes.

List of fungal strains used

Six fungi Candida albicans NCIM 3100, Monococcus purpureus MTCC 1090, Aspergillus niger MTCC 1344, Trichophyton rubrum MTCC 3272, Aspergillus fumigates MTCC 1811, Aspergillus parasites MTCC 2796 were grown in Pharmaceutical Biotechnology Laboratory, KMCH College of pharmacy, Coimbatore. For the study, fresh 24hr broth cultures were used.

ANTI FUNGAL SCREENING By

Kirby-Bauer Method

Sabourand’s Dextrose agar medium was prepared by dissolving 1 gm of peptone and 4 gm of dextrose in 100 ml of distilled water and agar-agar 1-2 gm for solubilisation, then kept it for sterilization in autoclave for 121°C for 15 min. The Petri plates were cleaned, sterilized and marked. These medium (Sabourand’s Dextrose agar) were poured into petri-plates under aseptic conditions and allowed to solidify. Standardized fungal inoculum was spread uniformly over the surface of medium by using a sterile non-absorbent cotton swab and finally the swab was passed around the edge of the medium. The inoculated petri plates were closed with the lid and allowed to dry at room temperature. The sample impregnated discs and standard discs were placed on the inoculated agar medium. All petriplates were incubated at 29°C for 24 - 48 hours. After the incubation, diameter of zone of inhibition produced by the sample and standard was measured. The details are tabulated in table no: 6.3.
DISC DIFFUSION METHOD

Micro organism : *Staphylococcus aureus* (Gram positive)
Concentrations of Synthesized compounds : 100µg/disc
Standard disc : Ciprofloxacin 5µg/disc

Fig: 6.1 Activity of compounds against *Staphylococcus aureus*
**DISC DIFFUSION METHOD**

<table>
<thead>
<tr>
<th>Micro organism</th>
<th>Escherichia Coli (Gram negative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations of Synthesized compounds</td>
<td>100µg/disc</td>
</tr>
<tr>
<td>Standard disc</td>
<td>Ciprofloxacin 5µg/disc</td>
</tr>
</tbody>
</table>

**Fig: 6.2** Activity of compounds against *Escherichia Coli*
**DISC DIFFUSION METHOD**

<table>
<thead>
<tr>
<th>Micro organism</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentrations of synthesized compounds</td>
<td>100µg/disc</td>
</tr>
<tr>
<td>Standard disc</td>
<td>Clotrimazole 5µg/disc</td>
</tr>
</tbody>
</table>

Fig: 6.3 Activity of compounds against *Candida albicans*. 
Table: 6.1 THE IN-VITRO ANTI-BACTERIAL ACTIVITY (GRAM POSITIVE STRAINS) DATA IN ZONE OF INHIBITION (mm) AND PERCENTAGE INHIBITION BY DISC DIFFUSSION METHOD

<table>
<thead>
<tr>
<th>S.NO</th>
<th>COMPOUND CODE</th>
<th>Diameter of zone of inhibition (mm)/percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Micrococcus luteus</td>
</tr>
<tr>
<td>1</td>
<td>B1P</td>
<td>12(54%)</td>
</tr>
<tr>
<td>2</td>
<td>B1PB</td>
<td>14(63%)</td>
</tr>
<tr>
<td>3</td>
<td>B1M</td>
<td>15 (68%)</td>
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<tr>
<td>4</td>
<td>B1Br</td>
<td>-</td>
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<tr>
<td>5</td>
<td>B12C</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>B14C</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>B1AZ</td>
<td>12 (54%)</td>
</tr>
<tr>
<td>8</td>
<td>B12N</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>B14N</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>B1AP</td>
<td>14 (63%)</td>
</tr>
<tr>
<td>11</td>
<td>B1T</td>
<td>12 (54%)</td>
</tr>
<tr>
<td>12</td>
<td>B1B</td>
<td>14 (63%)</td>
</tr>
<tr>
<td>Std</td>
<td>CIPROFLOXACIN</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>
Table: 6.2 THE \textit{IN-VITRO} ANTI-BACTERIAL ACTIVITY (GRAM NEGATIVE STRAINS) DATA IN ZONE OF INHIBITION (mm) AND PERCENTAGE INHIBITION BY DISC DIFFUSSION METHOD

<table>
<thead>
<tr>
<th>S.NO</th>
<th>COMPOUND CODE</th>
<th>Diameter of zone of inhibition (mm) /percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>\textbf{Escherichia coli}</td>
</tr>
<tr>
<td>1</td>
<td>B1P</td>
<td>15(100%)</td>
</tr>
<tr>
<td>2</td>
<td>B1PB</td>
<td>14(93%)</td>
</tr>
<tr>
<td>3</td>
<td>B1M</td>
<td>13(86%)</td>
</tr>
<tr>
<td>4</td>
<td>B1Br</td>
<td>10(66%)</td>
</tr>
<tr>
<td>5</td>
<td>B12C</td>
<td>7(46%)</td>
</tr>
<tr>
<td>6</td>
<td>B14C</td>
<td>10(66%)</td>
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<tr>
<td>7</td>
<td>B1AZ</td>
<td>12(80%)</td>
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<td>8</td>
<td>B12N</td>
<td>8(53%)</td>
</tr>
<tr>
<td>9</td>
<td>B14N</td>
<td>7(46%)</td>
</tr>
<tr>
<td>10</td>
<td>B1AP</td>
<td>9(60%)</td>
</tr>
<tr>
<td>11</td>
<td>BIT</td>
<td>7(46%)</td>
</tr>
<tr>
<td>12</td>
<td>B1B</td>
<td>15(100%)</td>
</tr>
<tr>
<td>Std</td>
<td>CIPROFLOXACIN</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>
Table: 6.3 THE *IN-VITRO* ANTI-FUNGAL ACTIVITY DATA IN ZONE OF INHIBITION (mm) AND PERCENTAGE INHIBITION BY DISC DIFFUSSION METHOD

<table>
<thead>
<tr>
<th>S.N O</th>
<th>COMPOUND CODE</th>
<th>Diameter of zone of inhibition (mm)/ percentage of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Candida albicans</td>
</tr>
<tr>
<td>1</td>
<td>B1P</td>
<td>10(50%)</td>
</tr>
<tr>
<td>2</td>
<td>B1PB</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>B1M</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>B1Br</td>
<td>10(50%)</td>
</tr>
<tr>
<td>5</td>
<td>B12C</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>B14C</td>
<td>10(50%)</td>
</tr>
<tr>
<td>7</td>
<td>B1AZ</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>B12N</td>
<td>12(60%)</td>
</tr>
<tr>
<td>9</td>
<td>B14N</td>
<td>14(70%)</td>
</tr>
<tr>
<td>10</td>
<td>B1AP</td>
<td>-</td>
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<tr>
<td>11</td>
<td>B1T</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>B1B</td>
<td>-</td>
</tr>
<tr>
<td>Std</td>
<td>CLO-TRIMAZONE</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>DMSO</td>
<td>-</td>
</tr>
</tbody>
</table>
Fig: 6.4 The *in-vitro* anti-bacterial activity (gram positive strains) by disc diffusion method
Fig: 6.5 The *in-vitro* anti-bacterial activity (gram negative strains) by disc diffusion method
Fig. 6.6 The *in-vitro* anti-fungal activity by disc diffusion method
MINIMUM INHIBITORY CONCENTRATION (MIC)\textsuperscript{88}

MIC is the lowest concentration of an anti-microbial agent that will inhibit the visible growth of a microorganism. Minimum inhibitory concentrations are important in diagnostic laboratories to confirm resistance of microorganisms to an anti-microbial agent and also to monitor the activity of new anti-microbial agents. The activity of the drug is based on inhibition of microbial growth as indicated by measurement of turbidity of a suspension of a suitable microorganism in a fluid medium to which graded amount of test compound have been added. These changes in turbidity were compared with that of a reference compound.

Procedure for determination of minimum inhibitory concentration for synthesized compounds against bacteria by serial dilution method

The serial dilution of compound solutions were made from the stock (1000 µg/ml) by using Muller Hinton broth using the method described below.

- The tubes were labelled 1 to 8 and 1 ml of Muller Hinton broth was added to the first 5 tubes and 8th tube, and then added 0.5 ml Muller Hinton broth to 6th and 7th tubes.
- 1 ml of different synthesized compounds was added to the 1st tube, mixed and transferred 1 ml serially up to tube 6. Mixed and transferred 0.5 ml to the 7th tube so that each tube, 1 to 7 contained 1 ml diluted extracts. The 8th tube served as the control.
- With a standardized micro pipette, added a drop of the diluted broth culture approximately 0.01 ml of the test organism to all tubes, including the control, gently mixed and incubated at 37\textdegree C for 18 hrs.
- After incubation the turbidity was observed. The highest dilution of particular compounds showing no turbidity and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC\textsuperscript{[149]}. The details are tabulated in Table no: 6.4 & 6.5
Procedure for determination of minimum inhibitory concentration for synthesized compounds against fungi by serial dilution method

The serial dilution of compound solutions were made from the stock (1000 µg/ml) by using Sabourand’s dextrose broth using the method described below.

- The tubes were labelled 1 to 8 and 1 ml of Sabourand’s dextrose broth was added to the first 5 tubes and 8th tube, and then added 0.5 ml Muller Hinton broth to 6th and 7th tubes.
- 1 ml of different synthesized compounds was added to the 1st tube, mixed and transferred 1 ml serially up to tube 6. Mixed and transferred 0.5 ml to the 7th tube so that each tube, 1 to 7 contained 1 ml diluted extracts. The 8th tube served as the control.
- With a standardized micro pipette, added a drop of the diluted broth culture approximately 0.01ml of the test organism to all tubes, including the control, gently mixed and incubated at 27°C for 24 - 48 hrs.
- The highest dilution of particular compounds showing no turbidity was observed and recorded. This was taken as the end point, and this dilution was considered to contain the concentration of drug equivalent to MIC. The details are tabulated in Table no:6.6
Table: 6.4 THE *IN-VITRO* ANTI-BACTERIAL ACTIVITY (GRAM POSITIVE STRAINS) DATA IN MINIMUM INHIBITORY CONCENTRATION

<table>
<thead>
<tr>
<th>S.NO</th>
<th>COMPOUND CODE</th>
<th>Minimum inhibitory concentration (µg/ml)</th>
<th>Micrococcus luteus</th>
<th>Staphylococcus aureus</th>
<th>Bacillus subtilis</th>
<th>Corny bacterium</th>
<th>Bacillus lintus</th>
<th>Staphylococcus albus</th>
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</thead>
<tbody>
<tr>
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<td>25</td>
<td>6.25</td>
<td>25</td>
<td>25</td>
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<tr>
<td>2</td>
<td>B1PB</td>
<td></td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
<td>25</td>
<td>25</td>
<td>6.25</td>
</tr>
<tr>
<td>3</td>
<td>B1M</td>
<td></td>
<td>12.5</td>
<td>6.25</td>
<td>25</td>
<td>25</td>
<td>NT</td>
<td>6.25</td>
</tr>
<tr>
<td>4</td>
<td>B1Br</td>
<td></td>
<td>NT</td>
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<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>25</td>
</tr>
<tr>
<td>5</td>
<td>B12C</td>
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<td>25</td>
<td>NT</td>
<td>NT</td>
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<td>NT</td>
</tr>
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<td>6</td>
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<td>NT</td>
<td>NT</td>
</tr>
<tr>
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<td>6.25</td>
<td>12.5</td>
<td>6.25</td>
<td>12.5</td>
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<td>B12N</td>
<td></td>
<td>NT</td>
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<td>NT</td>
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<td>NT</td>
<td>NT</td>
</tr>
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<td>B14N</td>
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<td>NT</td>
<td>NT</td>
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<td>25</td>
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<td>12</td>
<td>B1B</td>
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</table>

NT=NOT TESTED
Table: 6.5 THE \textit{IN-VITRO} ANTI-BACTERIAL ACTIVITY (GRAM NEGATIVE STRAINS) DATA IN MINIMUM INHIBITORY CONCENTRATION

<table>
<thead>
<tr>
<th>S.NO</th>
<th>COMPOUND CODE</th>
<th>Minimum inhibitory concentration (µg/ml)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Escherichia coli</td>
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<tr>
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<td>B1M</td>
<td>6.25</td>
</tr>
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<td>4</td>
<td>B1Br</td>
<td>12.5</td>
</tr>
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<td>5</td>
<td>B12C</td>
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<td>B1B</td>
<td>6.25</td>
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<td>Std</td>
<td>CIPROFLOXACIN</td>
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<td>DMSO</td>
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</tbody>
</table>

NT= NOT TESTED
### Table: 6.6 THE *IN-VITRO* ANTIFUNGAL ACTIVITY DATA IN MINIMUM INHIBITORY CONCENTRATION

<table>
<thead>
<tr>
<th>S.N O</th>
<th>COMPOUND CODE</th>
<th>Minimum inhibitory concentration (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Candida albicans</strong></td>
</tr>
<tr>
<td>1</td>
<td>B1P</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>B1PB</td>
<td>NT</td>
</tr>
<tr>
<td>3</td>
<td>B1M</td>
<td>NT</td>
</tr>
<tr>
<td>4</td>
<td>B1Br</td>
<td>25</td>
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<td>5</td>
<td>B12C</td>
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<td>B14C</td>
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<tr>
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</tr>
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<tr>
<td>Std</td>
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</tbody>
</table>

NT=NOT TESTED
**IN-VITRO ANTI-OXIDANT SCREENING OF SYNTHESIZED COMPOUNDS**

- In-vitro anti-oxidant screening of synthesized compounds were done by using three methods
  - DPPH method
  - FRAP method
  - ABTS method

** Determination of DPPH (1-1-diphenyl 2-picryl hydrazyl) radical-scavenging activity:**

**Procedure:**

The free radical-scavenging activity of the synthesized compounds was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical DPPH. 0.1 mM solution of DPPH in ethanol was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations (0.1–5 mg/ml). Thirty minutes later, the absorbance was measured at 517 nm. Ascorbic acid was used as the reference compound. Lower absorbance of the reaction mixture indicated higher free radical-scavenging activity. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula:

\[
\text{% inhibition} = \left( \frac{A_0 - A_t}{A_0} \right) \times 100
\]

Where \( A_0 \) was the absorbance of the control (blank, without compounds) and \( A_t \) was the absorbance in the presence of the compounds. All the tests were performed in triplicate and the graph was plotted with the mean values.

**Ferric reducing antioxidant power (FRAP) assay:**

FRAP assay is based on the ability of anti-oxidants to reduce Fe3+ to Fe2+ in the presence of 2,4,6-tri(2-pyridyl)- s-triazine (TPTZ), forming an intense blue Fe2+-TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the anti-oxidant content (Benzie and Strain, 1996). 0.2 ml of the compound is added to 3.8 ml of FRAP reagent (10 parts of 300
mM sodium acetate buffer at pH 3.6, 1 part of 10.0 mM TPTZ solution and 1 part of 20.0 mM FeCl₃. 6H₂O solution) and the reaction mixture is incubated at 37°C for 30 min and the increase in absorbance at 593 nm is measured. FeSO₄ is used for calibration. The antioxidant capacity based on the ability to reduce ferric ions of sample is calculated from the linear calibration curve and expressed as mmol FeSO₄ equivalents per gram of sample. BHT, BHA, ascorbic acid, quercetin, catechin or trolox can be used as a positive control.

**ABTS radical scavenging assay:**

The ABTS assay was employed to measure the anti-oxidant activity of the derivatives. ABTS was dissolved in de-ionized water to 7 mM concentration, and potassium persulphate added to a concentration of 2.45 mM. The reaction mixture was left to stand at room temperature overnight (12–16 h) in the dark before usage. 0.5 ml of derivatives were diluted with 0.3 ml ABTS solution and made up to the volume with methanol. Absorbance was measured spectrophotometrically at 745 nm.

The assay was performed at least in triplicates. Fresh stocks of ABTS solution were prepared every five days due to self-degradation of the radical. The assay was first carried out on Ascorbic acid, which served as a standard. The percentage of inhibition was measured by the following formula:

\[
\% \text{ inhibition} = \frac{(A_0 - A_t)}{A_0}
\]

Where \(A_0\) was the absorbance of the control (blank, without compounds) and \(A_t\) was the Absorbance in the presence of the compounds. All the tests were performed in triplicate and the graph was plotted with the mean values.
Table: 6.7 DPPH METHOD

<table>
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<tr>
<th>S.No</th>
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<th>% Inhibition</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt;</th>
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<td></td>
<td></td>
<td>25µg</td>
<td>50 µg</td>
</tr>
<tr>
<td>1</td>
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<td>36.48</td>
</tr>
<tr>
<td>2</td>
<td>B1PB</td>
<td>49.76</td>
<td>60.33</td>
</tr>
<tr>
<td>3</td>
<td>B1M</td>
<td>28.93</td>
<td>35.39</td>
</tr>
<tr>
<td>4</td>
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<tr>
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<td>B1AZ</td>
<td>19.78</td>
<td>28.68</td>
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<td>B12N</td>
<td>55.67</td>
<td>66.92</td>
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<td>B14N</td>
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<tr>
<td>10</td>
<td>B1AP</td>
<td>39.36</td>
<td>67.28</td>
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<tr>
<td>11</td>
<td>B1T</td>
<td>22.46</td>
<td>29.76</td>
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<td>12</td>
<td>B1B</td>
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<tr>
<td>Std</td>
<td>Ascorbic acid</td>
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[Graphs showing percentage inhibition vs. concentration for each compound]
Table: 6.8 FRAP METHOD

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<td>Std</td>
<td>Ferrous sulphate</td>
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**B1P**

\[ y = -0.020x + 3.93 \quad R^2 = 0.996 \]

**B1PB**

\[ y = -0.015x + 2.32 \quad R^2 = 0.949 \]

**B1M**

\[ y = -0.020x + 3.93 \quad R^2 = 0.996 \]

**B1Br**

\[ y = -0.006x + 3.93 \quad R^2 = 0.996 \]

**B12C**

\[ y = -0.020x + 3.93 \quad R^2 = 0.996 \]

**B14C**

\[ y = -0.002x + 3.91 \quad R^2 = 0.731 \]
### Table: 6.9 ABTS METHOD

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<tr>
<th>S.No</th>
<th>Compound</th>
<th>% Inhibition</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>25 µg</td>
<td>50 µg</td>
</tr>
<tr>
<td>1</td>
<td>B1P</td>
<td>50.76</td>
<td>61.33</td>
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<td>B1PB</td>
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<td>B1Br</td>
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<tr>
<td>Std</td>
<td>Ascorbic acid</td>
<td>58.77</td>
<td>69.94</td>
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IN-VITRO CYTOTOXICITY SCREENING BY MTT ASSAY\textsuperscript{90,91}

The mouse embryonic fibroblasts cell line (NIH 3T3) was obtained from National Centre for Cell Science (NCCS), Pune, and grown in Dulbecco's modified Eagles medium containing 10% fetal bovine serum (FBS). All cells were maintained at 37\textdegree{}C, 5% CO\textsubscript{2}, 95% air and 100% relative humidity. Maintenance cultures were passaged weekly, and the culture medium was changed twice a week.

Cell treatment procedure

The monolayer cells were detached with trypsin-ethylenediaminetetraacetic acid (EDTA) to make single cell suspensions and viable cells were counted using a hemocytometer and diluted with medium with 5% FBS to give final density of 1x10\textsuperscript{5} cells/ml. one hundred microlitres per well of cell suspension were seeded into 96-well plates at plating density of 10,000 cells/well and incubated to allow for cell attachment at 37\textdegree{}C, 5% CO\textsubscript{2}, 95% air and 100% relative humidity. After 24 h the cells were treated with serial concentrations of the extracts and fractions. They were initially dissolved in neat dimethylsulfoxide (DMSO) and further diluted in serum free medium to produce five concentrations. One hundred microlitres per well of each concentration was added to plates to obtain final concentrations of 100, 50, 25, 12.5 and 6.25 µM. The final volume in each well was 200 µl and the plates were incubated at 37\textdegree{}C, 5% CO\textsubscript{2}, 95% air and 100% relative humidity for 48h. The medium containing without samples were served as control. Triplicate was maintained for all concentrations.

MTT assay

MTT is a yellow water soluble tetrazolium salt. A mitochondrial enzyme in living cells, succinate-dehydrogenase, cleaves the tetrazolium ring, converting the MTT to an insoluble purple formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells.

After 48h of incubation, 15µl of MTT (5mg/ml) in phosphate buffered saline (PBS) was added to each well and incubated at 37\textdegree{}C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilised in 100µl of DMSO and then measured the absorbance at 570 nm using micro plate reader. The % cell inhibition was determined using the following formula.
% cell Inhibition = 100 - Abs (sample)/Abs (control) x100.

Nonlinear regression graph was plotted between % Cell inhibition and Log_{10} concentration and IC50 was determined using GraphPad Prism software.
### Table: 6.10 IC₅₀ VALUES OF COMPOUND

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<th>S.No</th>
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<th>Percentage of Cell Inhibition(%)</th>
<th>IC₅₀ Values(µM)</th>
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PHOTOGRAPHS OF MOUSE EMBRYONIC FIBROBLASTS CELL LINE (NIH 3T3) INHIBITION BY THE COMPOUND (0.1 µM -100 µM)

- Compound **B1P**

**Fig: 6.7**

**Fig: 6.8**
Compound **B1AZ**

Fig: 6.9

Fig: 6.10
7.1 CHEMISTRY

In the present work 12 different N-Mannich bases were synthesised in 4 steps.

Step: 1

6-bromobenzod[\textit{d}]thiazol-2-amine has been synthesized by precooled solution of 4-bromoaniline and ammonium thiocyanate in glacial acetic acid were stirred, to this mixture add bromine in glacial acetic acid for cyclization to form 6-bromobenzod[\textit{d}]thiazol-2-amine.

Step: 2

Ethyl [2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene](cyano)acetate was synthesized by coupling diazonium salt with ethylcyanoacetate in presence of sodium acetate. The diazonium salt of 6-bromobenzod[\textit{d}]thiazol-2-amine was synthesized by diazotization of 6-bromobenzod[\textit{d}]thiazol-2-amine with sodium nitrite in concentrated hydrochloric acid at 0-5 °C.

Step: 3

5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one was synthesized by cyclising ethyl[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene](cyano)acetate with hydrazine hydrate using ethanol as solvent.

Step: 4

N-Mannich bases of 5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one were synthesized by refluxing 5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one with 12 different aromatic amines and 90% formaldehyde using ethanol as solvent.

The yield of the synthesised compounds was found to be in the range of 57-95%. The purity of all the newly synthesized compounds were checked by melting point, TLC analysis and the structures were confirmed by UV, IR, NMR and MASS spectral data. The physicochemical data and analytical data’s were given in the table no: 5.2, 5.3, 5.4&5.5.
7.2 DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

7.2.1 MELTING POINT ANALYSIS

Melting points of all the newly synthesised compounds were checked and uncorrected and the values were given in table no: 5.2&5.4.

7.2.2 THIN LAYER CHROMATOGRAPHY ANALYSIS

The reaction time and purity of the compounds were determined by running TLC and a single spot was obtained. Rf Values of all the newly synthesised compounds were determined and given in table no: 5.2&5.4.

7.2.3 SOLUBILITY

Solubility was checked for all the newly synthesised compounds and found that all the compounds were soluble in semi polar solvent.

7.3 CHARACTERIZATION OF SYNTHESISED COMPOUNDS

7.3.1 INFRARED SPECTRAL ANALYSIS

The structures of intermediates (BTZ) confirmed by the presence of characteristic peaks in the region 3573.45 cm\(^{-1}\), 1663.3 cm\(^{-1}\), 669.17 cm\(^{-1}\) associated for –NH\(_2\), C=N-, C-S-C stretching respectively. The compound BTZE confirmed by the stretching of ester group in the region 1702.84 cm\(^{-1}\) for keto of acetyl group. The compound BTZP confirmed the presence of –NH\(_2\) and –NH- groups by the peaks at 3385.42 cm\(^{-1}\) and 3385 cm\(^{-1}\) respectively and also showed the disappearance of the characteristic bands of the acetyl carbonyl group and carboxylic acid ester.

All the N-Mannich derivative compounds showed the characteristic peaks in the region 3372.82 cm\(^{-1}\) for associated NH of amines, 840- 790 cm\(^{-1}\) for Ar, CH=CH stretching, 1640- 1620 cm\(^{-1}\) and 1600-1400 cm\(^{-1}\) for C=O stretching. Compound B12N containing NO\(_2\) group showed absorption bands at 1550-1500, for the N=O stretching. The peak at 771 cm\(^{-1}\) could be assigned to C-Cl stretching in the compound B12C. Presence of hydroxyl group was confirmed by the appearance of peak at 1165.76 cm\(^{-1}\) in the compound B1P. Presence of carboxylic acid group was confirmed by the appearance of peak at 1771 cm\(^{-1}\).
group was confirmed by the appearance of broad peak at 3127.01 cm\(^{-1}\) in the compound B1PB.

### 7.3.2 NUCLEAR MAGNETIC RESONANCE SPECTRAL ANALYSIS

The structures of the twelve new compounds (BTZE, BTZP, B1P, B1PB, B1M, B1Br, B12C, B14C, B1AZ, B12N, B14N, B1AP, B1T and B1B) were confirmed by \(^1\)H-NMR spectra. The \(^1\)H-NMR spectra of BTZE showed the presence of the peaks for the ethyl group, while the pyrazolinone –NH\(_2\) proton signal appeared at \(\delta\) 6.63 ppm. All the synthesized compounds showed multiplets in the range \(\delta\) 7.5-8.04 for the protons of aromatic ring and a doublet at \(\delta\) 4.5 which may be assigned to –CH\(_2\)– linkage. The spectrum of B1P revealed a singlet at \(\delta\) 9.1 ppm which may be assigned to -OH proton. The spectrum of B1M revealed a singlet at \(\delta\) 3.3 ppm which may be assigned to –OCH\(_3\) protons.

### 7.3.3 MASS SPECTRAL ANALYSIS

Electron impact mass spectral analysis was carried out on four randomly selected compounds B1T, B1AZ, B1B, and B14C. Mass spectrums of the compounds were in full agreement with their molecular weights.

### 7.4 BIOLOGICAL EVALUATION

#### 7.4.1 ANTIMICROBIAL STUDIES

All the newly synthesised compounds at 100µg/disc were screened for their preliminary anti-microbial activity against six gram positive bacteria strains, six gram negative bacteria stains and six fungi strains by disc diffusion method using muller hinton agar media for bacteria and potato dextrose agar media for fungi, and determination of Minimum Inhibitory Concentration (MIC) for selective synthesized compounds was done by 2 fold serial dilution method muller hinton broth for bacteria and potato dextrose broth for fungal.

**ANTI BACTERIAL ACTIVITY**

**PRELIMINARY ANTI-BACTERIAL SCREENING BY DISC DIFFUSION METHOD**

Muller Hinton agar media was used for screening anti-bacterial activity of synthesized compound. Reference standard Ciprofloxacin (5µg/disc), negative control DMSO
(DiMethyl Sulfoxide) and synthesized compounds (100µg/disc) were used. Observations were made for zone inhibition around the tested drugs and compared with that of reference standard. The bacterial zone of inhibition values are given in the table no: 6.1 & 6.2. The result of anti-bacterial activity showed most of the synthesized compounds were moderate to significant activity against *streptococcus aureus* and *streptococcus albus* (gram positive strain) at 100µg/disc compare with that of standard ciprofloxacin 5µg/disc concentration level. Especially the substitution of electron donating group attached at the para position of N-methyl benzenamine ring imparted significant gram positive as well as gram negative anti-bacterial activity to the resulting N-mannich base containing pyrazolone derivatives (B1P, B1M, B1AZ, B1AP, B1T and B1B) compared with electron withdrawing group attached at the para position of N-methyl benzenamine ring (B1Br, B12C, B14C, B12N, B14N). Particularly, the 2nd position of pyrazolone ring containing bulky group link through –CH2-NH- bridge (B1AZ and B1B) showed anti-bacterial activity against all tested gram positive and gram negative strains at 100µg/disc level. On the other hand, the presence of electron withdrawing group attached at the *para* position of N-methyl benzenamine ring display poor or loss of anti-bacterial activity against both gram positive and gram negative bacterial strains except the synthesized compound B1PB. All the synthesized compounds showed moderate to significant activity towards E.coli(gram negative bacteria), especially the compound B1P and B1B at 100µg/disc showed equal anti-bacterial activity as that of standard ciprofloxacin at 5µg/disc concentration level. All the synthesised compounds completely devoid activity against *vibrio cholera* and *salmonella paratyphi* (gram negative bacteria) except the compound B1PB, B1AZ and B1B.

**MINIMUM INHIBITORY CONCENTRATION SCREENING BY 2-FOLD DILUTION METHOD**

Based on the preliminary anti bacterial activity screening result, active compounds were selected for determining minimum inhibitory concentration (MIC) by 2-fold serial dilution method. Muller Hinton broth media was used for determining MIC value of synthesized compound. Series of 10-15 dilutions to final concentrations of 100-1.56 µg/ml are prepared.

The MIC of all the synthesised compounds was found at 6.25µg/ml, 12.5µg/ml, 25µg/ml, 50µg/ml concentration levels for tested gram positive and gram negative strains. The minimum inhibitory concentration values are given in table no: 6.4 & 6.5.
ANTI FUNGAL ACTIVITY

PRELIMINARY ANTI-FUNGAL SCREENING BY DISC DIFFUSION METHOD

The result of anti-fungal activity showed that the synthesised compound 100µg/disc were poor or devoid anti fungal activity for most of the tested fungal strains compare to the standard clo-trimozole at 5µg/disc concentration level. The synthesized compounds (B1PB, B1Br, B12C, B12N and B14N), substitution of electron withdrawing group attached at the para position of N-methyl benzenamine ring imparted moderate anti-fungal activity, particularly aspergillus niger, aspergillus fumigates, and aspergillus parasites to the resulting N-mannich base containing pyrazolone derivatives. On the other hand, the synthesized compound B1AZ and B1B showed moderate activity against most of the tested fungal strains. The fungal zone of inhibition value is given in the table no: 6.3.

MINIMUM INHIBITORY CONCENTRATION SCREENING BY 2-FOLD DILUTION METHOD

Based on the preliminary anti-fungal activity screening result, active compounds were selected for determining minimum inhibitory concentration (MIC) by 2-fold serial dilution method. Sabourand’s dextrose broth media was used for determining MIC value of synthesized compound. Series of 10-15 dilutions to final concentrations of 100-1.56µg/ml are prepared.

The MIC of all the synthesised compounds was found at 6.25µg/ml, 12.5µg/ml, 25 µg/ml, 50µg/ml concentration levels for tested fungal strains. The minimum inhibitory concentration values are given in table no: 6.6.

7.4.2 IN-VITRO ANTI OXIDANT ACTIVITIES

All newly synthesized compounds were screened for in-vitro anti-oxidant activity by DPPH, FRAP and ABTS assay method at the concentration of 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml. DMSO used as a solvent, ascorbic acid used as a standard for DPPH and ABTS, ferrous sulphate is used as a standard for FRAP method.

DPPH

The result of in-vitro anti-oxidant activity by DPPH method indicates that among the screened compounds, compound B12N, B14N, B1Br, B12C, B1PB, B14C and B1AP have
significant anti-oxidant activity with EC\textsubscript{50} value 22.45, 22.9, 23.58, 24.15, 25.12, 25.13 and 31.75 respectively. The other synthesized compounds showed mild anti-oxidant activity. The DPPH \textit{in-vitro} anti-oxidant assay method result review that the synthesized compounds containing substitution of electron with drawing group attached at the para position of N-methyl benzenamine ring imparted significant anti-oxidant property to the resulting N-mannich base containing pyrazolone derivatives (B12N, B14N, B1Br, B12C, B1PB, B14C and B1AP). The highest anti-oxidant activity due to the compound with high lipophilic value, lowest electron withdrawing power, highly polarisability. The percentage inhibition values were given in the **table no: 6.7**.

**FRAP**

Among the synthesised derivatives B1P, B1PB, B1M, and B1AZ are having the R\textsuperscript{2} value of 0.949, 0.984, 0.988 and 0.885 near to the standard ferrous sulphate value of 0.996 indicating that compounds showed good anti-oxidant activity. The R\textsuperscript{2} values were given in the **table no: 6.8**.

**ABTS**

The result of \textit{in-vitro} anti-oxidant activity by ABTS method shows that among the screened compounds, compound B12C, B14C, B1Br, B12N, B14N, B1P and B1AP have significant anti-oxidant activity with EC\textsubscript{50} value 22.05, 22.49, 22.83, 23.70, 24.30, 24.62 and 30.97 respectively. The other synthesized compounds showed mild anti-oxidant activity. The ABTS \textit{in-vitro} anti-oxidant assay method review that the synthesized compounds containing substitution of electron with drawing group attached at the para position of N-methyl benzenamine ring imparted significant anti-oxidant property to the resulting N-mannich base containing pyrazolone derivatives (B12C, B14C, B1Br, B12N, B14N, B1P and B1AP). The percentage inhibition values were given in the **table no: 6.9**.

**7.4.3 IN-VITRO CYTOTOXIC ACTIVITY**

Six of the newly synthesized compounds (B1P, B1PB, B14C, B1AZ, B1T, B1B) were screened for \textit{in vitro} cytotoxic activity against mouse embryonic fibroblasts cell line (NIH 3T3) by MTT assay in DMSO and their IC\textsubscript{50} values are 26.µM, 15.34µM, 10.95µM, 20.24 µM, 14.88 µM, >100µM respectively. Among the tested compounds B1B showed least cytotoxic activity. The IC\textsubscript{50} values were given in the **table no: 6.10**.
I. SUMMARY

8.1 EXPERIMENTAL

In the present work twelve title compounds were synthesised by subject 5-amino-4-[2-(6-bromo-1,3-benzo	hiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (BTZP) to manich reaction with 12 different amines. The intermediate 5-amino-4-[2-(6-bromo-1,3-benzo	hiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (BTZP) was synthesized from ethyl[2-(6-bromo-1,3-benzo	hiazol-2-yl)hydrazinylidene](cyano)acetate (BTZE) by cyclising with hydrazine hydrate. Ethyl[2-(6-bromo-1,3-benzo	hiazol-2-yl)hydrazinylidene](cyano)acetate was synthesized by coupling through diazonium salts of 6-bromobenzo[d]thiazol-2-amine with ethylcyanoacetate. The compound diazonium salts of 6-bromobenzo[d]thiazol-2-amine was formed by reacting 6-bromobenzo[d]thiazol-2-amine (BTZ) with sodium nitrite in concentrated hydrochloric acid, which in turn was synthssized from 4-bromo aniline and thiourea. The yield was found to be 55-75% in all the stage.

Most of the newly synthesized compounds were found to be soluble in semi polar solvents. Thin layer chromatography was used to confirm the reaction time and purity of the compounds synthesized. Melting point was determined by one end open glass capillary tubes and was uncorrected. The UV spectrum values observed between 400-800nm and IR spectra of synthesized compounds were exhibited 3372.82cm⁻¹ for associated NH of amines, 840-790cm⁻¹ for Ar, CH=CH stretching, 1640-1620cm⁻¹ and 1600-1400cm⁻¹ for C=O stretching. The ¹H NMR spectra all the synthesized compounds showed multiplets in the range δ 7.5-8.04 for the protons of aromatic ring In addition, ¹H NMR spectra of these compounds signals arising from possible –CH₂– structure at 3.3 ppm. The Ar-NH– proton structure of synthesized was also supported by ¹H-NMR spectra. Mass spectrums of the synthesized compounds were in full agreement with their molecular weights and studies showed satisfactory results.
8.2 BIOLOGICAL STUDIES

8.2.1 ANTI-BACTERIAL ACTIVITY

Disc Diffusion Method


✓ Among the synthesized compounds, compound B1P, B1PB, B1AZ, B1T and B1B at 100µg/disc was found to be significant active against most of the screened bacteria when compared with standard drug (Ciprofloxacin 5µg/disc).

✓ Among the synthesized compounds, Compound B1P, B1PB, and B1B at 100µg/disc demonstrated more zone of inhibition against *Escherichia coli* when compared with standard drug Ciprofloxacin (5µg/disc).
MIC STUDIES

- Based on the preliminary anti-bacterial activity screening result, active compounds were selected for determining minimum inhibitory concentration (MIC) by 2-fold serial dilution method.
- Among the screened compounds, compound B1P, B1PB, B1M, and B1B was found to possess anti-bacterial activity at 6.25µg/ml concentrations level for Staphylococcus aureus, Escherichia Coli.

8.2.2 ANTI-FUNGAL ACTIVITY

Disc Diffusion Method

All the newly synthesized compounds were screened for their preliminary anti fungal activity against Six fungal strains, Candida albicans NCIM 3100, Monococcus purpureus MTCC 1090, Aspergillus niger MTCC 1344, Trichophyton rubrum MTCC 3272, Aspergillus fumigates MTCC 1811, Aspergillus parasites MTCC 2796 by disc diffusion method at a concentration of 100µg/disc.

- Among the synthesized compounds, compound B12N, B14N was found to be significant active against most of the screened fungi when compared with standard drug (Clotrimazole 5µg/disc).

- Among the synthesized compounds, Compounds B1PB, B1Br, B12C, B1AZ, B12N, B14N and B1B demonstrated more zone of inhibition against aspergillus fumigates when compared with standard drug Clotrimazole (5µg/disc).
MIC STUDIES

- Based on the preliminary anti-fungal activity screening result, active compounds were selected for determining minimum inhibitory concentration (MIC) by 2-fold serial dilution method.

- Among the synthesized compounds, compound B14C was found to possess anti-fungal activity at 6.25µg/ml concentration level for aspergillus niger.

8.2.3 IN-VITRO ANTI-OXIDANT ACTIVITIES

All newly synthesized compounds were screened for in-vitro anti-oxidant activity by DPPH, FRAP and ABTS assay method at the concentration of 25µg/ml, 50µg/ml, 75µg/ml and 100µg/ml. DMSO used as a solvent, ascorbic acid used as an standard for DPPH and ABTS, ferrous sulphate is used as an standard for FRAP method.

DPPH

The result of in-vitro anti-oxidant activity by DPPH method shows that among the screened compounds, compound B12N, B14N, B1Br, B12C, B1PB, B14C and B1AP have significant anti-oxidant activity with EC_{50} value 22.45, 22.9, 23.58, 24.15, 25.12, 25.13 and 31.75 respectively. The other synthesized compounds showed mild anti-oxidant activity.

FRAP

Among the synthesised derivatives B1P, B1PB, B1M, and B1AZ are having the R^2 value near to the standard ferrous sulphate acid showed good anti-oxidant activity.

ABTS

The result of in-vitro anti oxidant activity by ABTS method shows that among the screened compounds, compound B12C, B14C, B1Br, B12N, B14N, B1P and B1AP have significant anti oxidant activity with EC_{50} value 22.05, 22.49, 22.83, 23.70, 24.30, 24.62 and 30.97 respectively. The other synthesized compounds showed mild anti oxidant activity.
8.2.4 IN-VITRO CYTOTOXICITY ACTIVITY

✓ Six compounds were screened for in-vitro cytotoxicity testing and five compounds shown activity in the tested concentrations 0.1, 1.0, 10, 100µM and one compound doesn’t shown activity in tested concentrations 0.1, 1.0, 10, 100µM.

II. CONCLUSION

✓ Twelve N-mannich bases of 5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (BTZP) were prepared.

✓ The structure of the compounds were characterized by UV, IR, NMR and Mass spectral data, and evaluated for their in-vitro anti-microbial, in-vitro anti-oxidant and in-vitro cytotoxicity activity.

✓ The compounds containing substitution of electron donating groups present at phenyl ring (B1P, B1M, B1AZ, B1AP, B1T and B1B) imparted significant anti-bacterial activity against compared with electron withdrawing group attached compounds (B1Br, B12C, B14C, B12N, B14N). Particularly, the 2nd position of pyrazolone ring containing bulky group link through –CH2-NH- Bridge (B1AZ and B1B) showed anti-bacterial activity and anti-fungal against all tested strains at 100µg/disc level. The compounds containing electron withdrawing group (B1PB, B1Br, B12C, B12N and B14N) imparted moderate anti fungal activity, particularly aspergillus niger, aspergillus fumigates, and aspergillus parasites to the resulting N-mannich base containing pyrazolone derivatives.

✓ The DPPH and ABTS in-vitro anti-oxidant methods result of the synthesised compound review that the electron withdrawing groups attached at the para position of N-methyl benzenamine ring compounds (B12N, B14N, B1Br, B12C, B1PB, B14C and B1AP) showed better anti-oxidant activity.

✓ All the tested compounds showing the in-vitro cytotoxicity activity except B1B compound but this compound showing moderate to significant anti microbial activity against all the tested strains. So this compound may consider for further modification for its biological activity.
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<th>Structure and IUPAC Name</th>
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<td>1</td>
<td>B1P</td>
<td>5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2-[(4-hydroxyphenyl)amino]methyl]-2,4-dihydro-3H-pyrazol-3-one</td>
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<td>2</td>
<td>B1PB</td>
<td>4-[(3-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-5-oxo-4,5-dihydro-1H-pyrazol-1-yl)methyl]amino]benzoic acid</td>
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<td>3</td>
<td>B1M</td>
<td>5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2-[(4-methoxyphenyl)amino]methyl]-2,4-dihydro-3H-pyrazol-3-one</td>
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<td>B1Br</td>
<td>5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2-[(4-bromophenyl)amino]methyl]-2,4-dihydro-3H-pyrazol-3-one</td>
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<td>5</td>
<td>B12C</td>
<td>5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2-[(2-chlorophenyl)amino]methyl]-2,4-dihydro-3H-pyrazol-3-one</td>
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<td>6</td>
<td>B14C</td>
<td>5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2-[(4-chlorophenyl)amino]methyl]-2,4-dihydro-3H-pyrazol-3-one</td>
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INTRODUCTION

1.1 BENZOTHIAZOLE$^{1-31}$

Benzothiazole is a heterocyclic compound, weak base, having varied biological activities and still of great scientific interest now a days. Being a heterocyclic compound, benzothiazole finds use in research as a starting material for the synthesis of larger, usually bioactive structures. Its aromaticity makes it relatively stable, although as a heterocyclic, it has reactive sites, which allow for functionalization. Benzothiazole is a colorless, slightly viscous liquid with a melting point of 2°C, and a boiling point of 227-229°C, the density of benzothiazole is 1.644gm/ml, and molecular mass is 139.19g/mol. Benzothiazole has no household use. It is used in industry and research.

A large number of therapeutic agents are synthesized with the help of benzothiazole nucleus. During recent years there have been some interesting developments in the biological activities of benzothiazole derivatives. These compounds have special significance in the field of medicinal chemistry due to their remarkable pharmacological potentialities$^1$.

Despite numerous attempts to develop new structural prototype in the search for more effective antimicrobials, benzothiazole still remain as one of the most versatile class of compounds against microbes$^2$ and therefore, are useful substructures for further molecular exploration. Benzothiazole derivatives have attracted continuing interest because of their varied biological activities viz. antitumor$^3$, antitubercular$^4$, immunosuppressive activities$^5$, anticonvulsant$^6$, anti-inflammatory$^7$, antidiabetic$^8$. Substituted 2-arylbenzothiazoles have emerged in recent years as an important pharmacophores in non-invasive diagnosis of Alzheimer’s disease. Recently benzothiazole derivatives have been evaluated as potential amyloid-binding diagnostic agents in neurodegenerative disease and as selective fatty acid amide hydrolase inhibitors$^9$. Azomethine linkage has also shown an array of biological activities viz. antimicrobial$^{10}$. 
SYNTHESIS

Many methods had been reported in literature. Some methods were listed in this study.

1) **Scheme-1:** Solvent free synthesis

Synthesis of 2-substituted benzothiazoles by condensation of 2-aminothiophenol with various saturated and olefinic fatty acids under microwave in solvent free condition (path-A) with the use of catalyst P₄S₁₀ in (path-B), the reaction was successful in terms of yield and was completed within 3-4 min¹¹.

2) **Scheme-2:** Synthesis of cyanosubstituted conjugated benzothiazoles

They explored synthesis of benzothiazole based organic nano-particles. The elaboration of conjugated system was performed by reacting equimolar quantities of 4 and 5 in dry THF and terbutyl alcohol at 50ºC while a small amount of terbutylammonium hydroxide was slowly dropped in mixture¹².
3) **Scheme-3:** Suzuki-Miyaura coupling reaction

Development of microwave promoted Suzuki-Miyaura reaction 2-chlorobenzothiazole with phenyl boronic acid was carried out using Pb(PPh₃)₄ as a catalyst. This reaction provides the adduct 6a with excellent regioselectivity. The bis adduct 2,6-diphenyl benzothiazole (7) by the catalysis of 2,6-dichloro benzothiazole with excess of phenyl boronic acid.¹³

![Scheme-3](image)

4) **Scheme-4:** Solid phase synthesis-

Conversion of resin bound isothiocynate 3 was to N-acyl, N-phenyl thioureas in general structure 4, X=H of 4 is cyclized to 2-acyl aminobenzothiazole 5 by treatment with 6 equivalent of bromine in acetic acid. Finally the desired compound 6 were obtained by treatment of 5 with 4% hydrazine monohydrate in ethanol.¹⁴
5) Scheme-5

Development of simple procedure to prepare a series of pyrimido[2,1-\textit{b}]benzothiazoles by the conjugation addition of the imino nitrogen of 2-aminobenzothiazoles to alkyne $\beta$-carbon atom of acetylinic acid followed by ring closure\textsuperscript{15}.

6) Scheme-6

Synthesis of substituted 2-mercaptobenzothiazoles by varying substituent’s at 4, 5, and 6-position in the benzothiazole ring system. The synthesis of final compounds involves two steps- 1) Substituted anilines were converted to its hydrochloride salts. 2) This aniline hydrochloride salt was then cyclized to substituted 2-mercaptobenzothiazoles by reacting with carbon disulphide in presence of sulfur in an alkaline medium\textsuperscript{16}.
7) Scheme-7

Synthesis of new 2-substituted benzothiazole derivatives by refluxing benzothiazolyl carboxyhydried with different aryl acids in phosphoryl chloride\textsuperscript{17}.

![Scheme-7]

8) Scheme-8

Synthesis of 2-aryl substituted benzothiazole derivatives by refluxing o-aminothiophenols with substituted benzoic acids in presence of polyphosphoric acid at 220°C\textsuperscript{18}.

![Scheme-8]
BIOLOGICAL ACTIVITY

1) Anti-microbial activity

Microbes are causative agents for various types of disease like pneumonia, amebiasis, typhoid, malaria, common cough and cold various infections and some severe diseases like tuberculosis, influenza, syphilis, and AIDS as well. Various approaches were made to check the role of benzothiazole moiety as antimicrobial agent from the discovery of molecule to the present scenario.

synthesis of series of pyrimido [2,1-b] benzothiazoles by conjugation addition to imino nitrogen of 2-aminobenzothiazoles to alkyne $\beta$-carbon atom of acetylenic acid followed by ring closure and synthesized compounds are studied for antimicrobial activity against *E.coli* and *Enterobacter* as test organisms at conc 100$\mu$g per disc using vancomycine and meropenam as standard drug$^{19}$. Synthesis of new benzothiazole and benzisoxazole from 2-amino 5/6-hydroxybenzothiazole, 6-hydroxy-3-methyl-1, 2-benzisoxal and different dihaloalkanes and screened for their antimicrobial activity against *Staphylococcus aureus*, and *E. coli* by disc diffusion method and anti fungal activity against *Aspergillus flavus*, and *Candida albicans*. Ciprofloxacin (10$\mu$g/ml) and fluconazole (10$\mu$g/ml) were used as standard drug for antibacterial and antifungal activity respectively$^{20}$.some new 2-mercapto- benzothiazoles and coredleted the effect on antimicrobial potency by varying the substituents in benzene part of the benzothiazole ring system. Anti-microbial screening was performed against *E. coli*, *S. aureus*, *C.albicans* and antifungal activity against *Aspergillus flavus* and *Candida albicans* at conc. 100$\mu$g/ml using agar plate Kirby-Bauer disc diffusion method in DMF as solvent. Ofloxacine (100$\mu$g/ml) and griciofulvin (100$\mu$g/ml) were used as standard drug for antibacterial and antifungal activity respectively$^{21}$. synthesis of some new 2-substituted benzothiazole derivatives by refluxing benzothiazolylcarboxyhydrazide with different aryl acids in phosphoryl chloride and screened the derivative for antimicrobial activity against *B. subtilis*, *E. coli* and *P.aeruginosa* by disc diffusion method at conc. 100$\mu$g/ml. The activity was compared to antibiotic ciprofloxine$^{22}$. Synthesis of series of benzothiazole-2-yl-dithiocarbamates along with copper complexes via reaction of suitable alkyl or heteroaryl halide with sodium salt of benzothiazole-2-yl-dithiocarbamic acid followed by complexation with copper sulphate and selected derivatives checked for their schistosomicidal activity against*Schistosoma mansoni* $^{23}$. 
2) Anti-cancer activity

Refluxing of o-aminophenols with substituted benzoic acid in presence of polyphosphoric acid at higher temperature to get aryl substituted benzothiazoles and evaluated them against Human Cervical Cancer cell lines as anticancer drugs. Synthesis of benzothiazole containing phthalimide and studied their anti-cancer activity on human carcinoma cell lines. Synthesis carbon 11 labeled fluorinated 2-aryl benzothiazoles used for protein emission tomography (PET) to image tyrocinekinese in cancer. Synthesis of benzothiazole derivatives and evaluated for in vitro cytotoxic activity against HL-60 and U-937 cell lines using 5-flurouracil, and cisplatin as std. drug. In silico pharmacokinetic study revealed that benzothiazole dimere were free from teratoginicity, irritation and sensitivity properties than monomers. The QSAR study showed that increase in hydrogen donor count is conductive for cytotoxic activity of benzothiazole derivatives against HL-60 cell lines. Synthesis of fluorinated analogues of 2-(4-aminoaryl) benzothiazoles which successfully block C-oxidation. Fluorinated benzothiazole analogue 2-(4-amino-3-methylphenyl)-5-fluorobenzothiazole (5F203, NSC 703786) (1d), exhibit selective and potent anticancer activity. It is the favored analogue for clinical consideration possessing enhanced efficacy in vitro and superior potencies against human breast and ovarian tumor xenografts implanted in nude mice Its lysylamide prodrug, (Phortress, NSC710305), (1e) is under phase I clinical trials at the United Kingdom.
3) Anthelmentic activity

Synthesis of flurobenzothiazole comprising sulfonamide pyrazole derivitives. They screened synthesized for anthelmentic activity by using earthworms (*Peritumaposthum*). Albendazole was used as standard drug. The compounds were evaluated by time taken for complete paralysis and death of worms

![Chemical structure](attachment:image.png)

4) Anti-diabetic activity

Synthesis of 2-amino[5’(4-sulphonylbenzylidine)-2,4-thiazolidinedione]-7-chloro-6-flurobenzothiazole series and screened for their antidiabetic activity on albino rat by alloxan induced tail tipping method

![Chemical structure](attachment:image.png)

5) Cyclooxygenase inhibitor activity

Pyrazolones and pyrazolinones rank among the more venerable non-steroidal anti-inflammatory agents. Phenylbutazone and its congeners incorporating a pyrazoline-3, 5-dione structure are more potent anti-inflammatory agents. In the recent years a number of Benzo-thiazole derivatives have been synthesized and found to display anti-inflammatory activity. Synthesis of series of 2-[(2-alkoxy-6-pentadecylphenyl) methyl] thio-1-Hbenzimidazoles/benzothiazole from anacardic acid (pentadecyl salicylic acid) and investigated their ability to inhibit human cyclooxygenase enzyme.
1.2 PYRAZOLONES\textsuperscript{32-40}

Pyrazolone moiety (a five-membered lactam ring containing two nitrogens and ketone in the same molecule or alternatively a derivative of pyrazole possessing an additional carbonyl/hydroxy group) has gaining the focus of medicinal chemists for over last 100 years because of the outstanding pharmacological properties shown by several of its derivatives. Due to use of such ring system as the core structure in many drug substances, covered wide range of pharmacological applications. Soon after the discovery of phenyl hydrazine A by Emil Fischer in 1883, Ludwig Knorr (Fischer’s assistant) attempted to synthesize a quinoline derivative. The product however, isolated after methylation was found to be a pyrazolone derivative and was named as antipyrine or phenazone B. Because of its promising antipyretic and analgesic activities antipyrine was launched by Hoechst Pharmaceuticals. For the next 20 years, antipyrine became the most widely used drug in the world, proving highly successful for treating fever and flu like infections, until acetylsalicylic acid (aspirin) began to outsell it\textsuperscript{32}.

Since the introduction of antipyrine, the first pyrazolone derivative used in the management of pain, inflammation and fever into clinical use in 1884, great attention has been focused on pyrazole derivatives as potent anti-inflammatory, analgesic and antipyretic agents. As a result, a large number of pyrazoles have been obtained and some have gained application on the clinical level.
Interest in this field has been intensified after the discovery of the natural pyrazole C-glycoside pyrazofurin 4-hydroxy-3-β-D-ribofuranosyl-1H-pyrazole-5-carboxamide. This antibiotic was reported to possess a broad spectrum of antimicrobial and antiviral activities in addition to being active against several tumor cell lines.

Pyrazolone, as a prominent structural motif, is found in numerous pharmaceutically active compounds. Due to the easy preparation and rich biological activity, pyrazolone framework plays an essential role and represents an interesting template for combinatorial and medicinal chemistry with having a wide range of bioactivities such as antimicrobial, anticancer, anti-inflammatory, analgesic, anti-pyretic, anti-bacterial, anti-tubercular activities. It has been found that these compounds have hypoglycemic activity, and are also known as inhibitors and deactivators of liver alcohol dehydrogenase and oxidoreductases. It has been shown in vivo that some of the pyrazole derivatives have appreciable antihypertensive activity. These compounds also exhibit properties such as cannabinoid hCB1 and hCB2 receptor, inhibitors of p38 Kinase, CB1 receptor antagonists.

Pyrazolin-5-one derivative, 3-methyl-1-phenyl-2-pyrazolin-5-one has been reported to be a promising candidate for the treatment of neonatal hypoxic-ischemic encephalopathy and paraquat poisoning due to its free radical scavenging activity.

Recently an important natural mediator of inflammation Leucettamine B was isolated and since then attempts to synthesis this compound and its analogues were continued.

A new pyrazolone compound III, Edaravone (3-methyl-1-phenyl-2-pyrazolin-5-one, also known as MCI-186), has been developed as a promising drug for brain ischemia and has also been reported to be effective for myocardial ischemia. More recently, a series of pyrazolone derivatives have been synthesized as potent inhibitors of protease-resistant prion protein accumulation for the treatment of fatal neurodegenerative diseases.
Pyrazolo[3,4-\(d\)]pyrimidine derivatives have been found to possess antitumor and antileukemia activity and pyrazolo [4,3-\(e\)]-1,2,4-triazolo[1,5-c] pyrimidine derivatives have been found to be highly potent and selective human A3, A2 and A2B adenosine receptor antagonists. Among the already marketed COX-2 inhibitors that comprise the pyrazole nucleus, celecoxib; 4-[5-(4-methylphenyl)-3-(trifluorophenyl)-1H-pyrazol-1-yl] benzene sulfonamide proved to be potent and GI safe anti-inflammatory and analgesic agent.

A number of methods have been developed for the preparation of pyrazolone derivatives. These methods are diverse, but frequently involve the condensation of a \(\beta\)-keto ester or \(\beta\)-keto aldehyde with substituted or unsubstituted hydrazines. These methodologies, although utilized for the preparation of a variety of pyrazolones, often require the use of refluxing conditions and lengthy reaction time, ideally 3-10 hrs.

**CHEMISTRY OF PYRAZOLONE**

**REACTIONS WITH ELECTROPHILIC REAGENTS**

**Oxidation at nitrogen**

The preparation of 1-hydroxypyrazoles can employ per acidic conditions or basic conditions. When it is the pyrazolyl anion it reacts with the oxidising agent, dibenzoyl peroxide.

**Alkylation at nitrogen**

3(5)-Substituted pyrazoles which have an N-hydrogen, can in principle give rise to two isomeric N-alkyl pyrazoles, after loss of proton from nitrogen and there is further reaction producing an N, N\(^1\)-disubstituted quaternary salt.

**Acylation at nitrogen**

The introduction of an acyl or phenylsulfonyl group onto a pyrazole nitrogen is usually achieved in the presence of a weak base such as pyridine; such process proceed via imine
nitrogen acylation, the N$^+$-H-deprotonation. Since acylation, unlike alkylation, is reversible, the more stable product is obtained.

The reactivity of the 3- and 5-azolones centres mainly on their ability to react with electrophiles such as halogens or to nitrate or undergo Vilsmeier formylation, the example is the formylation of antipyrine once used as an analgesic. Many dyestuffs have been synthesized via coupling of aryl diazonium cations with 5-pyrazolones at C-4 tartazine is such an example.

Pyrazole reacts with cyanamide very efficiently to produce an N-derivative which can be utilized by reaction with primary or secondary amines to synthesise guanidines.

Pyrazolones also condense with aldehydes in aldol-type processes, or react with other electrophiles such as carbon disulphide, in each case reaction presumably proceeding via the enol tautomer, or its anion. In basic solution oxazol-3-ones alkylate either or nitrogen.
Aminopyraole undergoes substituent-N-acetylation and easy electrophilic bromination at C-4. Diazotization and a subsequent Sandmeyer reaction provides route to azidopyrazoles.

\[
\begin{align*}
\text{Me} & \quad \text{NBS, AlBN} \quad \text{Br} \\
\text{Me} & \quad \text{CHCl}_3, \text{refux} \\
\text{Ph} & \quad \text{Pb(OAc), BF}_3\text{Et}_2\text{O} \\
\text{Ph} & \quad \text{MeOH, 0\degree C}
\end{align*}
\]

\[
\text{73\%} \quad 59\%
\]

Diazotisation of 4-aminopyrazoles, then deprotonation yields stable diazopyrazoles.

\[
\begin{align*}
\text{Me} & \quad \text{NaNO}_2 \quad \text{aq.HCl, 5\degree C} \\
\text{Me} & \quad 2\text{N NaOH}
\end{align*}
\]

\[
\text{85\%}
\]

**SYNTHESIS**

Many methods are available for synthesizing pyrazoline derivatives. Most of the methods make use of and \(\alpha, \beta\)-unsaturated carbonyl compounds. 2-pyrazolines were synthesized by reacting \(\alpha, \beta\)-unsaturated aldehydes or ketones with aryl hydrazine.

Different solvents and widely different experimental conditions were employed to get the product. Included in these were the reactants being refluxed in ethanol maintaining reaction time varying from 10 min to 3 hrs.

Eg: -acrylaldehyde and hydrazine gives 1,2- pyrazolines.

Pyrazolines may be oxidized to pyrazoles by bromine or mercuric oxide. If either carbon atom of the double bond is attached to a halogen atom, then a pyrazole is obtained.
1-Phenyl, 2-pyrazolines was synthesized from the reaction between acrolein and phenyl hydrazine.

\[
\begin{align*}
H_2C=CHCHOH + HN-NH_2 &\rightarrow H_2C=CCCC=NN-NH-CN\text{--}\text{phenyl} \\
\end{align*}
\]

The phenyl hydrazide that was initially formed was then cyclized under specific reaction conditions to yield the product.

**From 1,3-dicarbonyl compounds and hydrazines or hydroxylamine:**

Pyrazoles can be made from a 1,3-dicarbonyl component and a hydrazine or hydroxylamine respectively.

\[
\begin{align*}
\text{3-methylpentane-2,4-dione} &\rightarrow \text{pyrazole} \\
\text{-2H}_2\text{O} \\
\end{align*}
\]

**1.3 MANNICH BASES**

In 1912 Mannich and Krosche discovered the property of formaldehyde to bind an amine with a carbon acid *via* a methylene bridge (Mannich, Kather, 1919). This method was utilized to obtain pharmaceutical products by implication of acid components, which were recognized like substances with therapeutic action.

The Mannich reaction consists on the condensation of a CH-activated compound with a primary or a secondary amine and a non-enolizable aldehyde or ketone to afford β-amino carbonyl derivatives known as Mannich bases.
Ketonic amines prepared from the condensation of a ketone or a primary or secondary amine. The product formed is referred to as a Mannich base which can act as the equivalent of an alpha, beta unsaturated ketone in synthesis. The so called mannich reaction refers to a family of transformations that involve three fundamental reagents: a primary or secondary amine, an aldehyde, and an active methylene compound. These reagents condense, with the release of water, to form a Mannich base.

The Mannich reaction is an example of nucleophilic addition of an amine to a carbonyl group followed by elimination of a hydroxyl anion to the Mannich base. The Mannich base is an electrophile which reacts in step two in a second nucleophilic addition with a carbanion generated from a compound containing an acidic proton. The Mannich reaction is also considered a condensation reaction.

**REACTION MECHANISM**

The Mannich Reaction has a two part reaction mechanism:

1. Formation of the mannich base electrophile in a nucleophilic addition.

In the second step of the reaction a carbanion is generated from a CH acidic compound under the influence of a base which then attacks the iminium salt in a second nucleophilic addition.
INTRODUCTION

Modern theoretical consideration permits inclusion of acid -NH, -OH or –SH containing compounds as qualified active hydrogen compounds susceptible to the mannich amino methylation process.

\[
\begin{align*}
\text{H} & - \text{C} - \text{H} + \text{NH}_4^+\text{Cl}^- + \text{CH}_3\text{COR}' \rightarrow \text{H}_2\text{N} - \text{C} - \text{C} - \text{COR}'
\end{align*}
\]

If it is the primary or secondary amine then it may combine with one or two additional molecules of the aldehyde and active compound.

\[
\begin{align*}
\text{H}_2\text{N-CH}_2\text{-CH}_2\text{-CO-R} + \text{HCHO} & \rightarrow \text{HN-(CH}_2\text{-CH}_2\text{-COR)}_2 \rightarrow \text{N-(CH}_2\text{CH}_2\text{COR)}_3
\end{align*}
\]

If the active hydrogen compound has two or three active hydrogen’s the mannich base may condense with one or two additional molecules of aldehyde and ammonia or amine.

Example:

\[
\begin{align*}
\text{H}_2\text{N-CH}_2\text{-CH}_2\text{-CO-R} + \text{HCHO} & \rightarrow \text{H}_2\text{N-CH}_2\text{CHCOR} \rightarrow \text{H}_2\text{N-CH}_2\text{-C-COR}
\end{align*}
\]

1.4 ANTIMICROBIAL AGENTS

An anti-microbial is a substance that kills or inhibits the growth of microorganisms such as bacteria, fungi, or protozoans. Antimicrobial drugs either kill microbes (microbicidal) or prevent the growth of microbes (microbiostatic). Disinfectants are antimicrobial substances used on non-living objects or outside the body.

1.4.1 ANTIBACTERIAL AGENTS

The synthetic or naturally occurring agents, which can kill or inhibit the growth of bacterial cells, are called antibacterial agents.

The year 1935 was important in chemotherapy of systemic bacterial infections. The discovery of antimicrobial activity of penicillin turned the attention of investigators to antibiotics as potentially useful chemotherapeutic compounds. In 1940’s and 1950’s
streptomycin, tetracyclines, chloramphenicol, polymyxin and Bacitracin greatly increased the range of effectiveness of antibacterial chemotherapy.

**Mechanism of action**

The goal is to limit toxicity to the host and maximize chemotherapeutic activity affecting invading microbes only. The mechanisms of action of antibacterial agents are

1. **Inhibition of cell wall synthesis**

   Inhibition of cell wall synthesis leads to bacterial cell lysis and death. Since animal cells do not have a cell wall, they are unaffected by such agents. E.g. Penicillins and cephalosporins.

   ![Methicillin sodium](image)

2. **Inhibition of protein synthesis**

   Most of the antibacterial agents that inhibit protein synthesis interact with the bacterial ribosome. The difference between composition of bacterial and mammalian ribosomes gives the compounds their selectivity.

   E.g. Netimicin, Amikacin.

   ![Chloramphenicol](image)

3. **Inhibition of bacterial metabolism**

   The antimetabolites are synthetic compounds that interfere with bacterial synthesis of folic acid. Inhibition of folate synthesis leads to cessation of cell growth and some case, to bacterial cell death. The principal antibacterial antimetabolites are sulphonamides and trimethoprim.
4. **Inhibition of nucleic acid synthesis**

Inhibition of nucleic acid function prevents cell division and the synthesis of essential enzymes. Agents acting in this way include Nalidixic acid and Proflavin.

\[
\text{Nalidixic acid}
\]

5. **Alteration of cell wall membrane permeability**

Some antibacterial agents behave as cationic, surface active compounds that disrupt the permeability of both the outer and cytoplasmic membranes of gram negative bacteria. E.g. peptide antibiotics, polymyxin-B, Amphotericin-B.

**DRUGS USED IN THE TREATMENT**

1. **Sulphonamides and related drugs**

Sulphonamides were the first anti-microbial agents (AMAs) effective against pyrogenic bacterial infections. A free amino group in para position is required for antibacterial activity. They act as antimetabolites.

\[
\text{Sulphamethoxazole}
\]
2. **β-Lactum antibiotics**

These are antibiotics having a β-lactum ring. The two major groups are Penicillins and Cephalosporins. Monobactams and carbapenems are the newer additions.

![Penicillins](image)

3. **Tetracyclines**

These are a class of antibiotics having a nucleus of four cyclic rings. All are obtained from soil actinomycetes.

![Tetracycline](image)

4. **Quinolones and Fluoroquinolones**

These are entirely synthetic antimicrobials having a quinolone structure that are active against gram negative and gram positive bacteria.

![Ciprofloxacin](image)

5. **Miscellaneous**

This includes aminoglycosides (E.g. streptomycin) and macrolide antibiotics (E.g. Erytromycin).
1.4.2 ANTIFUNGAL AGENTS

Classification

ACCORDING TO CHEMICAL STRUCTURE:

1) Antifungal antibiotics:-
   a) Polyenes: Amphotericin B, Nystatin, Natamycin
   b) Heterocyclic Benzofurans: Griseofulvin

2) Synthetic Antifungal drugs:-
   i) Azoles:
      (a) Imidazole derivatives: Clotrimazole, Econazole, Miconazole,
          Ketoconazole, Oxiconazole
      (b) Triazole derivatives: Fluconazole, Intraconazole
   ii) Allylamines: Terbinafine, Naftifine
   iii) Thiocarbamate; Tolnaftate
   iv) Derivatives of Unecyclenic acid: Ointment “Zincudin” & Undecin
   v) Pyrimidine Antagonists: Flucytosine
   vi) Echinocandin: Caspofungin, Micafungin, Anidulafungin
   vii) Antifungal drugs with other chemical structure:
      (a) Degudinium chloride
      (b) Iodine Drugs: KI
      (c) Drugs of salicylic acid
**ACCORDING TO MODE OF ACTION:**

1) Alter cell permeability: Azoles, Polyenes, Terbinafine
2) Disrupt microtubule function: Griseofulvin
3) Ergosterol binding: Amphotericin
4) DNA-RNA inhibitors: Flucytosine
5) Ergosterol synthesis inhibitors/P450 inhibitors: Ketoconazole, Itraconazole, Fluconazole, Voriconazole
6) B(1-3) glucan inhibitors: Caspofungin, Micafungin, Anidulafungin.

**ACCORDING TO SITE OF ACTION:**

1) Systemic Antifungal Drugs
   i) For Systemic Mycoses: Amphotericin B, Flucytosine, Azoles, Echinocandins
   ii) For Mucocutaneous Mycoses: Griseofulvin, Terbinafine
2) Topical Antifungal Drugs: Nystatin, Topical Azoles, Topical Allylamine

**1.5 CANCER:**

The term carcinoma is the medical term for a malignant tumor derived from epithelial cells. It was Celsius who translated *carcino* into the Latin *cancer*, also meaning crab. Galen used "*oncos*" to describe all tumors, the root for the modern word oncology.

Hippocrates described several kinds of cancers. He called benign tumors “*oncos*”, Greek for swelling and malignant tumors “*carcinos*”, Greek for crab or crayfish. This name comes from the appearance of the cut surface of a solid malignant tumor; with a roundish hard centre surrounded by a pointy projection, vaguely resembling the shape of crab. He later added the suffix -*oma*, Greek for swelling, giving the name *Carcinoma*.

Since it was against Greek tradition to open the body, Hippocrates only described and made drawings of outwardly visible tumors on the skin, nose, and breasts. Treatment was based on the humor theory of four bodily fluids (black and yellow bile, blood, and phlegm). According to the patient's humor, treatment consisted of diet, blood-letting, and/or laxatives. Through the centuries it was discovered that cancer could occur anywhere in the body, but
humor-theory based treatment remained popular until the 19th century with the discovery of cells.

Cancer is the gross distortion of the cell behavior caused by numerous gene mutations and numerous abnormalities in the production of functioning proteins. The specific abnormalities vary greatly, depending on the type of cancer as well as the type of tissue from which cancer has originated.

Thus, there is not a single description of cancer or oncogenesis, because cancer is not a single disease. It is really a class of diseases all pertaining to unlimited cell growth that is potentially fatal to the organism.

Cancer initiates from a single cell that has been transformed due to particular changes in its DNA. Some events such as exposure to radiation or exposure to chemical carcinogen, creates a change in genome. This may be a DNA mutation, or epigenetic modification. In comparison with normal cell, a neoplastic cell is hyper responsive to growth factors, under responsive to growth inhibitors, and has an increase in metabolic transport capabilities.

Broadly, carcinomas are the cancers of epithelial cells; sarcomas are cancers of connective tissue or muscle cells, and leukemias are cancers of the blood or lymph systems. In the normal human population, over 90% of all human cancers are carcinomas.
Different types of cancers

- Uterus
- Bladder
- Melanoma
- Ovary
- Brain and CNS
- Breast
- Colorectal
- Cervix
- Melanoma
- Kidney
- Esophagus
- Leukemia
- Non-Hodgkin lymphoma
- Multiple myeloma
- Pancreas
- Prostate
- Stomach

Causes of Cancer

Cancer is a diverse class of diseases which differ widely in their causes and biology. The common thread in all known cancers is the acquisition of abnormalities in the genetic material of the cancer cell and its progeny. Research into the pathogenesis of cancer can be divided into three broad areas of focus. The first area of research focuses on the agents and events which cause or facilitate genetic changes in cells destined to become cancer. Second, it is important to uncover the precise nature of the genetic damage, and the genes which are affected by it. The third focus is on the consequences of those genetic changes on the biology of the cell, both in generating the defining properties of a cancer cell, and in facilitating additional genetic events, leading to further progression of the cancer. Chemical carcinogens, Ionizing radiation, Hormonal imbalances, Immune system dysfunction, Heredity (45) and others.

New Research in Cancer Treatment

- Genetically engineered bacteria and viruses
- Anti-cancer vaccines
- Cancer markers
- Cancer growth retarders / inhibitors
2.1 BENZOTHIAZOLE

Anti-cancer activity

1. Malcolm F.G. Stevens et al (2001)\textsuperscript{46}, has been reported a series of sulfamate salt derivatives of the potent and selective 2-(4-aminophenyl) benzothiazole antitumor agents has been prepared and their evaluation as potential prodrugs for parenteral administration carried out. The sodium salts of [4-(1,3-benzothiazol-2-yl)phenyl] sulfamic acid (fig1) was found to be markedly less active than their parent amines against sensitive human tumour cell lines \textit{in vitro} (MTT assay) at IC\textsubscript{50} in the range <0.0001–0.001 Mm.

![Fig 1](image1)

2. Albert Sun Chi Chan et al (2008)\textsuperscript{47}, has been reported the ‘one pot’ condensation reaction for the synthesis of phthalic imide derivative (fig 2 benzothiazole containing phthalimide), exhibiting \textit{in vitro} cytotoxic potential on human cancer cell lines in One Step ATP lite assay at 25 µg/mL concentration.

![Fig 2](image2)

3. Yuichi Sugano et al (2005)\textsuperscript{48}, has been reported synthesized as a biologically stable derivative containing no nitro group. The highly potent derivative (fig 3) exhibited excellent \textit{in vivo} inhibitory effect on tumor growth at 13ng/mL concentration.

![Fig 3](image3)
4. Hoyun lee et al (2009)\textsuperscript{49}, has been reported, to design and synthesis isatin-benzothiazole analogs to examine their anti-breast cancer activity. Compounds examined were quite effective on all the cancer cell lines examined the compounds 4-chloro-1-dimethylaminomethyl-3-(6-methyl-benzothiazol-2-ylimino)-1, 3-dihydroindol-2one (fig4) emerged as the most active compounds of this series.

![Fig 4](image)

5. Roman lesyk et al (2010)\textsuperscript{50} has been reported reactions of (benzothiazole-2yl)hydrazine with trithiocarbonyl diglycolic acid or 6-methyl-2-aminobenzothiazole with 2-carbethoxy methylthio-4-one have yielded starting 3- or 2-substituted 4-thiazolidiones which have been subsequently utilized in a knoevenagel condensation for obtaining a series of 5-arylidene derivative es.\textit{in vitro} anticancer activity of the synthesized compounds (fig5) and (fig6) were tested the anti cancer activity on leukemia, melanomia, lung, colon, renal, and prostate and breast cancers cell lines.

![Fig 5](image) ![Fig 6](image)

**Anti-microbial activity**

6. Samir bondock et al (2009)\textsuperscript{51} has been reported synthesis of polyfunctionally substituted heterocyclic incorporating benzothiazole moiety via its reactions with some N-
nucleophiles. Representative compounds (fig7) of the synthesized products were tested and evaluated as antimicrobial agents.

![Fig 7](image)

7. **Samir bondock et al (2010)** has been reported in an attempt to find a new class of antimicrobial agents, a series of thiazole, thiophene, pyrazole and other related products containing benzothiazole moiety were prepared. These compounds (fig8) were screened for their antibacterial activity and antifungal.

![Fig 8](image)

8. **Balram soni et al (2010)** has been reported as benzothiazole has proven to be good antimicrobial agent, a novel series of schiff bases of benzothiazole derivatives were synthesized. All the synthesized compounds (fig9) were screened for their antimicrobial activity.

![Fig 9](image)

9. **Barot Hitesh k et al (2010)** has been reported synthesized 2-amino-7-chloro-6-fluoro benzothiazole by using 4-fluoro-3-chloroaniline and potassium thiocyanate. The title compound was screened for anti-bacterial (agar diffusion method) and anti oxidant activity.
(ferric ion reduction method) and inhibition of denaturation of protein. Most of the compounds (fig10) have shown promising activity.

![Fig 10](image)

10. **Sohail saeed et al (2010)**\(^{55}\) has been reported five series of thiourea derivatives bearing benzothiazole moiety (20 compounds) were efficiently synthesized and evaluated for antimicrobial and anticancer activities. In preliminary MTT cytotoxicity studies, the thiourea derivatives (fig11) were found most potent.

![Fig 11](image)

R=H, NO\(_2\), NH\(_2\), Br

R\(_1\)=4-Nitrophenyl, 2-thiophene, n-butyl

**Anti-inflammatory activity**

11. **Paramashivappa et al (2003)**\(^{56}\), has been reported a series of 2-[(2-alkoxy-6-pentadecyl phenyl) methyl] thio-1-Hbenzimidazoles/ benzothiazole from anacardic acid (pentadecyl salicylic acid) and investigated their ability to inhibit human cyclooxygenase enzyme-2. The active compounds were screened for cyclooxygenase-1 (COX-1) inhibition. Compound 2-(2-methoxy-6-pentadecylbenzylthio)benzo[d]thiazole (fig 12) is more than 470-fold selective towards COX-2 compared to COX-1.
12. **Young-Rae et al (2011)**, has been reported the effects of a benzothiazole analog, SPA0537 (fig 13) exerts anti-inflammatory effects in rheumatoid FLS through the inhibition of the NF-κB pathway. Therefore, it may have therapeutic value for the treatment of rheumatoid arthritis.

13. **Russol et al (1994)**, has been reported synthesis of a series of substituted analogues based on the novel 4H thieno[2’,3’:4,5]pyrimido [2,1-b]benzothiazole and 4H-thieno[2’,3’:4,5]pyrimido [2,1-b]benzoxazole r&g systems was synthesized.Synthesized compounds were evaluated for their potential analgesic activity in phenylquinone-induced writhing test in mice and for their potential antiinflammatory activity in carrageenan-induced rat-paw oedema test, in acetic-acid peritonitis assay and in cotton oil-induced mouse-ear oedema test. 9,10,1 1,12-Tetrahydro-12H benzothieno [2’,3’:4,5]pyrimido [2,1-]benzoxazol-l2-one (fig 14) is the most active derivative in the series in all performed tests. It showed remarkable analgesic and antiinflammatory activities associated with an excellent gastric tolerance.
14. Venkatesh et al (2009) 59, has been reported a series of some novel 2-amino benzothiazole derivatives were synthesized and evaluated for anti-inflammatory activity. The anti-inflammatory activities of new compounds were determined by \( \lambda \)-Carrageenan-induced mice paw edema method using diclofenac sodium as a standard. Among the compounds tested three compounds Bt2 (5- chloro-1,3-benzothiazole-2-amine), and Bt7 (6-methoxy-1,3-benzothiazole- 2-amine) (fig 15) were the most active compounds in these series when compared with diclofenac sodium.

\[ \text{SN} \quad \text{NH}_2 \]

\[ R = 5\text{Cl Bt2} \]
\[ R = 6\text{-OCH}_3 \text{ Bt7} \]

Fig 15

15. Muttu et al (2010) 60, has been reported synthesize a series of various substituted benzothiazole derivatives containing 7-chloro-6-fluoro-N (substituted hydrozones) - benzothiazole. The compounds MIIIc, MIIIf and MIIIJ at dose 5 mg/kg and 10mg/kg body.wt were evaluated for anti-inflammatory activity using carragennan induced paw edema method. The selected compounds have shown significant anti-inflammatory activity as compared to the standard drug. Compound MIIIJ (fig 16) (10 mg/kg body weight) has shown more significant result when compared with standard drug.

\[ \text{SN} \quad \text{NH}_2 \]

\[ \text{Cl} \quad \text{F} \quad \text{N} \quad \text{OCH}_3 \quad \text{OCH}_3 \quad \text{OCH}_3 \]

Fig 16
Anthelmentic activity

16. Sreenivasa M et al (2009) has been reported synthesis of 6-flurobenzothiazole comprising sulfonamide pyrazole derivatives. They screened synthesized for anthelmentic activity by using earthworms (Peritum posthum). Albendazole was used as standard drug. The compounds were evaluated by time taken for complete paralysis and death of worms.

![Figure 17](image17.png)

Anti-diabetic activity

17. Pattan S et al (2005) has been reported synthesis of 2-amino [5’ (4-sulphonylbenzylidine)-2,4-thiazolidinedione]-7-chloro-6-flurobenzothiazole series and screened for their antidiabetic activity on albino rat by alloxan induced tail tipping method.

![Figure 18](image18.png)

Microsomal triglyceride transfer protein (MTP) inhibitors

18. Chi B et al (2009) has been reported synthesis of triamide derivatives based on benzothiazoletemplate. A series of these compounds showed potent enterocyte-specific microsomal triglyceride transfer protein (MTP) inhibitors. Inhibition of MTP by small molecules, therefore lead to reduction in plasma triglycerides and cholesterol level.

![Figure 19](image19.png)
Anti alzheimers activity

19. Serdons K et al (2009)\textsuperscript{64} has been reported a F-labeled 2-(4’-fluorophenyl)-1-3-benzothiazoles. They evaluated it as amyloid imaging agent in Alzheimers disease in comparison with [11C]PIB (11C labeled 6-hydroxy-2-(4”-N- [11C] methylaminophenol)-1,3-benzothiazole and showed excellent characteristics comparable with those of [11C]PIB, namely good affinity for amyloid plaques present in human Alzheimers disease.

\[\text{Fig 20}\]

2.2 PYRAZOLONE

Anti- Mycobacterial Activity

20. S.Guniz Kucukguzel et.al. \textsuperscript{65} has been reported Synthesis, characterization of novel coupling products and 4-arylhydrazono-2-pyrazoline-5-ones as potential anti-mycobacterial agents. Novel coupling products and 4-arylhydrazono-2-pyrazoline-5-ones were synthesized and evaluated for anti-mycobacterial activity against \textit{Mycobacterium tuberculosis} H37Rv and \textit{Mycobacterium avium}. fig 21 was found to be the most potent derivatives of the series by an MIC value of 6.25µg/ml.

\[\text{Fig 21}\]

21. Daniele Castagnolo \textit{et.al.} \textsuperscript{66} has been reported Synthesis, biological evaluation and SAR study of novel pyrazole analogues as inhibitors of \textit{Mycobacterium tuberculosis} two series of pyrazole derivatives were synthesized by parallel solution- phase synthesis and were assayed as inhibitors of \textit{Mycobacterium tuberculosis} (MTB), which is the causative agent of tuberculosis. One of these compounds showed high activity against MTB (MIC = 4µg/ml). The newly synthesized pyrazoles were also computationally investigated to analyze their fit properties to the
pharmacophoric model for anti-tubercular compounds previously built by us and to refine structure–activity relationship analysis. The pyrazole derivative, $8g$, with the p-bromophenyl group at N$_1$ position showed to be very active.

![Chemical Structure of 8a-k](image)

Where R = -Br, R$_1$ = -CH$_3$.

**Fig 22**

**22. Daniele Castagnolo et al.,**$^{67}$ has done Synthesis, biological evaluation, and SAR study of novel pyrazole analogues as inhibitors of Mycobacterium tuberculosis: Part 2. Synthesis of rigid pyrazolones. The newly synthesized pyrazolones were also computationally investigated to analyze if their properties fit the pharmacophoric model for anti-tubercular compounds previously built by us. The results are in agreement with those reported by us previously for a class of pyrazole analogues and confirm the fundamental role of the p-chlorophenyl moiety at C4 in the anti-mycobacterial activity. Compounds 5f–g, bearing N-Me-piperazine and morpholine moieties proved to be very active with MIC 4 µg/ml.

![Chemical Structure of 5A-K](image)

Where 5f R = -Cl, R$_1$ = N-Me-piperazine, 5g R = -Cl R$_1$ = Morpholine.

**Fig 23**
Anti-Bacterial Activity

23. Amar R. Desai et al.,\textsuperscript{68} has been reported microwave induced and conventional synthesis of quinazolinones and 3-methyl-1H-5-pyrazolones by Niementowski reaction to synthesize 3-substituted/2,3-disubstituted 4(3H) quinazolinones instead of the 2-substituted derivatives. All synthesized compounds were screened for their antibacterial activity against gram positive and gram negative bacteria and showed good to significant activity, as well as antifungal activity against Candida albicans ATCC 10231 and C. krusei GO3. The compounds 3a, 3b, 3e, 3g and 3h showed significant activity against E.Coli and P. aeruginosa at 100 µg/ml concentration.

![Fig 24](image)

Anti-Fungal Activity

24. R. Venkat Ragavan et al.,\textsuperscript{69} has been reported Synthesis of some novel bioactive 4-oxy/thio substituted-1H-pyrazol-5(4H)-ones via efficient cross-Claisen condensation. α-Oxy/thio substituted-β-keto esters were synthesized and converted in situ into 4-oxy/thio substituted-1H-pyrazol-5(4H)-ones by the addition of hydrazine derivatives and screened for their antibacterial, antifungal activities against Aspergillus flavus, Aspergillus fumigates, Candida albicans, Penicillium marneffei and Trichophyton mentagrophytes in DMSO by serial plate dilution method and compounds 1a–c, 1e–g, 1i, 1j and 1m–p were active.

![Fig 25](image)

Where \( X \quad R_1 \quad R_2 \quad R_3 \)

1a, O -Ph -CH$_2$OPh -H
25. **S. Guniz Kucukguzel et al.**\(^7\) has been described Synthesis, characterization and pharmacological properties of some 4-arylhydrazono-2-pyrazoline-5-one derivatives obtained from heterocyclic amines compounds were tested in vitro against Gram-positive and Gram-negative bacterial strains, two mycobacterial strains and a fungus, *Candida albicans*. Compound 22 was found to be more active against *Staphylococcus aureus* than the other compounds at a concentration of 15.6 μg/ml.

\[
\begin{align*}
\text{NH} & \quad \text{O} \\
\text{CH}_3 & \quad \text{R}
\end{align*}
\]

Where Compound 22 \( R = -C_6H_5 \).

**Fig 26**

**Anti-Cancer Activity**

26. **Magda M.F. Ismail et al.**,\(^7\) has been done Synthesis and docking studies of novel benzopyran-2-ones with anticancer activity Novel series of 7-substituted-benzopyran-2-ones was synthesized by incorporating heterocyclic rings as oxadiazole, triazole, pyrazole or pyrazolin-5-one to benzopyran-2-one nucleus at p-7 via methylene-oxy (or) acetoxy linker. Among 12, compound 9a exhibited broad spectrum antitumor activity showing full panel median growth inhibition (GI50) \( \frac{1}{4} \) 5.46 mM. According to docking results using Mol soft ICM 3.4-8c program, the target compounds may act through inhibition of topoisomerase 1, where camptothecin is used as ligand.

\[
\begin{align*}
\text{HN} & \quad \text{O} \\
\text{O} & \quad \text{R}
\end{align*}
\]

Where Compound 9a \( R = -\text{ph}, R_1 = -\text{CH}_3 \).

**Fig 27**
27. Rahat Khan et al.,\textsuperscript{72} has been studied Synthesis and preliminary evaluation of brominated 5-methyl-2,4-dihydropyrazol-3-one and its derivatives as cytotoxic agents. The cytotoxicity studies of the synthesized compounds revealed that compound 4,4-dibromo-2-(2,4-dinitro-phenyl)-5-methyl-2,4-dihydropyrazol-3-one (2c) had shown tremendous bioactivity against brine shrimp. However, the compounds 4,4-dibromo-5-methyl-2,4-dihydropyrazol-3-one (2a), 4,4 dibromo-2-(2,4-dibromo-phenyl)-5-methyl-2,4-dihydropyrazol-3-one (2b) and 4,4-dibromo-2-(dinitro-phenyl)-5-methyl-2,4-dihydropyrazol-3-one (2c) showed higher activity leaving the other compounds almost inactive.

![Fig 28](image)

28. Xiao Hong Wang et al.,\textsuperscript{73} has done A cell based screen for anticancer activity of 13 pyrazolone derivatives against four human tumour cell lines HepG2, OVCAR3 KB and Multi drug resistance (MDR) KBv 200 cell lines invitro and in vivo. The 50% inhibitory concentration (IC\textsubscript{50}) values were determined by MTT assay. Out of 13 compounds screened compound 9 exhibits remarkable effect of which IC\textsubscript{50} values are (3.24± 0.28), (2.58± 0.61), (3.81± 0.02) and (3.45 ± 0.03) µg/ml in HepG2, OVCAR3 KB and Multi drug resistance (MDR) KBv 200 cell lines respectively (p> 0.05).

![Fig 29](image)

29. Hari Pado Devnath et al.,\textsuperscript{74} has been reported Synthesis of some pyrazolone derivatives from ciprofloxacin and study of their cytotoxicity. Three pyrazolone derivatives with
pyrazole ring extension were synthesized from ciprofloxacin by treating the parent compound with hydrazine derivatives. These derivatives showed potential cytotoxicity against brine shrimp nauplii than the ciprofloxacin. The substituted benzene ring by –NO₂ group of the 5-Cyclopropyl-2-(2,4-dinitrophenyl)-8-fluoro-7-piperazin-1-yl-2,5-dihydro pyrazolo [4,3-c]quinolin-3-one, 1b show better activity than unsubstituted benzene.

![Fig 30](image)

Where 1b: R = -C₆H₃(NO₂)₂.

30. G. Mariappan et.al.⁷⁵ has been reported synthesis and bioactivity evaluation of pyrazolone derivatives 3-Methyl-4-substituted benzylidene-pyrazol-5-ones are synthesized by the condensation of 3-methyl-pyrazol-5-one with substituted aliphatic and aromatic aldehydes. Among the synthesized derivatives 5, 6, 7 and 10 are found to have a potent anti-inflammatory response whereas compounds 1, 4, 5, 8 and 10 have an effective analgesic response. There is no remarkable difference in bioactivity of pyrazolones derived from aliphatic and aromatic aldehydes.

![Fig 31](image)

Where, R = 3-Cl.

**Non steroidal anti-inflammatory activity**

31. Soad A.M. El-Hawash et.al.⁷⁶ has been prepared “Non steroidal anti-inflammatory agents—part 2 anti-inflammatory, analgesic and antipyretic activity of some substituted 3-pyrazolin-5-ones and 1,2,4,5,6,7-3H-hexahydroindazol-3-ones.” Anti-inflammatory, analgesic and antipyretic activities recorded for some of new 3-pyrazolin-5-ones. A structure–activity
relationship (SAR) comparative study indicated that some compounds from 3-pyrazolin-5-one (2, 6–8, 10) series exhibited pronounced anti-inflammatory, analgesic and antipyretic activities relative to indomethacin.

![Figure 32](image)

32. Amol Gadakh et al., 77 has been described an efficient synthesis of 3-trifluoromethyl-1-(3,4-difluorophenyl)-1H-pyrazol-5(4H)-one (3) and their Knoevenagel condensation reaction with 1,4-diphenyl-1H-pyrazole-3-carbaldehydes, 4,4-oxo-4H-chromene-3-carbaldehydes and 2-chloro quinoline-3-carbaldehydes have been described by using conventional and non-conventional techniques. Comparison of conventional and non-conventional techniques like Microwave, Ultrasonic assisted reactions showed that, the later procedure require shorter reaction time, good yield and was applicable for larger set of substrates emphasizing the importance of eco-friendly conditions.

![Figure 33](image)

33. Angela Antochi et al., 78 has been studied the synthesis and modelling of the obtaining process of 1-[2'-(theophyllin-7-y1) sulfonyl-4-chlor-phenoxyacetyl]-3-methyl-5-pyrazolone starting from 4-chlor-2-(theophyllin-7'-yl)sulfonyl-phenoxyacetyl hydrazide and concluded the optimum reaction time is 18.35 hours. The optimum ratio of the reactants hydrazide and ethyl acetoacetate is of 1/2.49 and the output for 1-[2'-(theophyllin-7-y1) sulfonyl-4-chlor-phenoxyacetyl]-3-methyl-5-pyrazolone tends toward optimum when all the considered variables remain in the limits of the initially established variation domain.
34. Mohamed A. Saleh et al., 79 has been reported Synthesis of Novel 3H-Quinazolin-4-ones Containing Pyrazolinone, Pyrazole and Pyrimidinone Moieties from the diazonium salt of 3-(4-aminophenyl)-2-methyl-3H-quinazolin-4-one and its 6-bromo derivative reacted with some active methylene compounds, namely ethyl acetoacetate, ethyl cyanoacetate and acetylacetone, to afford the corresponding hydrazono quinazolinone derivatives and they are tested for Non steroidal anti-inflammatory activity.

Where Compound 9a R = H, X = H, 9b R = H, X = Br, 9c R = Ph, X = H, 9d R = Ph, X = Br.

35. Ruoquin Ma et al. 80 has been developed An efficient one-pot method to generate structurally diverse and medicinally interesting pyrazolone derivatives in good to excellent yields of 51–98% under microwave irradiation and solvent-free conditions. 4-arylidenepyrazolone derivatives 1 are synthesized using as starting materials substituted aldehydes 5 or imine and 2-pyrazolin-5-ones 4, the latter generally being obtained by the Knorr condensation of β-ketoesters 2 with substituted hydrazines 3.
2.3 Mannich Bases

Sheela Joshi et al.,\(^8\) has been reported “In vitro study of some medicinally important Mannich bases derived from anti-tubercular agent”, Biologically active Mannich bases with heteroaromatic ring system have been synthesised employing Mannich reaction of isonicotinyl hydrazide with various sulphonamides/secondary amines and Comparing antibacterial activity of newly synthesized Mannich bases with sulphonamides observed that Mannich (Fig 37) is significantly better than its corresponding sulphonamide in checking the growth of P. multocida.

\[ R = \text{-COCH}_3\text{Na (H}_2\text{O). Fig 37} \]
Since resistance of pathogenic bacteria towards available antibiotics is rapidly becoming a major worldwide problem, the design of new compounds to deal with resistant bacteria has become one of the most important areas of anti-microbial research today.

During the past decades, large numbers of therapeutic agents are synthesized with the help of benzothiazole nucleus because of their pharmacological properties such as anti-microbial, anti-tumor, anti-tubercular, immunosuppressive, anti-convulsant, anti-inflammatory, anti-diabetic, etc.

Moreover, the benzothiazole structure containing sulphur and nitrogen in five membered ring systems which is similar to that of first anti-microbial agent penicillin and its derivatives ring system.

![Penicillin and Benzothiazole](image)

The literature review shows that many researchers have synthesised 2-substituted benzothiaozle as a target structure and evaluated its biological activities. e.g.

1. **Barot Hitesh.k.et.al., (2010)** has been reported synthesis and characterisation of various substituents of 2-amino-7-chloro-6-fluoro benzothiazole. The results suggest that among the compounds tested 2-(2-(3,4,5-trimethoxybenzylidene)hydrazinyl)-N,N-2-hydroxydiethyl-6-fluorobenzo[d]thiazol-7-amine (fig 3.2) have exhibited higher activity.

![Substituted Benzothiazole](image)

2. **Roman lesyk.et.al., (2010)** has been reported the anti-cancer activity of (benzothiazole-2-yl) hydrazine with trithiocarbonyl diglycolic acid or 6-methyl-2-
aminobenzothiazole with 2-carbethoxymethylthio-4-one have yielded starting 3- or 2-substituted 4-thiazolidiones.

![Chemical Structure](image)

**Fig. 3.3**

On the other hand, pyrazolone derivatives have engrossed substantial attention from organic and medicinal chemists for many years as they belong to a class of compounds with proven utility in medicinal chemistry. Antipyrine was the first pyrazolone derivative for management of pain inflammation and fever. In addition, many pyrazolone derivatives have special ability to scavenger reactive oxygen species (ROS) and to influence processes involve in free radical injury, large numbers of therapeutic agents are synthesized with the help of pyrazolone nucleus because of their pharmacological properties anti-microbial\(^{34}\), anti-cancer\(^{35}\), anti-inflammatory\(^{36}\), analgesic\(^{36}\), anti-pyretic\(^{36}\), anti-bacterial\(^{37}\), anti-tubercular\(^{38}\), anti-oxidant activities, etc.,.

The literature review shows that many researchers have synthesised pyrazolone derivatives as a target structure and evaluated its biological activities, e.g.

1. **S. Guniz Kucukgu et.al.**,\(^{65}\) has been reported Synthesis, characterization of novel coupling products and 4-arylhydrazono-2-pyrazoline-5-ones and screened for their anti-mycobacterial against *Mycobacterium tuberculosis* H37Rv and *Mycobacterium avium*.

![Chemical Structure](image)

**Fig. 3.4**
2. Soad A.M. El-Hawash et. al. \[76\] has been reported synthesis and anti-inflammatory, analgesic and antipyretic activity of some substituted 3-pyrazolin-5-ones and 1,2,4,5,6,7-3H-hexahydroindazol-3-ones

![Fig. 3.5](image)

From the above literatures, some of the compounds bearing the pharmacophore groups showed in the fig 3.6 have been reported to responsible for anti-microbial activity and anti-cancer activity moreover the pyrazolone has been reported as anti-oxidant properties. With this background the aim of our present study is to couple benzothiazole and pyrazolone through azomethine protons (-NHN=CH) linkage to constitute important new class of compound, have potent anti-microbial, anti-oxidant properties with less side effects.

**THE PROPOSED STRUCTURE**
The plan of the present work can be summarized as follows:

- Literature review and scheme development.
- To synthesis some novel Benzothiazole nucleus containing pyrazolone, having active hydrogen in 2\textsuperscript{nd} position (NH) and subject to N-Mannich reaction to form title compounds.
- To determine physicochemical properties of synthesized compounds by Melting point, TLC and solubility.
- Characterization of synthesized compounds by various analytical techniques like UV, IR, \textsuperscript{1}HNMR and Mass Spectral studies.
- Screening of synthesized compounds for their \textit{in-vitro} anti-microbial activity against gram positive and gram negative bacteria and fungal by Disc diffusion method and determination of Minimum inhibitory concentration (MIC) by 2-fold serial dilution method.
- Screening of synthesized compounds for their \textit{in-vitro} anti-oxidant property by DPPH, FRAP and ABTS methods.
- Screening of synthesized compounds for \textit{in-vitro} cytotoxic activity by MTT assay method.
DETERMINATION OF PHYSICOCHEMICAL PROPERTIES OF SYNTHESIZED COMPOUNDS

MELTING POINT ANALYSIS

Melting points of the synthesized compounds were determined in a one end fused capillary tube method by using Thermionic Model–C-LMP- 1 CAMPVEEL Serial. No. 0712022 melting point apparatus, and were uncorrected.

THIN LAYER CHROMATOGRAPHY ANALYSIS

Purity of the compounds was checked by TLC using silica gel G (0.5mm thickness) coated over glass plate (12 x 20 cm). For the determination $R_f$ value the dried silica gel G coated over glass plate were used.

Preparation of TLC plate: By using distilled water silica gel G slurry is prepared and poured on to a glass plate which is maintained on a level surface. The slurry is spread uniformly on the surface of the glass plate. After setting, the plates are dried in an oven at 50°C or 15 minutes for activating the TLC plate

Chromatogram was developed by ascending technique when solvent front travelled appropriate distance; plates were taken out and dried. The location of spot was detected by using iodine chamber.

$$R_f = \frac{\text{Distance travelled by solute}}{\text{Distance travelled by solvent}}$$

SOLUBILITY

Solubility the solubility of synthesised compound was tested in different solvents such as polar, semi polar, and non polar solvents.

Polar solvent: A solvent in whose molecules there is either a permanent separation of positive and negative charges, or the centres of positive and negative charges do not coincide; these solvents have high dielectric constants, are chemically active, and form coordinate covalent bonds; examples are water and carboxylic acids etc.
Semi polar solvent: Semi-polar solvents may induce a certain degree of polarity in non-polar molecules and may thus act to improve the miscibility of polar and non-polar liquids; examples are alcohols and ketones etc.

Nonpolar solvent: a liquid solvent without significant partial charges on any atoms, as in the hydrocarbons, or where the polar bonds are arranged in such a way that the effects of their partial charges cancel out, as in carbon tetrachloride. Liquid hydrocarbons are the most common examples.

The following table 5.1 indicates the meanings of the terms used in statements of approximate solubilities.

**Table: 5.1**

<table>
<thead>
<tr>
<th>Descriptive term</th>
<th>Approximate volume of solvent in millilitres per gram of solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very soluble</td>
<td>less than 1</td>
</tr>
<tr>
<td>Freely soluble</td>
<td>from 1-10</td>
</tr>
<tr>
<td>Soluble</td>
<td>from 10-30</td>
</tr>
<tr>
<td>Sparingly soluble</td>
<td>from 30-100</td>
</tr>
<tr>
<td>Slightly soluble</td>
<td>from 100-1000</td>
</tr>
<tr>
<td>Very slightly soluble</td>
<td>from 1000-10,000</td>
</tr>
<tr>
<td>Insoluble or practically insoluble</td>
<td>more than 10,000</td>
</tr>
</tbody>
</table>
SPECTRAL STUDIES
CONFIRMATION OF THE STRUCTURE OF SYNTHESIZED COMPOUNDS

ULTRA VIOLET SPECTRAL ANALYSIS

The maximum absorbance or \( \lambda_{\text{max}} \) of synthesized compounds were determined at 0.01% w/v concentration in ethanol by using Shimadzu 2000, UV1700 ultraviolet Spectrophotometer at KMCH College of pharmacy. The maximum absorbance was measured in nm.

INFRARED SPECTRAL ANALYSIS

The IR Spectra of the synthesized compounds were recorded at KMCH college of pharmacy by JASCO-FT/IR -1700, Serial no B016861016 spectrophotometer in KBr disc. The IR value was measured in cm\(^{-1}\).

NUCLEAR MAGNETIC RESONANCE SPECTRAL ANALYSIS

The NMR Spectra of the synthesized compounds were recorded at IIT Madras by Bruker 300 MHz FT- NMR using TMS (Tetra Methyl Silane) as internal standard. The PMR (Proton Magnetic Resonance) spectroscopic values are measured in \( \delta \) ppm in DMSO-d\(_6\). Compounds were particulars of work done on DSX-300/AV-III 400/DRX-500/AV-III 500(S)/(L) AV-700 NMR spectrometer.

MASS SPECTRAL ANALYSIS

The Mass Spectra of the synthesized compounds were recorded at IIT Madras in MS (EI) JEOL GC MATE 700 EV.
METHODOLOGY

Step I: Synthesis of 6-bromobenzo[d]thiazol-2-amine (BTZ) \(^{84}\)

To glacial acetic acid (150ml), precooled to 5°C, ammonium thiocyanate (0.06 mol, 4.56gm) and 4-bromoaniline (0.06mol, 10.26gm) were added. The mixture was placed in dry ice then addition of bromine (0.02mol, 1ml of bromine dissolved in 10ml of glacial acetic acid) from a dropping funnel at such rate that temperature does not rise above 5°C, stirring was continued for an additional 6 hrs at 0-10°C and neutralized with aqueous ammonia solution, kept it in overnight, filtered, washed with water and dried, recrystallised from ethanol to obtain grey color precipitate of 6-bromo benzo [d] thiazol-2-amine (BTZ). The purity of compounds was established by single spot on TLC plate. The solvent system used for TLC was chloroform: methanol (7:3) and Yield: 95.06%w/w, m.p: 204-206°C, Rf: 0.786. Table: 5.2.

\[
\text{Br} \quad \text{NH}_2 \\
\begin{array}{c}
\text{NH}_4\text{SCN} \\
\text{gl. Acetic acid, Br}_2 \\
\text{stirring, 6hrs} \\
\text{0-10°C}
\end{array} \\
\begin{array}{c}
\text{S} \\
\text{N} \\
\text{Br} \\
\text{6-bromobenzo[d]thiazol-2-amine}
\end{array}
\]

Step II: Synthesis of Ethyl [2-(6-bromo-1, 3-benzothiazol-2-yl) hydrazinylidene](cyano)acetate (BTZE) \(^{85}\)

Dissolve 6-bromo benzo [d] thiazol-2-amine (BTZ) (0.05 mol, 11.45gm) in concentrated HCl (20ml) and water (10 ml), cooled to 0-5°C under dry ice and precooled solution of sodium nitrite (1.5gm in 10ml of water) was added to it dropwise during 10mts. The reaction mixture was stirred for 40 minutes. The ice cold mixture of ethyl cyano acetate (0.01mol, 1.12gm) and saturated solution of sodium acetate (0.05mol, 4.1gm) in ethanol (50ml), was added dropwise with stirring to a solution of diazonium salt compound over 15 mts. The stirring was continued for 30 mts at 0-5°C and the reaction mixture then stirred for 2.30 hrs at room temperature. The product was collected and recrystallised from ethanol to give grayish brown color solid of Ethyl [2-(6-bromo-1, 3-benzothiazol-2-yl) hydrazinylidene](cyano)acetate (BTZE). The purity of compounds was established by single spot on TLC plate. The solvent system used was benzene: chloroform: water (40:35:25) and Yield: 58.07%w/w, m.p: 174-176°C, Rf: 0.446. Table: 5.2
Step III: Synthesis of 5-amino-4-[2-(6-bromo-1, 3-benzothiazol-2-yl) hydrazinylidene]-2, 4-dihydro-3H-pyrazol-3-one (BTZP) 

A mixture of ethyl cyano[2-(4-phenyl-1,3-thiazol-2-yl)hydrazinylidene]acetate, (0.005mole, 1.5gm) and hydrazine hydrate (0.01 mole, 0.32ml) in 30 ml of ethanol was heated under reflux for 6 hrs. The solvent was concentrated and the product obtained was allowed to cool, filtered, washed with water, dried and recrystallized from ethanol to get brownish red colour solid of 5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one(BTZP). The purity of compounds was established by single spot on TLC plate. The solvent system used for TLC was benzene: chloroform: water (40:35:25) and Yield: 67%w/w, m.p: 211-213˚C, Rf: 0.685 Table: 5.2.

Step IV: Synthesis of Mannich Bases

A mixture of 5-amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one (0.05 mole, 1.73 gm) and 90% formaldehyde (6 ml) were refluxed with different aromatic amines (0.1mole) in ethanol (30 ml) for appropriate time and the reaction was monitored by TLC. The resulting mixture was poured in to crushed ice. The solid obtained was filtered, dried and recrystallized from ethanol. The physicochemical properties and the synthesized compounds were given in the Table: 5.4.
SCHEME

4-Bromobenzenamine + NH₄SCN

Glacial CH₃COOH
0-5°C

Stirring for 6 hrs
Br₂

6-Bromo-1,3-benzothiazol-2-amine

CNCH₂COOCH₂CH₃

CH₃COONa

Stirring 2.30 hrs
NaNO₂/HCl
0-5°C

Ethyl [2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene](cyano)acetate

NH₂NH₂, H₂O
C₂H₅OH

Reflux on water bath 6 hrs
60°C

5-Amino-4-[2-(6-bromo-1,3-benzothiazol-2-yl)hydrazinylidene]-2,4-dihydro-3H-pyrazol-3-one

90% HCHO
C₂H₅OH

Reflux on water bath 4-6 hrs
60°C

MANNICH BASE
LIST OF AMINES USED IN MANNICH BASE

R =

1. \( \text{H}_2N-\text{H}_2\text{O}_\text{OH} \) 4-amino phenol

2. \( \text{H}_2N-\text{H}_2\text{COOH} \) 4-amino benzoic acid

3. \( \text{H}_2N-\text{H}_2\text{OCH}_3 \) 4-methoxy aniline

4. \( \text{H}_2N-\text{H}_2\text{Br} \) 4-bromo aniline

5. \( \text{H}_2N-\text{H}_2\text{Cl} \) 2-chloro aniline

6. \( \text{H}_2N-\text{H}_2\text{Cl} \) 4-chloro aniline

7. \( \text{H}_2N-\text{N}==\text{N}-\text{H}_2 \) 4-amino azo benzene

8. \( \text{H}_2N-\text{H}_2\text{O}_\text{NO}_2 \) 2-nitro aniline

9. \( \text{H}_2N-\text{H}_2\text{NO}_\text{2} \) 4-nitro aniline

10. \( \text{H}_2N-\text{H}_2\text{N} \) 2-amino pyridine

11. \( \text{H}_2N-\text{H}_2\text{H}_2\text{C} \) 2-methyl aniline

12. \( \text{H}_2N-\text{H}_2\text{Br} \) 6-bromobenz[d]thiazol-2-amine
Table: 5.2 PHYSICOCHEMICAL PROPERTIES OF INTERMEDIATES

<table>
<thead>
<tr>
<th>Cpd code</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Rf Value</th>
<th>Melting point °C</th>
<th>CLog P</th>
<th>Polarizability</th>
<th>Percentage Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTZ</td>
<td>C&lt;sub&gt;7&lt;/sub&gt;H&lt;sub&gt;5&lt;/sub&gt;BrN&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>229</td>
<td>0.78</td>
<td>204-206</td>
<td>2.68</td>
<td>19.54</td>
<td>95%</td>
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<tr>
<td>BTZE</td>
<td>C&lt;sub&gt;12&lt;/sub&gt;H&lt;sub&gt;9&lt;/sub&gt;BrN&lt;sub&gt;4&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>353</td>
<td>0.44</td>
<td>174-176</td>
<td>4.78</td>
<td>30.23</td>
<td>57%</td>
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<td>BTZP</td>
<td>C&lt;sub&gt;10&lt;/sub&gt;H&lt;sub&gt;7&lt;/sub&gt;BrN&lt;sub&gt;6&lt;/sub&gt;O&lt;sub&gt;2&lt;/sub&gt;S</td>
<td>339</td>
<td>0.68</td>
<td>211-212</td>
<td>1.02</td>
<td>28.32</td>
<td>62%</td>
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</table>
### Table: 5.3 SPECTRAL DATA OF THE INTERMEDIATES

<table>
<thead>
<tr>
<th>Cpd code</th>
<th>$\lambda_{\text{max}}$</th>
<th>(IR) $\nu_{\text{max}}$ (KBr/cm$^{-1}$)</th>
<th>NMR ($\delta$ ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BTZ</td>
<td>484</td>
<td>3573.45 (NH$_2$ Stretching), 825.026 (Ar,CH=CH Stretching), 1663.3 (C=N Stretching), 669.178 (C=S-C Stretching).</td>
<td>-</td>
</tr>
</tbody>
</table>
| BTZE     | 574                    | 1527.35 (C=N Stretching), 1400.07 (C-N Stretching), 1702.84 (COOR Stretching), 3442.31 (-NH-Stretching), 639.84 (C=S-C Stretching). | 6.63(m,3H,Ar)  
6.60(s,1H,NH=N- proton of hydrazone)  
3.60(s,3H, -CH$_3$ proton of ethyl) |
| BTZP     | 498                    | 3385.00 (NH Stretching), 1676 (C=O Stretching), 1528.38 (C=N Stretching), 3385 (NH$_2$ Stretching), 615.43 (C=S-C Stretching). | 6.7-6.75(m,3H,Ar)  
6.60(s,1H,NH=N- proton of hydrazone)  
7.76(s,1H,NH proton of pyrazolone)  
6.55(s,2H,NH$_2$) |
Table: 5.4  PHYSICOCHEMICAL DATA OF SYNTHESIZED DERIVATIVES

<table>
<thead>
<tr>
<th>Compound code</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Rf Value</th>
<th>Melting point °C</th>
<th>CLog P</th>
<th>Polarisability</th>
<th>%Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1P</td>
<td>C_{17}H_{14}BrN_{7}O_{2}S</td>
<td>460</td>
<td>0.46</td>
<td>209-211</td>
<td>2.53</td>
<td>41.41</td>
<td>95%</td>
</tr>
<tr>
<td>B1PB</td>
<td>C_{18}H_{14}BrN_{7}O_{3}S</td>
<td>488</td>
<td>0.44</td>
<td>189-191</td>
<td>3.28</td>
<td>43.20</td>
<td>72%</td>
</tr>
<tr>
<td>B1M</td>
<td>C_{18}H_{16}BrN_{7}O_{2}S</td>
<td>474</td>
<td>0.72</td>
<td>219-221</td>
<td>3.30</td>
<td>43.30</td>
<td>72%</td>
</tr>
<tr>
<td>B1Br</td>
<td>C_{17}H_{13}Br_{2}N_{7}OS</td>
<td>523</td>
<td>0.66</td>
<td>229-231</td>
<td>4.36</td>
<td>43.69</td>
<td>75%</td>
</tr>
<tr>
<td>B12C</td>
<td>C_{17}H_{13}BrClN_{7}OS</td>
<td>478</td>
<td>0.74</td>
<td>250-251</td>
<td>4.21</td>
<td>42.69</td>
<td>72%</td>
</tr>
<tr>
<td>B14C</td>
<td>C_{17}H_{13}BrClN_{7}OS</td>
<td>478</td>
<td>0.46</td>
<td>219-221</td>
<td>4.21</td>
<td>42.68</td>
<td>72%</td>
</tr>
<tr>
<td>B1AZ</td>
<td>C_{23}H_{18}BrN_{9}OS</td>
<td>548</td>
<td>0.71</td>
<td>240-242</td>
<td>5.69</td>
<td>51.94</td>
<td>69%</td>
</tr>
<tr>
<td>B12N</td>
<td>C_{17}H_{13}BrN_{8}O_{3}S</td>
<td>489</td>
<td>0.56</td>
<td>239-241</td>
<td>3.74</td>
<td>42.78</td>
<td>77%</td>
</tr>
<tr>
<td>B14N</td>
<td>C_{17}H_{13}BrN_{8}O_{3}S</td>
<td>489</td>
<td>0.43</td>
<td>220-222</td>
<td>3.59</td>
<td>42.77</td>
<td>71%</td>
</tr>
<tr>
<td>B1AP</td>
<td>C_{16}H_{13}BrN_{8}OS</td>
<td>445</td>
<td>0.46</td>
<td>221-223</td>
<td>2.67</td>
<td>39.91</td>
<td>72%</td>
</tr>
<tr>
<td>BIT</td>
<td>C_{18}H_{16}BrN_{7}OS</td>
<td>458</td>
<td>0.72</td>
<td>261-263</td>
<td>3.69</td>
<td>42.50</td>
<td>73%</td>
</tr>
<tr>
<td>B1B</td>
<td>C_{18}H_{12}Br_{2}N_{8}OS_{2}</td>
<td>580</td>
<td>0.58</td>
<td>190-192</td>
<td>5.05</td>
<td>48.97</td>
<td>77%</td>
</tr>
</tbody>
</table>

Solubility: partially soluble in ethanol
Table: 5.5 SPECTRAL DATA OF THE SYNTHESIZED COMPOUNDS

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Cpd Code</th>
<th>R</th>
<th>λ\textsubscript{max} (nm)</th>
<th>(IR) (\nu)\textsubscript{max} (KBr/cm\textsuperscript{-1})</th>
<th>NMR (δ ppm)</th>
<th>Molecular ion (m/z)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1P</td>
<td>-N(\text{H})-(\text{OH})</td>
<td>784</td>
<td>1. 1587.7 (C=N),</td>
<td>6.60 (s, 1H, NH=N-proton of hydrazone)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. 832.13 (Ar, CH=CH),</td>
<td>4.8 (d, -CH\textsubscript{2}- methylene),</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. 1598.7 (C=O),</td>
<td>7.5-7.7 (m, Ar- H),</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4. 3141.47 (NH aliphatic),</td>
<td>6.63 (s, 2H, NH\textsubscript{2}),</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>5. 1165.76 (OH),</td>
<td>9.1 (s, 1H, OH).</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>6. 636.53 (C-Br),</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1PB</td>
<td>-N(\text{H})-(\text{COOH})</td>
<td>594</td>
<td>1. 1588.09 (C=N),</td>
<td>6.62 (s, 1H, NH=N-proton of hydrazone)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2. 808.02 (Ar, CH=CH),</td>
<td>7.78-8.04 (m, Ar-H),</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. 3127.01 (COOH),</td>
<td>6.92 (s, 2H, NH\textsubscript{2}),</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>4. 691.35 (C-Br),</td>
<td>4.72 (d, -CH\textsubscript{2}- methylene),</td>
<td></td>
</tr>
<tr>
<td>Cpd code</td>
<td>R</td>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>(IR) $\nu_{\text{max}}$ (KBr/cm$^{-1}$)</td>
<td>NMR ($\delta$ ppm)</td>
<td>Molecular ion (m/z)</td>
</tr>
<tr>
<td>----------</td>
<td>--------------------</td>
<td>-----------------------------</td>
<td>------------------------------------------</td>
<td>---------------------------------------------------------------------------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>B1M</td>
<td>$\text{HN-} \text{OCH}_3$</td>
<td>487</td>
<td>1. 1526.33 (C=N), 2. 862.02 (Ar,CH=CH), 3. 3118.33 (NH aliphatic), 4. 675.92 (C-Br).</td>
<td>6.63(s,1H,NH=N-proton of hydrazone), 3.3(s,3H, (OCH$_3$), 7.7-8.14 (m, Ar-H), 6.73(s,2H,NH$_2$), 4.8 (d, -CH$_2$- methylene),</td>
<td>-</td>
</tr>
<tr>
<td>B1Br</td>
<td>$\text{HN-Br}$</td>
<td>575</td>
<td>1. 1660.63(C=N) 2. 768.49(Ar CH=CH), 3. 3121.22 (NH aliphatic) 4. 1674.87 (C=O), 5. 676.17 (C-Br).</td>
<td>6.90(s,1H,NH=N-proton of hydrazone), 7.5-7.7 (m, Ar-H), 6.7(s,2H,NH$_2$), 4.95 (d, -CH$_2$- methylene),</td>
<td>-</td>
</tr>
<tr>
<td>B12C</td>
<td>$\text{HN-Cl}$</td>
<td>564</td>
<td>1. 697.12(ArCH=CH), 2. 1676.8 (C=O), 3. 771.38 (C-Cl).</td>
<td>6.60(s,1H,NH=N-proton of hydrazone), 8.00 (m, Ar-H), 4.7(s,2H,NH$_2$), 4.9 (d, -CH$_2$- methylene),</td>
<td>-</td>
</tr>
<tr>
<td>Cpd Code</td>
<td>R</td>
<td>$\lambda_{\text{max}}$ (nm)</td>
<td>(IR) $\nu_{\text{max}}$ (KBr/cm$^{-1}$)</td>
<td>NMR ($\delta$ ppm)</td>
<td>Molecular ion (m/z)</td>
</tr>
<tr>
<td>---------</td>
<td>----</td>
<td>----------------</td>
<td>--------------------------------</td>
<td>------------------</td>
<td>------------------</td>
</tr>
</tbody>
</table>
| B14C    | | 518            | 1.  775.24(ArCH=CH),  
2.  1676.80 (C=O),  
3.  697.01 (C-Cl). | 6.40(s,1H,NH=N-proton of hydrazone),  
7.04-7.64 (m, Ar-H),  
6.5(s,2H,NH$_2$),  
4.62 (d, -CH$_2$- methylene), | 478.75  
Base peak 478.47 |
| B1AZ    | | 439            | 1.  1491 (C=N),  
2.  830.20(Ar,CH=CH),  
3.  3363.25( NH aliphatic),  
4.  1655.60 (C=O), | 6.80(s,1H,NH=N-proton of hydrazone),  
7.83-8.0 (m, Ar-H),  
4.72 (d, -CH$_2$- methylene), | 548.42  
Base peak 548.50 |
| B12N    | | 729            | 1.  1669.09 (C=N),  
2.  840.81(Ar, CH=CH),  
3.  1603.52 (C=O),  
4.  3359.39( NH aliphatic),  
5.  1510.95 (-NO$_2$). | 6.63(s,1H,NH=N-proton of hydrazone),  
7.78-8.02 (m, Ar-H),  
6.67(s,2H,NH$_2$),  
4.82 (d, -CH$_2$- methylene), | - |
<table>
<thead>
<tr>
<th>Cpd Code</th>
<th>R</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>(IR) $\nu_{\text{max}}$ (KBr/cm$^{-1}$)</th>
<th>NMR (δ ppm)</th>
<th>Molecular ion (m/z)</th>
</tr>
</thead>
</table>
| **B14N** | ![R-B14N](image) | 654 | 1. 1669.09 (C=N),  
2. 840.81 (Ar, CH=CH),  
3. 1603.52 (C=O),  
4. 3359.39 (NH aliphatic),  
5. 1510.95 (-NO$_2$). | | 6.60 (s, 1H, NH=N-proton of hydrazone),  
7.73-8.14 (m, Ar-H), |
| **B1AP** | ![R-B1AP](image) | 498 | 1. 1593.6 (C=N),  
2. 843.42 (Ar CH=CH),  
3. 1671 (C=O),  
4. 6.56 (s, 1H, NH=N-proton of hydrazone),  
7.78-8.04 (m, Ar-H),  
6.53 (s, 2H, NH$_2$),  
4.72 (d, -CH$_2$- methylene), | | |
| **B1T** | ![R-B1T](image) | 474 | 1. 926.54 (Ar CH=CH),  
2. 1674.87 (C=O),  
3. 1600.63 (C=N),  
4. 1293.36 (C-CH$_3$) | 6.5 (s, 1H, NH=N-proton of hydrazone),  
7.7-7.8 (m, Ar-H),  
6.6 (s, 2H, NH$_2$),  
4.7 (d, -CH$_2$- methylene),  
3.5 (s, -CH$_3$) | 458.33  
Base peak 458.99 |
| **B1B** | ![R-B1B](image) | 483 | 1. 1511.11 (C=N),  
2. 3390.00 (NH aliphatic),  
3. 1673.91 (C=O),  
4. 686.17 (C-Br Streching), | 6.9 (s, 1H, NH=N-proton of hydrazone),  
7.78-8.04 (m, Ar-H),  
6.93 (s, 2H, NH$_2$),  
4.72 (d, -CH$_2$- methylene), | 580.28  
Base peak 580.28 |
IR Spectra of compounds

- **Compound BTZ**

- **Compound BTZE**
**Compound BTZP**

![Compound BTZP diagram]

**Compound B1P**

![Compound B1P diagram]
**Compound B1PB**

![Compound B1PB IR spectrum](image)

**Compound B1M**

![Compound B1M IR spectrum](image)
**Compound B1Br**

![Graph of Compound B1Br]

**Compound B12C**

![Graph of Compound B12C]
- Compound B14C

- Compound B1AZ
Compound B12N

Compound B14N
- **Compound B1AP**

![Compound B1AP Spectrogram](image1)

- **Compound B1T**

![Compound B1T Spectrogram](image2)
Compound B1B
 Compound BTZP
Compound B1P
Compound B1PB
Compound B1M
Compound B1Br
Compound B14C
Compound B1AZ
Compound B12N
Compound B1AP
Compound B1T
Compound B1B
EXPERIMENTAL WORK

DEPT. OF PHARMACEUTICAL CHEMISTRY

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MASS SPECTROGRAM

- Compound **B1AZ**
Compound B1B
Compound B1T
Compound B14C