CHAPTER III
CHAPTER III

Synthesis and biological evaluation of 3-mercapto / -thione- substituted - 1,2,4-triazoles

3.1 Various approaches for synthesis of mercapto / thione-1,2,4-triazoles

Mercapto / thione-1,2,4-triazoles were synthesized using various approaches due to their high medicinal value. Most of the routes describes synthesis of 5-substituted-3-mercapto / thione-1,2,4-triazoles, 4,5-disubstituted-3-mercapto / thione-1,2,4-triazoles and 2,4,5-trisubstituted-1,2,4-triazole-3-thiones. Few of the examples from each class of compounds are listed here.

5-Substituted-3-mercapto / thione-1,2,4-triazoles

The reaction of carboxylic acid chlorides and thiosemicarbazide had produced acylthiosemicarbazides, which without purification were cyclized in alkaline media to yield the corresponding 2,4-dihydro-3H-1,2,4-triazole-3-thiones.¹

\[
\text{R-COCI} + \text{H}_2\text{N'}-\text{NH}_2 + \text{NH}_2\text{S} \rightarrow \text{R} + \text{N-N-SH} \rightarrow \text{R-N-N-SH}
\]

The 3-mercaptotriazoles were prepared by reaction of acid halides with a lead (II) thiocyanate and hydrazine hydrate (15%) in a solvent at -70 to +200°C.²

\[
\text{R-O-X} + \text{Pb(SCN)$_2$} \rightarrow \text{R-N-N-SH}
\]

In addition, the triazolethiones can be prepared readily from the thermolysis of thiosemicarbazones.³

\[
\text{NNHCSNH$_2$} \rightarrow \text{R-N-N-SH}
\]

Condensation of carbamate with thiosemicarbazide in boiling pyridine via initial nucleophilic attack of the amino group to the ester carbonyl without attack at the carbonyl of the pyrazine ring followed by cyclization had produced triazolylquinoxaline.⁴
4,5-Disubstituted-3-mercapto/thione-1,2,4-triazoles

The reaction of hydrazine or substituted hydrazines with suitable electrophiles is the most common method for the preparation of the triazoles. Two examples where hydrazines provide the 4-amino-5-substituted-1,2,4-triazoles are described below.\(^5\)

\[
\begin{align*}
\text{NH}_2\text{CSNHNH}_2 + \text{RHNCOOCH}_3 & \rightarrow \text{NH}_2\text{CSNHNH}_2 \\
\text{RHNCOOCH}_3 + \text{NH}_2\text{CSNHNH}_2 & \rightarrow \text{RHNCONHNH}_2
\end{align*}
\]

4,5-Disubstituted-3-alkylthio-1,2,4-triazole derivatives were produced by the reaction of phenyl isothiocyanate with 2-cyanoacetohydrazide via intermediate I and cyclization with α-bromo ethyl acetate to yield desired triazoles.\(^5\)

\[
\begin{align*}
\text{CONHNH}_2 + \text{PhNCS} + \text{NaH} & \rightarrow \text{EtOOC}-\text{Br} \\
\text{EtOOC}-\text{Br} + \text{NNHCOCH}_2\text{CN} & \rightarrow \text{EtOOC}-\text{S}-\text{S}-\text{N} - \text{N} - \text{CN}
\end{align*}
\]

The cyclodehydration of thiosemicarbazides in alkaline medium afforded 4,5-disubstituted-1,2,4-triazoline-3-thiones.\(^6\) It has been reported that these triazoles exist mainly in a thione form.

\[
\begin{align*}
\text{RCONHNCSNHR} + \text{NaOH} & \rightarrow \text{N} - \text{SNa} \\
\text{N} - \text{SNa} + \text{Dilute HCl} & \rightarrow \text{N} - \text{SH} \\
\text{N} - \text{SH} & \rightarrow \text{N} - \text{S}
\end{align*}
\]

4,5-Disubstituted-2,4-dihydro-1,2,4-triazole-3-thiones were obtained by the action of primary amines on thiosemicarbazones of ester.\(^2\)
2.5-Disubstituted-3-mercapto/-thione-1,2,4-triazoles

5-Aryl-1,2-dihydro-2-(2-hydroxyethyl)-3H-1,2,4-triazole-3-thiones were prepared from the reaction of 2-hydrazoneethanol with aryl isothiocyanate in anhydrous benzene and in the presence of \( p \)-toluenesulfonic acid.\(^7\)

Kidwai et al. have synthesized new antifungal azoles including 2,5-disubstituted-1,2,4-triazole-3-thiones derivatives from substituted hydrazide using various solid supports under microwave irradiation.\(^8\)

2.4,5-Trisubstituted-1,2,4-triazole-3-thiones

The oxidative cyclization of aldehyde thiosemicarbazones with ferric chloride solutions gave 1,2,4-triazoline and 1,3,4-thiadiazoline.\(^9\)

The photochemistry behavior of some substituted aldehyde thiosemicarbazones have been investigated in methanol at 254 nm and cyclized to furnish the 5-thioxo-1,2,4-triazolines.\(^10\) The first step of the photoreaction of aldehyde thiosemicarbazones depicted as the cyclization to the 1,2,4-triazolidinethiones, the second step as the photo oxidation of give triazole.
The 2,4-dimethyl-5-(2-fluorophenyl)-5-phenyl-1,2,4-triazoline-3-thione was prepared in low yield by heating a methanolic solution of 2-fluorobenzophenone and 2,4-dimethylthiosemicarbazide in the presence of KOH.

3.2 Chemical reactivity of mercapto/thione-1,2,4-triazoles

▶ Alkylation

Alkylation of mercapto/thione-1,2,4-triazoles could occur either on S or N or on both S and N depending upon reaction conditions.

The reaction of 1,2,4-triazoline-5-thione with acrylonitrile afforded the N-substituted adduct.

Triazoles when reacted with α-halo ketones like RCOCH₂R₂ (R₂ =Cl, Br) it gave potential fungicidal N-alkylated derivatives.

Catalytic vinylation of triazoles by acetylene over Cd(OAc)₂ or CuCl at 15 atm. gave mixtures containing two different divinylated triazoles.
When triazole was reacted with $R_1C≡CCOPh$ it gave N-alkylated derivative with 55-88% yield.$^{14}$

![Reaction scheme](image)

The alkylation of triazole with alkyl halide in refluxing ethanol gave 32-99% yields of 5-alkylthio-1,2,4-triazoles which had moderate bacteriostatic activity and diuretic activity that increased with the size of alkyl group.$^{15}$

![Reaction scheme](image)

Addition reaction of epichlorohydrin with the corresponding triazoles gave S-alkylated product.$^{16}$

![Reaction scheme](image)

When 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine reacted with triazoles in alkaline medium both the S-alkylated and the N-alkylated isomers were obtained.$^{17}$

![Reaction scheme](image)

**Synthesis of disulfides**

When 3-methyl-1,2,4-triazole-5-thione was allowed to react with diethyl azodicarboxylate the corresponding disulfide was obtained.$^6$ Similarly, the other disulfides were prepared from corresponding triazoles.$^6$
Synthesis of Mannich base derivatives

It has been found that the Mannich and double Mannich reaction were carried out starting from \(s\text{-triazolo}[3,4-b]benzothiazol-3\text{-thiol}\) to prepare some biologically active Mannich bases.\(^{18}\)

Synthesis of thiazolotriazoles

The reaction of triazole with 1,2-dibromoethane and with \(\alpha\)-haloketones leading to the formation of thiazolotriazole.\(^{19}\)

The facile and regioselective synthesis of 2-substituted-5-methylthiazolo[3,2-b]-1,2,4-triazoles proceeded via \(\text{H}_2\text{SO}_4\) catalyzed cyclization of the corresponding (propynylthio)triazoles.\(^{20}\)
Synthesis of triazolothiazines

The condensation of triazolinethiones with 3-aryl-2-propenoyl chlorides or 3-aryl-acryloyl chloride gave 5-aryl-5,6-dihydro-7H-[1,2,4]triazolo[5,1-b][1,3]thiazin-7-ones.\(^{21}\)

\[
\begin{align*}
\text{R}^N\text{N} = \text{S} & \quad + \quad \text{Cl} \quad \text{O} \quad \text{R}' \\
\text{R}^N\text{N} = \text{S} & \quad + \quad \text{Cl} \quad \text{O} \quad \text{R}' \\
\end{align*}
\]

The 1,2,4-triazolo[5,1-b][1,3]thiazin-7-ones have been first prepared by Peter et al. by cyclization of triazoles with diethyl ethoxymethylene malonate in fair to good yields.\(^{22}\) Also, Heindel et al. have synthesized this heterocyclic system by condensation of triazole with methyl propiolate, hydrolysis of the resulting S-acrylic esters to the corresponding S-acrylic acids and subsequent cyclization to N-2 or N-4.\(^{23}\)

Synthesis of triazolothiazepines

Triazolobenzothiazepinones were synthesized in a regioselective manner via reaction of triazole with 2-chloromethyl-benzoyl chloride in good yields.\(^{24}\)
3.3 Present work

3.3.1 Chemistry

As we have seen in Chapter I, the chemistry of 1,2,4-triazoles and their fused heterocyclic derivatives has received considerable attention in the last few decades, owing to their synthetic and effective biological importance. The 1H-1,2,4-triazole compounds possess important pharmacological activities such as, anti-inflammatory, CNS stimulants sedatives, antianxiety, antimicrobial agents, and antimycotic activity such as fluconazole, intraconazole, voriconazole. Also, there are known drugs containing the 1,2,4-triazole group. Moreover, sulphur containing heterocycles represent an important group of sulphur compounds that are promising for use in practical applications. Among these heterocycles, the mercapto/thione-substituted 1,2,4-triazole ring systems were reported with biological activities, such as anticancer, antitubercular, diuretic, antibacterial, antifungal, antymycobacterial, and hypoglycemic properties. As we have seen in Chapter I, several cyclic amine (e.g. substituted piperazine) derivatives were synthesized in the last decade, as useful chemotherapeutic agents for various diseases, such as Crivixan (Indinavir sulphate) and delavirdine (Rescriptor), powerful inhibitors for the protease and reverse transcriptase inhibitor of HIV enzymes, respectively. Some piperizinyl [1,2,4]triazole derivatives were reported as 5-HT$_{1A}$ serotonin receptor ligands.

In the present work, synthesis of 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazoles (8) and 2-[3-[4-(substituted)-1-cyclicamine]propyl]-5-(substituted)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (11) is described. The anticancer, antibacterial and antifungal activity of these derivatives were studied.

➢ Retro synthetic analysis

We had planned to prepare 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazole derivatives (8) as it contains three important parts from the structural point of view as shown in Figure 1.

(i) a pharmacophoric portion constituted by a substituted 1,2,4-triazole,

(ii) a terminal fragment constituted by a substituted cyclic amine like aryl piperazine which is again a pharmacophore and
(iii) a three carbon linker between these two substructures i.e. pharmacophores.

So idea was that the this structural future could have influence on their biological activity

![Figure 1](image)

The 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazole derivatives (8) could be synthesized by the N-alkylation of the corresponding cyclic amines (7) with 3-(3-chloropropyl)-sulfanyl-5-substituted-[1,2,4]triazoles (6). 3-(3-chloropropyl)-sulfanyl-5-substituted-1,2,4-triazoles (6) could be synthesized by S-alkylation of 5-substituted-[1,2,4]triazole-3-thiones (5) with 1-bromo-3-chloropropane. 5-Substituted-[1,2,4]triazole-3-thiones (5) could be synthesized by well known method from corresponding aryl carboxylic acids (1) via acid hydrazides (3) (Scheme 1).

Scheme 1: Retro synthetic analysis

**Synthesis of the 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazole derivatives (8)**

5-Substituted[1,2,4]triazole-3-thiones (5) were synthesized by following reported sequence of the reactions from corresponding aryl carboxylic acids (1) (Scheme 2). The aryl carboxylic acids (1) were converted to corresponding methyl esters (2) with catalytic amount of sulfuric acid in methanol. This was confirmed by disappearance of broad carboxylic acid peak in $^1$H NMR spectra and disappearance of broad ‘OH’ stretching of
carboxylic acid in IR spectra of the product. It was also supported by appearance of OCH$_3$ peak in $^1$H NMR spectra of methyl esters (2). The methyl esters (2) were converted to corresponding acid hydrazides (3) using hydrazine hydrate in methanol.\textsuperscript{25} This was confirmed by disappearance of OCH$_3$ peak and appearance of NH and NH$_2$ peaks in $^1$H NMR spectra of the product. It was also supported by the appearance of NH stretching and C=O stretching of acid hydrazide in IR spectra of the product. The acid hydrazides (3) were reacted with potassium thiocyanate and concentrated hydrochloric acid to produce corresponding thiosemicarbazides (4).\textsuperscript{26} Appearance of additional NH stretching in IR spectra and molecular weight had confirmed the formation of thiosemicarbazides (4). Thiosemicarbazides (4) were reacted with aqueous sodium hydroxide to produce 5-substituted[1,2,4]triazole-3-thiones (5).\textsuperscript{27} They can also exist in tautomeric thiol form. Here we found that they mainly exist in thione form which is also supported by some earlier reports.\textsuperscript{28} Some reports states that they mainly exist in thione-thiol tautomeric forms in solution but the thione structures dominate in the solid state.\textsuperscript{29,30} It has been reported that the crystal structure of these compounds corresponded to the thione form.\textsuperscript{31} The product was confirmed by appearance of characteristic broad peak for NH protons with chemical shift 13-14 ppm. Appearance of characteristic triazole NH stretching in IR spectra and molecular weight had further confirmed the formation of 5-substituted[1,2,4]triazole-3-thiones (5)

![Scheme 2 : Synthesis of 5-substituted[1,2,4]triazole-3-thiones](image-url)
The 5-substituted[1,2,4]triazole-3-thiones (5) were reacted with 1-bromo-3-chloropropane in presence of K₂CO₃ in acetonitrile at ambient temperature to obtain the corresponding 3-(3-chloropropyl)-sulfanyl-5-substituted[1,2,4]triazoles (6) (Scheme 3). The products were confirmed by appearance of characteristic S-CH₂ protons peak in ¹H NMR spectra. Mass and IR spectra had further supported the formation of 6. Now to obtain the 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazole derivatives (8), 3-(3-chloropropyl)-sulfanyl-5-substituted[1,2,4]triazoles (6) were reacted with cyclic amines (7) in presence of K₂CO₃ and catalytic amount of KI in acetonitrile (Scheme 3). After work up when product was analyzed it was not the desired 8 because characteristic cyclic amine CH₂ protons peaks were absent in ¹H NMR spectra. In some cases desired product 8 was formed but yield was less than 10% while other product was major.

So we have studied the particular example of 3-(3-chloropropyl)-sulfanyl-5-(4-tert-butylphenyl)-1,2,4-triazoles (6a). 6a was reacted with morpholine (7a) in presence of K₂CO₃ and catalytic amount of KI in acetonitrile (Scheme 4). A CH₂ peak as triplet with chemical shift 4.36 was observed in ¹H NMR spectrum of the product which is characteristic triazole N-CH₂ peak. Characteristic NH stretching of triazole at 3074 cm⁻¹ was absent in IR spectra and mass spectum of product was showing elimination of HCl. All the three observations were pointing structure of the product as 9a. This was further supported by some reports which states formation of linear ring rather than angular.¹⁹, ₃₂-₃₅
So we did further study with changing alkyl chain length and substituents on phenyl ring to observe that whether it favors formation of 8 or 9 (Scheme 5). The product 9 was obtained with carbon chain length 2, 3, 4 and 5. The different substituents on phenyl ring had also always favored the product 9. In no cases the desired 8 was obtained.

Synthesis of the 3-[3-[4-(substituted)-1-cyclicamine[propyl]thio-5-substituted[1,2,4]triazole derivatives (8) and 2-[3-[4-(substituted)-1-cyclicamine[propyl]-5-(substituted)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (11) using different approaches

So we have decided first to prepare 1-(3-chloropropyl)-4-substituted cyclic amines (10) by the reaction of cyclic amines (7) with 1-bromo-3-chloropropane in presence of activated zinc in THF at ambient temperature (Scheme 6).
The 5-substituted[1,2,4]triazole-3-thiones (5) were reacted with 1-(3-chloropropyl)-4-substituted cyclic amines (10) under reported method like KOH and a catalytic amount of KI in ethanol to obtain 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazole derivatives (8), but it was unsuccessful. Therefore we carried out the reaction using K$_2$CO$_3$ in acetone. In these experimental conditions, both the S-alkylated and the N-alkylated isomers were obtained (3:1). By careful literature work we found the methods for the selective N and S-alkylation of 4-amino-5-aryl-1,2,4-triazole-3-thiols. Now we planned to prepare 2-[3-[4-(substituted)-1-cyclicamine]propyl]-5-(substituted)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (11) in addition to 8 as this may lead some interesting results in biological activity point of view. Thus to obtain the target compounds 8 and 11 in shorter reaction time, good yields and selectivity, we did slight modifications in the reported methods. When the 5-substituted[1,2,4]triazole-3-thiones (5) and 1-(3-chloropropyl)-4-substituted cyclic amines (10) were reacted in the presence of triethyl amine in ethanol with a catalytic amount of tetra butyl ammonium iodide (TBAI). Exclusively 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazoles (8) were formed i.e. S-alkylation (Scheme 7). In the derivatives 8, a triplet for the methylene group of the propyl chain that connects the cyclic amine moiety and the triazole part of the molecule is observed with a chemical shift in the range of $\delta$ 3.25-3.15, which is typical for S–CH$_2$ connectivity.
Table 1: List of the synthesized compounds 8a-m with yields.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8a</td>
<td>4-C_4H_9-C_6H_4</td>
<td>O</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>8b</td>
<td>4-C_4H_9-C_6H_4</td>
<td>N-phenyl</td>
<td>66</td>
</tr>
<tr>
<td>3</td>
<td>8c</td>
<td>4-CH_3-C_6H_4</td>
<td>N-3-chlorophenyl</td>
<td>70</td>
</tr>
<tr>
<td>4</td>
<td>8d</td>
<td>4-CH_3-C_6H_4</td>
<td>N-ethyl</td>
<td>63</td>
</tr>
<tr>
<td>5</td>
<td>8e</td>
<td>4-Cl-C_6H_4</td>
<td>N-3-chlorophenyl</td>
<td>75</td>
</tr>
<tr>
<td>6</td>
<td>8f</td>
<td>4-Cl-C_6H_4</td>
<td>N-2-pyrimidyl</td>
<td>65</td>
</tr>
<tr>
<td>7</td>
<td>8g</td>
<td>4-F-C_6H_4</td>
<td>N-benzyl</td>
<td>70</td>
</tr>
<tr>
<td>8</td>
<td>8h</td>
<td>3-CH_3-C_6H_4</td>
<td>N-phenyl</td>
<td>66</td>
</tr>
<tr>
<td>9</td>
<td>8i</td>
<td>3-CH_3-C_6H_4</td>
<td>N-2-pyridyl</td>
<td>70</td>
</tr>
<tr>
<td>10</td>
<td>8j</td>
<td>3-Cl-C_6H_4</td>
<td>N-2-pyridyl</td>
<td>68</td>
</tr>
<tr>
<td>11</td>
<td>8k</td>
<td>2-Cl-C_6H_4</td>
<td>N-ethyl</td>
<td>60</td>
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<tr>
<td>12</td>
<td>8l</td>
<td>2-Cl-C_6H_4</td>
<td>N-2-pyridyl</td>
<td>65</td>
</tr>
<tr>
<td>13</td>
<td>8m</td>
<td>C_6H_5CH_2</td>
<td>N-2-pyridyl</td>
<td>73</td>
</tr>
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</table>

When the 5-substituted-1,2,4-triazole-3-thiones (5) and 1-(3-chloropropyl)-4-substituted cyclic amines (10) were reacted in the presence of K_2CO_3 in acetonitrile with a catalytic amount of TBAI. The 2-[3-[4-(substituted)-1-cyclic amine]propyl]-5-(substituted)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (11) were formed i.e. N-alkylation with the traces of 8 (Scheme 8). In the derivatives 11, a triplet for the methylene group of the propyl chain that connects the piperizine moiety and the triazole part of the molecule is observed with a chemical shift in the range of δ 4.45-4.35, which is typical for NCSN–CH_2 connectivity.\(^{17,37}\) Further the products 11a-h were confirmed by mass and IR spectroscopy and the details are given in Table 2.
Table 2: List of the synthesized compounds 11a-h with yields.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11a</td>
<td>4-C₆H₄-C₆H₄</td>
<td>N-phenyl</td>
<td>60</td>
</tr>
<tr>
<td>2</td>
<td>11b</td>
<td>4-C₆H₄-C₆H₄</td>
<td>N-3-chlorophenyl</td>
<td>68</td>
</tr>
<tr>
<td>3</td>
<td>11c</td>
<td>4-CH₃-C₆H₄</td>
<td>N-3-chlorophenyl</td>
<td>58</td>
</tr>
<tr>
<td>4</td>
<td>11d</td>
<td>4-Cl-C₆H₄</td>
<td>N-3-chlorophenyl</td>
<td>65</td>
</tr>
<tr>
<td>5</td>
<td>11e</td>
<td>4-F-C₆H₄</td>
<td>N-benzyl</td>
<td>66</td>
</tr>
<tr>
<td>6</td>
<td>11f</td>
<td>3,4-di OCH₃-C₆H₃</td>
<td>O</td>
<td>59</td>
</tr>
<tr>
<td>7</td>
<td>11g</td>
<td>3,4-di OCH₃-C₆H₃</td>
<td>N-ethyl</td>
<td>60</td>
</tr>
<tr>
<td>8</td>
<td>11h</td>
<td>4-OCH₃-C₆H₄</td>
<td>N-2-pyridyl</td>
<td>62</td>
</tr>
</tbody>
</table>

3.3.2 Biology

All the compounds 8a-m and 11a-h were tested for anticancer, antibacterial and antifungal activity

- **Anticancer activity**

As we have already seen in Chapter I that mercapto / thione-substituted 1,2,4-triazole are emerging as a potential anticancer agents. So the sensitivity of the human leukemic cell lines, HL-60 (myeloid leukemia) and U937 (leukemic monocyte lymphoma) to the synthetic triazole derivatives 8a-m and 11a-h was evaluated. The cytotoxic activities of the compounds were determined by measuring the number of live cells after 24 h of treatment (MTT assay) and IC₅₀ values of the most potent compounds are presented in Table 3. Other compounds had shown IC₅₀ values higher than 150 µM and were considered as inactive. Etoposide was used as a positive control in these assays and the IC₅₀ values obtained on HL-60 and U937 as 1.84 and 17.95 µM, respectively.

The test compounds, 11d, 8j and 8i were potent antiproliferative agents against HL-60 cells, with IC₅₀ values of 6.67 µM, 18.51 µM and 29.36 µM, respectively. The relative activities of these molecules were 3.63, 10.1 and 15.96 fold less active than the positive control. Interestingly, similar trend was observed even in U937 cells with an IC₅₀ of 28.19 µM, 49.13 µM and 52.33 µM, respectively, which was equivalent to 1.57, 2.73 and 2.92 fold less active, than Etoposide. The order of decreasing cytotoxic potency of these compounds against both HL-60 and U937 cells are 11d > 8j > 8i > 8f > 11h. On the
structure point of view, the compounds with chloro group at 3rd or 4th position of the phenyl ring had shown good cytotoxic activity \((11d, 8j, 8f)\). On the other hand, the compounds with piperizine containing N-3-chlorophenyl, N-2-pyrimidyl and N-2-pyridyl groups had shown good cytotoxic activity \((11d, 8j, 8i, 8f)\). It was interesting that S-alkylated isomer of \(11d\) i.e. \(8e\) had not shown anticancer activity.

### Table 3. Cytotoxic activity of triazole derivatives on HL-60 and U937 Cells

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cytotoxic activity* of test compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HL-60 Cells (§IC(_{50}) in µM)</td>
</tr>
<tr>
<td>8f</td>
<td>105.06±5.49</td>
</tr>
<tr>
<td>8i</td>
<td>29.36±2.23</td>
</tr>
<tr>
<td>8j</td>
<td>18.51±1.16</td>
</tr>
<tr>
<td>11d</td>
<td>6.67±0.55</td>
</tr>
<tr>
<td>11h</td>
<td>114.95±6.00</td>
</tr>
</tbody>
</table>

| Etoposide (Positive control) | 1.84±0.20 | 17.95±1.19 |

*Exponentially growing cells were treated with different concentrations of test compounds for 24 h and cell growth inhibition was analyzed through MTT assay. §IC\(_{50}\) is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control cultures in the absence of an inhibitor. The values represent the mean ± SE of five individual observations.

Overall it was observed from the results that cytotoxicity depends on the particular substituents on the phenyl ring and piperizine ring rather than \(S\) and \(N\) alkylation of triazoles. These findings suggest that the newly synthesized triazole have shown significant anti-proliferative activity on both the human leukemic cell lines. The potency of the compounds varied between the two cell lines suggesting that a structural property of these compounds as possible determinants of their biological activity.
Antibacterial activity

In the search of new antibacterial agents, all the 21 compounds 8a-m and 11a-h were evaluated for antibacterial activity and results are listed in Table 4.

Table 4: Antibacterial activity of 8a-m and 11a-h.

<table>
<thead>
<tr>
<th>MIC (µg/ml)</th>
<th>Gram-positive</th>
<th>Gram-negative</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Compound</strong></td>
<td>B.subtilis</td>
<td>S.aureus</td>
</tr>
<tr>
<td>8a</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8b</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8c</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8d</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8e</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8f</td>
<td>150</td>
<td>75</td>
</tr>
<tr>
<td>8g</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8h</td>
<td>150</td>
<td>75</td>
</tr>
<tr>
<td>8i</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8j</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8k</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8l</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>8m</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>11a</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>11b</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>11c</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>11d</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>11e</td>
<td>150</td>
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<tr>
<td>11f</td>
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<tr>
<td>11g</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>11h</td>
<td>150</td>
<td>150</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1.562</td>
<td>1.562</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>6.25</td>
<td>6.25</td>
</tr>
</tbody>
</table>

The minimum inhibitory concentrations (MIC) of synthesized compounds were tested against three representative Gram-positive organisms viz. Bacillus subtilis (MTCC 441), Staphylococcus aureus (MTCC 96), Staphylococcus epidermidis and Gram-negative organisms viz Escherichia coli (MTCC 443), Pseudomonas aeruginosa (MTCC 741).
and *Klebsiella pneumoniae* (MTCC 618). The compounds had not shown any remarkable antibacterial activity.

- **Antifungal activity**

In the search of new antifungal agents, all the 21 compounds 8a-m and 11a-h were evaluated for antifungal activity and the results are summarized in Table 5.

**Table 5:** Antifungal activity of 8a-m and 11a-h.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C.albicans</td>
</tr>
<tr>
<td></td>
<td>100 µg</td>
</tr>
<tr>
<td>8a</td>
<td>7 10 10 13 a</td>
</tr>
<tr>
<td>8b</td>
<td>- - 9 12 7 9</td>
</tr>
<tr>
<td>8c</td>
<td>- - 8 11 9 13</td>
</tr>
<tr>
<td>8d</td>
<td>- - - - -</td>
</tr>
<tr>
<td>8e</td>
<td>- - 8 11 7 10</td>
</tr>
<tr>
<td>8f</td>
<td>- - 7 10</td>
</tr>
<tr>
<td>8g</td>
<td>- 9 9 13</td>
</tr>
<tr>
<td>8h</td>
<td>- - 7 10 8 10</td>
</tr>
<tr>
<td>8i</td>
<td>- - 7 10</td>
</tr>
<tr>
<td>8j</td>
<td>- - 7 10</td>
</tr>
<tr>
<td>8k</td>
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</tr>
<tr>
<td>8l</td>
<td>- - 8 10</td>
</tr>
<tr>
<td>8m</td>
<td>- - 10 14</td>
</tr>
<tr>
<td>11a</td>
<td>- - 8 11</td>
</tr>
<tr>
<td>11b</td>
<td>7 9 - - -</td>
</tr>
<tr>
<td>11c</td>
<td>- - 7 10</td>
</tr>
<tr>
<td>11d</td>
<td>7 10 8 11 7 10</td>
</tr>
<tr>
<td>11e</td>
<td>8 11 9 13 7 10</td>
</tr>
<tr>
<td>11f</td>
<td>- - - - -</td>
</tr>
<tr>
<td>11g</td>
<td>- - - - -</td>
</tr>
<tr>
<td>11h</td>
<td>- - 7 10</td>
</tr>
<tr>
<td><strong>Amphotericin-B (50µg)</strong></td>
<td>23.5 21 23 22 9 12</td>
</tr>
</tbody>
</table>

* Concentration used: 100µg/150µg
* Negative Control: DMSO (No activity)
* Positive Control: Amphotericin-B (50µg)

\(^a\) not active
Most of the compounds had shown moderate activity especially against *C.rugosa*. The compounds 8b, 11d and 11e had shown comparatively more antifungal activity than other compounds. None of the compounds had shown higher activity than the standard Amphotericin-B.

### 3.3.3 Experimental

*General procedure for the preparation of methyl esters (2).*

\[
\text{RCOOCH}_3
\]

A catalytic amount of concentrated H\(_2\)SO\(_4\) was added to a solution of an appropriate aryl carboxylic acid 1 (0.15 mol) in 150 mL of methanol and the mixture was refluxed for 4 h. It was allowed to cool. The saturated solution of NaHCO\(_3\) was to the reaction mixture and was extracted with methylene dichloride (CH\(_2\)Cl\(_2\)) (2 X 100 mL). The combined organic layer was dried (Na\(_2\)SO\(_4\)) and concentrated to obtain pure methyl ester (2).

*General procedure for the preparation of acid hydrazides (3).*

\[
\text{RCONHNH}_2
\]

To a solution of an appropriate methyl ester 2 (0.1 mol) in 150 mL of methanol was added 95% hydrazine hydrate (0.2 mol), and the mixture was refluxed for 2-3 h upto reaction completed (TLC). It was allowed to cool, and the obtained solid was washed with methanol and dried (Na\(_2\)SO\(_4\)). The crude compounds were recrystallized from ethanol.

*General procedure for the preparation of thiosemicarbazides (4).*

\[
\text{RCONHNHCSNH}_2
\]

To a solution of an appropriate acid hydrazide 3 (0.02 mol) in 50 mL of methanol was added a solution of KSCN (0.03 mol) and 3 mL of HCl with constant stirring. The mixture was immediately

110
evaporated to dryness on a steam bath and heated for an additional 1 h with another 50 mL of methanol. The resulting solid was treated with distilled water and with a small amount of ethanol. The crude thiosemicarbazides (4) were used as such for next step.

*General procedure for the preparation of 5-substituted[1,2,4]triazole-3-thiones (5a-j).*

To a solution of an appropriate thiosemicarbazide 4 (0.01 mol) in 15 mL of methanol was added a solution of 10.0% NaOH (20 mL), and the reaction mixture was refluxed immediately for 5-6 h upto reaction was completed (TLC). The mixture was cooled and acidified with dilute HCl at pH 5-6. The resulting solid was filtered, washed with distilled water, and dried (Na$_2$SO$_4$). The crude compounds were recrystallized from ethanol.

*General procedure for the preparation of 3-(haloalkyl)-sulfanyl-5-substituted[1,2,4]triazoles (6a-f)*

The K$_2$CO$_3$ (0.005 mol) was added to the solution of 5-substituted[1,2,4]triazole-3-thione 5 (0.005 mol) in acetonitrile (20 mL) and stirred for 15 minutes. To the above mixture was added an appropriate dihalo compound 12 (0.006 mol) and mixture was stirred for 5 h at room temperature. After completion of the reaction water (20 mL) was added to the reaction mixture and was extracted with ethyl acetate (2 X 20 mL). The combined organic layer was dried (Na$_2$SO$_4$), concentrated and recrystallized from ethanol to obtain the pure product.

*General procedure for the preparation of cyclic products of triazoles (9a-f)*
To the solution of morpholine \(7a\) (0.001 mol) in acetonitrile (7 mL) was added \(K_2CO_3\) (0.001 mol) and stirred for 15 minutes. To above mixture an appropriate 3-(haloalkyl)-sulfanyl-5-substituted[1,2,4]triazoles \(6\) (0.001 mol) in acetonitrile (3 mL) and a catalytic amount of KI were added and was refluxed for 4 h. After completion of the reaction water (10 mL) was added to the reaction mixture and was extracted with ethyl acetate (2 X 10 mL). The combined organic layer was dried (\(Na_2SO_4\)), concentrated and subjected to flash column chromatography to obtain the pure product.

**General procedure for the preparation of 1-(3-chloropropyl)-4-substituted cyclic amines (10a-g).**

The activated zinc powder (0.002 mol) was added to a solution of cyclic amine \(7\) (0.002 mol) and 1-bromo-3-chloropropane (0.002 mol) in THF (20 mL) and stirred at room temperature for 2 h. After completion of the reaction, the mixture was filtered and the solid was washed with solvent ether (3 X 20 mL). The combined filtrate was treated with 10% \(NaHCO_3\) (20 mL), water (2 X 20 mL), dried (\(Na_2SO_4\)) and evaporated to give the crude product. The crude product was purified by flash column chromatography.

**General procedure for the preparation of 3-[3-[4-(substituted)-1-cyclic amine]propyl]thio-5-substituted[1,2,4]triazoles (8a-m)**

5-Substituted[1,2, 4]triazole-3-thione \(5\) (0.0005 mol) was refluxed with triethyl amine (0.001 mol) in ethanol (8 mL) for 15 minutes. 1-(3-Chloropropyl)-4-substituted cyclic amine \(10\) (0.0006 mol) and tetrabutyl ammonium iodide (TBAI) (10 mg) in ethanol (3 mL) were added to above mixture carefully and was refluxed for 4 h. After the reaction was completed, water (10 mL) was added and extracted with ethyl acetate (3 X 10 mL). The combined organic layer was
dried (Na$_2$SO$_4$) and concentrated. The crude product was subjected to flash column chromatograph to obtain pure product.

**General procedure for the preparation of 2-[3-[4-(substituted)-1-cyclic amine]propyl]-5-(substituted)-2, 4-dihydro-3H[1,2,4]triazole-3-thiones (11a-h)**

5-Substituted[1,2,4]triazole-3-thione 5 (0.0005 mol) was refluxed with K$_2$CO$_3$ (0.00075 mol) in acetonitrile (8 mL) for 15 minutes. 1-(3-Chloropropyl)-4-substituted cyclic amine 10 (0.0006 mol) and tetra butyl ammonium iodide (TBAI) (10 mg) in acetonitrile (3 mL) were added to above mixture carefully and was refluxed for 4 h. After the reaction was completed, water (10 mL) was added and extracted with ethyl acetate (3 X 10 mL). The combined organic layer was dried (Na$_2$SO$_4$) and concentrated. The crude product was subjected to flash column chromatograph to obtain pure product.

**Spectoscopic data for the synthesized compounds:**

**methyl 4-(tert-butyl)benzoate (2a)**

![Diagram of methyl 4-(tert-butyl)benzoate (2a)](image)

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.74 (d, $J$= 8.6 Hz, 2H, 2XAr-H); 7.35 (d, $J$= 8.6 Hz, 2H, 2XAr-H); 3.87 (s, 3H, OCH$_3$); 1.34 (s, 9H, C(CH$_3$)$_3$).

**methyl 4-methylbenzoate (2b)**

![Diagram of methyl 4-methylbenzoate (2b)](image)

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.89 (d, $J$= 7.6 Hz, 2H, 2XAr-H); 7.18 (d, $J$= 8.3 Hz, 2H, 2XAr-H); 3.87 (s, 3H, OCH$_3$); 2.39 (s, 3H, Ar-CH$_3$).

IR (KBr, cm$^{-1}$): 2953 (-C-H), 1724 (C=O), 1602, 1437 (C=C), 1279, 1110 (C-O), 712 (=C-H ben).
methyl 4-chlorobenzoate (2c)

\[
\text{IR (KBr, cm}^{-1}\text{): 2953 (C-H), 1735 (C=O), 1593, 1435 (C=C), 1297, 1254, 1053 (C-O), 749 (C-H ben).}
\]

\[
\text{^1H NMR (300 MHz, CDCl}_3\text{) \text{\delta: 7.95 (d, } J = 8.2 \text{ Hz, 2H, } 2\text{XAr-H); 7.40 (d, } J = 8.9 \text{ Hz, 2H, } 2\text{XAr-H); 3.91 (s, 3H, OCH}_3\text{).}
\]

\[
\text{methyl 4-fluorobenzoate (2d)}
\]

\[
\text{^1H NMR (300 MHz, CDCl}_3\text{) \text{\delta: 8.09-7.98 (m, 2H, } 2\text{XAr-H); 7.13-7.04 (m, 2H, } 2\text{XAr-H); 3.90 (s, 3H, OCH}_3\text{).}
\]

\[
\text{methyl 3-chlorobenzoate (2f)}
\]

\[
\text{^1H NMR (300 MHz, CDCl}_3\text{) \text{\delta: 8.00 (t, } J = 2.6 \text{ Hz, 1H, Ar-H); 7.92-7.87 (m, 1H, Ar-H); 7.53-7.48 (m, 1H, Ar-H); 7.36 (t, } J = 8.3 \text{ Hz, 1H, Ar-H); 3.92 (s, 3H, OCH}_3\text{).}
\]

\[
\text{methyl 3,4-dimethoxybenzoate (2i)}
\]

\[
\text{^1H NMR (300 MHz, CDCl}_3\text{) \text{\delta: 7.62 (dd, } J = 6.6, 2.2 \text{ Hz, 1H, Ar-H); 7.49 (d, } J = 2.2 \text{ Hz, 1H, Ar-H); 6.83 (d, } J = 8.8 \text{ Hz, 1H, Ar-H); 3.92 (s, 6H, } 2\text{XAr-OCH}_3\text{); 3.88 (s, 3H, OCH}_3\text{).}
\]

\[
\text{methyl 4-methoxybenzoate (2j)}
\]
4-(tert-butyl)-1-benzenecarbohydrazide (3a)

White solid. mp 148-150°C.

\[ ^1H \text{ NMR (300 MHz, CDCl}_3) \delta: 7.94 \text{ (d, } J= 9.1 \text{ Hz, 2H, 2XAr-H); 6.86 \text{ (d, } J= 9.1 \text{ Hz, 2H, 2XAr-H); 3.86 (s, 3H, Ar-OCH}_3); 3.84 (s, 3H, OCH}_3). \]

4-methyl-1-benzenecarbohydrazide (3b)

White solid. mp 118-120°C.

\[ ^1H \text{ NMR (300 MHz, CDCl}_3) \delta: 7.62 \text{ (d, } J= 8.4 \text{ Hz, 2H, 2XAr-H); 7.38-7.26 \text{ (broad, 1H, NH); 7.21 (d, } J= 8.4 \text{ Hz, 2H, 2XAr-H); 4.07-3.92 \text{ (broad, 2H, NH}_2); 2.41(s, 3H, Ar-CH}_3). \]

IR (KBr, cm\(^{-1}\)):
- 3304, 3221 (N-H), 3026 (=C-H), 1662 (C=O), 1615, 1563 (C=C), 1347 (C-N), 717, 678 (=C-H ben).
- ESI-MS (m/z): 151 (M+1)\(^+\).

4-chloro-1-benzenecarbohydrazide (3c)

White solid. mp 163-165°C.

\[ ^1H \text{ NMR (300 MHz, CDCl}_3) \delta: 9.80-9.54 \text{ (broad, 1H, NH); 7.84 (d, } J= 8.6 \text{ Hz, 2H, 2XAr-H); 7.37 (d, } J= 8.6 \text{ Hz, 2H, 2XAr-H); 4.55-3.58 \text{ (broad, 2H, NH}_2). \]
IR (KBr, cm\(^{-1}\)): 3299, 3198 (N-H), 3020 (=C-H), 1661 (C=O), 1615, 1571 (C=C), 1348 (C-N), 684 (=C-H ben).

4-fluoro-1-benzenecarbohydrazide (3d)

<table>
<thead>
<tr>
<th><img src="images/3d.png" alt="Image" /></th>
</tr>
</thead>
</table>

White solid. mp 121-123°C.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 9.67-9.42 (broad, 1H, NH); 8.24-7.95 (m, 2H, 2XAr-H); 7.21-7.09 (m, 2H, 2XAr-H); 4.34-3.63 (broad, 2H, NH\(_2\)).

3-chloro-1-benzenecarbohydrazide (3f)

<table>
<thead>
<tr>
<th><img src="images/3f.png" alt="Image" /></th>
</tr>
</thead>
</table>

White solid. mp 158-160°C.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 7.77-7.32 (m, 4H, 4XAr-H); 4.00-3.96 (broad, 2H, NH\(_2\)).

3,4-dimethoxy-1-benzenecarbohydrazide (3i)

<table>
<thead>
<tr>
<th><img src="images/3i.png" alt="Image" /></th>
</tr>
</thead>
</table>

White solid. mp 162-164°C.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 9.54-9.45 (broad, 1H, NH); 7.48 (m, 2H, 2XAr-H); 6.84 (d, \(J=8.8\) Hz, 1H, Ar-H); 3.84 (s, 6H, 2XAr-OCH\(_3\)); 3.39-3.10 (broad, 2H, NH\(_2\)).

4-methoxy-1-benzenecarbohydrazide (3j)

<table>
<thead>
<tr>
<th><img src="images/3j.png" alt="Image" /></th>
</tr>
</thead>
</table>

White solid. mp 128-130°C.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) \(\delta\): 7.69 (d, \(J=8.6\) Hz, 2H, 2XAr-H); 7.35-7.27 (broad, 1H, NH); 6.89 (d, \(J=8.6\) Hz, 2H, 2XAr-H); 4.00-3.91 (broad, 2H, NH\(_2\)); 3.84 (s, 3H, Ar-OCH\(_3\)).
3-[4-(tert-butyl)phenyl]-4,5-dihydro-1H-1,2,4-triazole-5-thione (5a)

White solid. mp 267-270°C.

\[ \text{\textsuperscript{1}H NMR (300 MHz, CDCl}_3\text{)} \delta: 13.45-13.02 \text{ (broad, 1H, NH)}；7.84 (d, J= 8.3 Hz, 2H, 2XAr-H); 7.43 (d, J= 8.3 Hz, 2H, 2XAr-H); 1.34 (s, 9H, C(CH}_3\text{)}_3\text{).} \]

ESI-MS (m/z): 234 (M+1)\textsuperscript{+}.

3-(4-methylphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (5b)

White solid. mp 237-239°C.

\[ \text{\textsuperscript{1}H NMR (300 MHz, CDCl}_3\text{)} \delta: 13.50-13.05 \text{ (broad, 1H, NH)}；7.80 (d, J= 8.7 Hz, 2H, 2XAr-H); 7.21 (d, J= 8.0 Hz, 2H, 2XAr-H); 2.39 (s, 3H, ArCH}_3\text{).} \]

IR (KBr, cm\textsuperscript{-1}): 3085 (N-H), 3016 (=C-H), 2915 (C-H), 1592, 1523 (C=C), 1460, 1234 (C-N), 818, 717 (=C-H ben).

ESI-MS (m/z): 192 (M+1)\textsuperscript{+}.

3-(4-chlorophenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (5c)

White solid. mp 240-242°C.

\[ \text{\textsuperscript{1}H NMR (300 MHz, CDCl}_3\text{)} \delta: 13.70-13.38 \text{ (broad, 1H, NH)}；7.92 (d, J= 8.6 Hz, 2H, 2XAr-H); 7.41 (d, J= 8.6 Hz, 2H, 2XAr-H). \]

3-(4-fluorophenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (5d)

White solid. mp 259-261°C.

\[ \text{\textsuperscript{1}H NMR (300 MHz, CDCl}_3\text{)} \delta: 8.17-7.86 \text{ (m, 2H, 2XAr-H)}；7.27-6.99 \text{ (m, 2H, 2XAr-H).} \]
3-(3-methylphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (5e)

White solid. mp 276-278°C.
\[\text{H NMR (300 MHz, CDCl}_3\text{)} \delta: 13.70-13.21 \text{ (broad, 1H, NH); 7.84-7.64 (m, 2H, 2XAr-H); 7.38-7.13 (m, 2H, 2XAr-H); 2.40 (s, 3H, Ar-CH}_3\text{).}\]

3-(3-chlorophenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (5f)

White solid. mp 259-261°C.
\[\text{H NMR (300 MHz, CDCl}_3\text{)} \delta: 13.52-13.26 \text{ (broad, 1H, NH); 7.92-7.56 (m, 4H, 4XAr-H).}\]
\[\text{ESI-MS (m/z): 212 (M}^+\text{).}\]

3-(2-chlorophenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (5g)

White solid. mp 227-229°C.
\[\text{H NMR (300 MHz, CDCl}_3\text{)} \delta: 13.80-13.21 \text{ (broad, 1H, NH); 7.29-7.23 (m, 4H, 4XAr-H).}\]

3-benzyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (5h)

White solid. mp 221-223°C.
\[\text{H NMR (300 MHz, CDCl}_3\text{)} \delta: 13.16-13.00 \text{ (broad, 1H, NH); 7.34-7.14 (m, 5H, 5XAr-H); 3.86 (s, 2H, Ph-CH}_2\text{).}\]
\[\text{IR (KBr, cm}^{-1}\text{): 3093 (N-H), 3027 (=C-H), 2927 (C-H), 1605 (C=C), 1481 (C-N), 793, 716 (=C-H ben).}\]
\[\text{ESI-MS (m/z): 192 (M}^+\text{).}\]
3-(3,4-dimethoxyphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (5i)

White solid. mp 180-182°C.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 13.56-13.11 (broad, 1H, NH); 7.65-7.43 (m, 2H, 2XAr-H); 6.88 (d, $J$ = 8.1 Hz, 1H, Ar-H); 3.88 (s, 6H, 2XAr-CH$_3$).

ESI-MS (m/z): 238 (M+1)$^+$. 

3-(4-methoxyphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (5j)

White solid. mp 175-177°C.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 13.75-13.28 (broad, 1H, NH); 7.98 (d, $J$ = 7.0 Hz, 2H, 2XAr-H); 7.44 (d, $J$ = 7.0 Hz, 2H, 2XAr-H); 3.84 (s, 3H, Ar-CH$_3$).

5-[4-(tert-butyl)phenyl]-4H-1,2,4-triazol-3-yl (3-chloropropyl) sulfide (6a)

White solid. mp 127-128°C.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.86 (d, $J$ = 8.3 Hz, 2H, 2XAr-H); 7.42 (d, $J$ = 8.3 Hz, 2H, 2XAr-H); 3.67 (t, $J$ = 6.0 Hz, 2H, Cl-CH$_2$CH$_2$); 3.32 (t, $J$ = 6.0 Hz, 2H, S-CH$_2$); 2.23 (quintet, $J$ = 6.0 Hz, 2H, CH$_2$-CH$_2$); 1.34 (s, 9H, C(CH$_3$)$_3$).

IR (KBr, cm$^{-1}$): 2960, 2928, 2861 (C-H), 1445 (C-N), 845, 757 (=C-H ben).

ESI-MS (m/z): 310 (M+). 

4-bromopentyl 5-[4-(tert-butyl)phenyl]-4H-1,2,4-triazol-3-yl sulfide (6b)

White solid. mp 114-116°C.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.87 (d, $J$ = 7.8 Hz, 2H, 2XAr-H); 7.43 (d, $J$ = 8.8 Hz, 2H, 2XAr-H); 4.16-4.08 (m, 1H, CH$_2$CH(Br)CH$_3$); 3.20 (t, $J$ = 6.8 Hz, 2H, S-CH$_2$); 2.05-1.87 (m, 4H, 2XCH$_2$);
1.71 (d, J = 6.8 Hz, 3H, CH-CH₃); 1.35 (s, 9H, C(CH₃)₃).
IR (KBr, cm⁻¹): 2961, 2864, 2715 (C-H), 1448, 1264 (C-N), 843, 725 (=C-H ben).
ESI-MS (m/z): 382 (M⁺).

5-bromopentyl 5-[4-(tert-butyl)phenyl]-4H-1,2,4-triazol-3-yl sulfide (6c)

White solid. mp 125-126°C.

1H NMR (300 MHz, CDCl₃) δ: 7.87 (d, J = 8.3 Hz, 2H, 2XAr-H); 7.37 (d, J = 8.3 Hz, 2H, 2XAr-H); 3.33 (t, 2H, J = 6.8 Hz, 2H, S-CH₂); 3.13 (t, J = 6.8 Hz, 2H, Br-CH₂CH₂); 1.90-1.80 (m, 2H, CH₂-CH₂CH₂); 1.78-1.68 (m, 2H, CH₂-CH₂CH₂); 1.61-1.48 (m, 2H, CH₂-CH₂CH₂); 1.32 (s, 9H, C(CH₃)₃).
IR (KBr, cm⁻¹): 3060 (N-H), 2954, 2862, 2784 (C-H), 1448 (C-N), 832, 745 (=C-H ben).
ESI-MS (m/z): 382 (M⁺).

2-bromoethyl 5-[4-(tert-butyl)phenyl]-4H-1,2,4-triazol-3-yl sulfide (6d)

White solid. mp 72-74°C.

1H NMR (300 MHz, CDCl₃) δ: 7.85 (d, J = 8.3 Hz, 2H, 2XAr-H); 7.44 (d, J = 8.3 Hz, 2H, 2XAr-H); 3.74-3.66 (m, 2H, S-CH₂); 3.59-3.51 (m, 2H, Br-CH₂); 1.35 (s, 9H, C(CH₃)₃).
IR (KBr, cm⁻¹): 2929, 2849, 2724 (C-H), 1448 (C-N), 832, 745 (=C-H ben).
ESI-MS (m/z): 340 (M⁺).

3-chloropropyl [5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl] sulfide (6e)

White solid. mp 129-130°C.

1H NMR (300 MHz, CDCl₃) δ: 7.81 (d, J = 8.3 Hz, 2H, 2XAr-H); 7.20 (d, J = 7.6 Hz, 2H, 2XAr-H); 3.67 (t, J = 6.0 Hz, 2H, Cl-CH₂CH₂); 3.32 (t, J = 6.8 Hz, 2H, S-CH₂); 2.40 (s, 3H, Ar-CH₃); 2.24 (quintet, J = 6.8 Hz, 2H, CH₂-CH₂CH₂).
IR (KBr, cm$^{-1}$): 3080 (N-H), 2926, 2856, 2709 (C-H), 1447, 1264 (C-N), 828, 744 (=C-H ben).
ESI-MS (m/z): 268 (M$^+$).

3-[(3-chloropropyl)sulfanyl]-5-(4-methoxyphenyl)-4H-1,2,4-triazole (6f)

White solid. mp 105-107°C.

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.93 (d, $J$ = 8.9 Hz, 2H, 2XAr-H); 6.89 (d, $J$ = 8.9 Hz, 2H, 2XAr-H); 3.84 (s, 3H, OCH$_3$); 3.70 (t, $J$ = 6.0 Hz, 2H, Cl-CH$_2$CH$_2$); 3.56 (t, $J$ = 6.0 Hz, 2H, S-CH$_2$); 2.31 (quintet, $J$ = 6.0 Hz, 2H, CH$_2$-CH$_2$CH$_2$).
IR (KBr, cm$^{-1}$): 3065 (N-H), 2928, 2852, 2754 (C-H), 1446, 1269 (C-N), 835, 758 (=C-H ben).
ESI-MS (m/z): 284 (M$^+$).

2-[4-(tert-butyl)phenyl]-6,7-dihydro-5H-[1,2,4]triazolo[5,1-b][1,3]thiazine (9a)

$^1$H NMR (300 MHz, CDCl$_3$) $\delta$: 7.84 (d, $J$ = 8.3 Hz, 2H, 2XAr-H); 7.34 (d, $J$ = 8.3 Hz, 2H, 2XAr-H); 4.36 (t, $J$ = 6.2 Hz, 2H, N-CH$_2$); 3.58 (t, $J$ = 6.2 Hz, 2H, S-CH$_2$); 2.13 (quintet, $J$ = 6.2 Hz, 2H, CH$_2$-CH$_2$CH$_2$); 1.26 (s, 9H, C(CH$_3$)$_3$).
IR (KBr, cm$^{-1}$): 2963, 2871 (C-H), 1610 (C=C), 1466 (C-N), 774, 708 (=C-H ben).
ESI-MS (m/z): 274 (M+1)$^+$. 

2-[4-(tert-butyl)phenyl]-5-methyl-5,6,7,8-tetrahydro[1,2,4]triazolo[5,1-b][1,3]thiazepine (9b)
1H NMR (300 MHz, CDCl₃) δ: 7.94 (d, J= 8.5 Hz, 2H, 2XAr-H); 7.38 (d, J= 8.3 Hz, 2H, 2XAr-H); 4.87-4.77 (m, 1H, N-CH(CH₃)CH₂); 2.85 (t, J= 5.7 Hz, 2H, S-CH₂); 2.38-1.77 (m, 4H, 2XCH₂); 1.70 (d, J= 7.2 Hz, 3H, CH-CH₃); 1.35 (s, 9H, C(CH₃)₃).
IR (KBr, cm⁻¹): 2958, 2868 (C=H), 1619 (C=C), 1462, 1325 (C=N), 844, 758 (=C=H ben). ESI-MS (m/z): 302 (M⁺).

2-[4-(tert-butyl)phenyl]-6,7,8,9-tetrahydro-5H-[1,2,4]triazolo[5,1-b][1,3]thiazocine (9c)

1H NMR (300 MHz, CDCl₃) δ: 7.98 (d, J= 8.3 Hz, 2H, 2XAr-H); 7.40 (d, J= 8.3 Hz, 2H, 2XAr-H); 4.56 (t, J= 5.3 Hz, 2H, N-CH₂); 2.91 (t, J= 5.3 Hz, 2H, S-CH₂); 2.06-1.70 (m, 4H, 2XCH₂); 1.48-1.37 (m, 2H, CH₂); 1.35 (s, 9H, C(CH₃)₃).
IR (KBr, cm⁻¹): 2961, 2869 (C=H), 1611 (C=C), 1471, 1361 (C=N), 846, 760 (=C=H ben). ESI-MS (m/z): 302 (M⁺).

2-[4-(tert-butyl)phenyl]-5,6-dihydro[1,2,4]triazolo[5,1-b][1,3]thiazole (9d)

1H NMR (300 MHz, CDCl₃) δ: 7.97 (d, J= 8.7 Hz, 2H, 2XAr-H); 7.38 (d, J= 6.8 Hz, 2H, 2XAr-H); 4.40 (t, J= 6.8 Hz, 2H, N-CH₂); 3.72 (t, J= 6.8 Hz, 2H, S-CH₂); 1.35 (s, 9H, C(CH₃)₃).
IR (KBr, cm⁻¹): 2955, 2865 (C=H), 1612 (C=C), 1460 (C=N), 779, 740 (=C=H ben). ESI-MS (m/z): 259 (M⁺).

2-(4-methylphenyl)-6,7-dihydro-5H-[1,2,4]triazolo[5,1-b][1,3]thiazine (9e)

1H NMR (300 MHz, CDCl₃) δ: 7.81 (d, J= 8.1 Hz, 2H, 2XAr-H); 7.12 (d, J= 7.9 Hz, 2H, 2XAr-H); 4.36 (t, J= 6.0 Hz, 2H, N-CH₂); 3.59 (t, J= 6.4 Hz, 2H, S-CH₂); 2.34
(s, 3H, Ar-CH₃); 2.14 (quintet, $J$= 6.0 Hz, 2H, CH₂-CH₂CH₂).

IR (KBr, cm⁻¹): 2966, 2838 (C-H), 1609 (C=C), 1469, 1364 (C-N), 826, 753 (=C-H ben).

ESI-MS (m/z): 231 (M⁺).

2-(4-methoxyphenyl)-6,7-dihydro-5H-[1,2,4]triazolo[5,1-b][1,3]thiazine (9f)

$^1$H NMR (300 MHz, CDCl₃) δ: 7.86 (d, $J$= 8.3 Hz, 2H, 2XAr-H); 6.79 (d, $J$= 8.3 Hz, 2H, 2XAr-H); 4.34 (t, $J$= 6.0 Hz, 2H, N-CH₂); 3.76 (s, 3H, OCH₃); 3.58(t, $J$= 6.0 Hz, 2H, CH₂-CH₂CH₂).

IR (KBr, cm⁻¹): 2954, 2856 (C-H), 1606 (C=C), 1473, 1368 (C-N), 837, 743 (=C-H ben).

ESI-MS (m/z): 247 (M⁺).

The spectral data for 10a-10c and 10e-10g is already given in the experimental part of Section B of Chapter I.

1-(3-chloropropyl)-4-ethylpiperazine (10d)

$^1$H NMR (300 MHz, CDCl₃) δ: 3.57 (t, $J$= 6.9 Hz, 2H, Cl-CH₂CH₂); 2.59-2.26 (m, 12H, 6XN-CH₂); 1.92 (quintet, $J$= 6.9 Hz, 2H, CH₂CH₂CH₂); 1.07 (t, $J$= 6.9 Hz, 3H, CH₂CH₃).

IR (KBr, cm⁻¹): 2957 (-C-H), 1371 (-C-N).

EI-MS (m/z): 191 (M⁺).

5-[4-(tert-butyl)phenyl]-4H-1,2,4-triazol-3-yl (3-morpholinopropyl) sulfide (8a)

Brown gummy liquid.

$^1$H NMR (200 MHz, CDCl₃) δ: 7.90 (d, $J$= 8.6, 2H, 2XAr-H); 7.42 (d, $J$= 8.6, 2H, 2XAr-H); 3.69-3.57 (m, 4H, 2XO-CH₂); 3.17 (t, $J$= 7.0 Hz, 2H, S-CH₂); 2.51-2.32
(m, 6H, 3XN-CH$_2$); 1.91 (quintet, $J$= 7.0 Hz, 2H, CH$_2$CH$_2$CH$_2$); 1.34 (s, 9H, C(CH$_3$)$_3$).
IR (KBr, cm$^{-1}$): 3152 (N-H), 2959, 2862, 2812 (-C-H), 1615 (C=C), 1456, 1329 (C-N), 1267, 1117 (C-O), 847, 756 (=C-H ben).
ESI-MS (m/z): 361 (M+1)$^+$.  
Anal. Calcd for C$_{19}$H$_{28}$N$_4$OS (360): C, 63.30; H, 7.83; N, 15.54. Found: C, 63.34; H, 7.81; N, 15.58.

5-[4-(tert-butyl)phenyl]-4H-1,2,4-triazol-3-yl [3-(4-phenylpiperazino)propyl] sulfide (8b)

White solid. mp 138-140$^\circ$C.
$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 7.90 (d, $J$= 8.3 Hz, 2H, 2XAr-H); 7.35 (d, $J$= 8.3 Hz, 2H, 2XAr-H); 7.16 (t, $J$ = 7.9 Hz, 2H, 2XAr-H); 6.83-6.75 (m, 3H, 3XAr-H); 3.26-3.16 (m, 4H, 2XN-CH$_2$); 3.13 (t, $J$= 6.8 Hz, 2H, S-CH$_2$); 2.76-2.68 (m, 4H, 2XN-CH$_2$); 2.64 (t, $J$= 6.6 Hz, 2H, N-CH$_2$); 1.99-1.94 (m, 2H, CH$_2$CH$_2$CH$_2$); 1.29 (s, 9H, C(CH$_3$)$_3$).
IR (KBr, cm$^{-1}$): 3448 (N-H), 2956 (-C-H), 1639 (C=C), 1495, 1420, 1225 (C-N), 768 (=C-H ben).
ESI-MS (m/z): 436 (M+1)$^+$.  
Anal. Calcd for C$_{25}$H$_{33}$N$_5$S (435): C, 68.93; H, 7.64; N, 16.08. Found: C, 68.96; H, 7.66; N, 16.06.

3-[4-(3-chlorophenyl)piperazino]propyl [5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl] sulfide (8c)

Yellow solid. mp 85-86$^\circ$C.
$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 7.88 (d, $J$= 7.8 Hz, 2H, 2XAr-H); 7.21 (d, $J$ = 7.8 Hz, 2H, 2XAr-H); 7.12 (t, $J$= 8.6 Hz, 1H, Ar-H); 6.85-6.67 (m, 3H,
3XAr-H); 3.28-3.04 (m, 6H, S-CH$_2$ & 2XN-CH$_2$); 2.67-2.45 (m, 6H, 3XN-CH$_2$); 2.40 (s, 3H, Ar-CH$_3$); 1.97 (quintet, $J$=7.0 Hz, 2H, CH$_2$CH$_3$CH$_2$).

IR (KBr, cm$^{-1}$): 2947, 2826 (-C-H), 1594 (C=C), 1486, 1328, 1274 (C-N), 834, 760 (=C-H ben).

ESI-MS (m/z): 428 (M$^+$).


3-(4-ethylpiperazino)propyl [5-(4-methylphenyl)-4H-1,2,4-triazol-3-yl] sulfide (8d)

Brown gummy liquid.

$^1$H NMR (200 MHz, CDCl$_3$) δ: 7.90 (d, $J$= 7.6 Hz, 2H, 2XAr-H); 7.16 (d, $J$= 8.3 Hz, 2H, 2XAr-H); 3.15 (t, $J$= 6.8 Hz, 2H, S-CH$_2$); 2.72-2.40 (m, 12H, 6XN-CH$_2$); 2.37 (s, 3H, Ar-CH$_3$); 2.02-1.93 (m, 2H, CH$_2$CH$_3$CH$_2$); 1.26 (t, $J$= 6.8 Hz, 3H, CH$_3$CH$_2$).

IR (KBr, cm$^{-1}$): 3406 (N-H), 2930, 2823 (-C-H), 1615 (C=C), 1450, 1327, 1267 (C-N), 830, 752 (=C-H ben).

ESI-MS (m/z): 346 (M+1$^+$).


3-[4-(3-chlorophenyl)piperazino]propyl [5-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl] sulfide (8e)

White solid. mp 72-74°C.

$^1$H NMR (200 MHz, CDCl$_3$) δ: 7.96 (d, $J$= 8.8 Hz, 2H, 2XAr-H); 7.33 (d, $J$= 8.1 Hz, 2H, 2XAr-H); 7.18-7.04 (m, 1H, Ar-H); 6.94-6.62 (m, 3H, 3XAr-
5-(4-chlorophenyl)-4H-1,2,4-triazol-3-yl 3-[4-(2-pyrimidinyl)piperazino]propyl sulfide (8f)

White solid. mp 128-129°C.

$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 8.24 (d, $J= 5.1$ Hz, 2H, 2XArkH); 7.99 (d, $J= 8.9$ Hz, 2H, 2XArk-H); 7.40 (d, $J= 8.9$ Hz, 2H, 2XArk-H); 6.47 (t, $J= 5.1$ Hz, 1H, Ar-H); 3.85-3.74 (m, 4H, 2XN-CH$_2$); 3.23 (t, $J= 7.4$ Hz, 2H, S-CH$_2$); 2.57-2.30 (m, 6H, 3XN-CH$_2$); 1.97 (quintet, $J= 7.4$ Hz, 2H, CH$_2$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 3171 (N-H), 2930, 2852 (-C-H), 1586, 1493 (C=C), 1448, 1260 (C-N), 752 (=C-H ben).

ESI-MS (m/z): 417 (M$^+$).

Anal. Calcd for C$_{19}$H$_{22}$ClN$_7$S (416): C, 54.87; H, 5.33; N, 23.57. Found: C, 54.91; H, 5.31; N, 23.54.

3-(4-benzylpiperazino)propyl [5-(4-fluorophenyl)-4H-1,2,4-triazol-3-yl] sulfide (8g)

Brown gummy liquid.

$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 8.08-7.94 (m, 2H, 2XAr-H); 7.33-7.16 (m, 5H, 5XAr-H); 7.11-6.97 (m, 2H, 2XAr-H); 3.54 (s, 2H, Ph-CH$_2$); 3.12 (t, $J= $...
6.0 Hz, 2H, S-CH$_2$); 2.72-2.43 (m, 10H, 5XN-CH$_2$); 1.97 (quintet, $J=6.0$ Hz, 2H, CH$_3$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 3405 (N-H), 2932, 2830 (-C-H), 1606 (C=C), 1455, 1326, 1226 (C-N), 847, 752 (=C-H ben).

ESI-MS (m/z): 412 (M+1)$^+$. 

Anal. Calcd for C$_{22}$H$_{26}$FN$_5$S (411): C, 64.21; H, 6.37; N, 17.02. Found: C, 64.18; H, 6.40; N, 17.05.

5-(3-methylphenyl)-4H-1,2,4-triazol-3-yl [3-(4-phenylpiperazino)propyl] sulfide (8h)

White solid. mp 75-77°C.

$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 7.88-7.74 (m, 2H, 2XArkH); 7.37-7.10 (m, 4H, 4XArk-H); 6.89-6.70 (m, 3H, 3XArk-H); 3.27-3.05 (m, 6H, S-CH$_2$ & 2XN-CH$_2$); 2.62-2.45 (m, 6H, 3XN-CH$_2$); 2.40 (s, 3H, Ar-CH$_3$); 1.95 (quintet, $J=7.0$ Hz, 2H, CH$_2$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 3421 (N-H), 2921, 2852 (-C-H), 1599 (C=C), 1456, 1328, 1234 (C-N), 741, 685 (=C-H ben).

ESI-MS (m/z): 394 (M+1)$^+$. 

Anal. Calcd for C$_{22}$H$_{27}$N$_5$S (393): C, 67.14; H, 6.92; N, 17.79. Found: C, 67.10; H, 6.95; N, 17.81.

5-(3-methylphenyl)-4H-1,2,4-triazol-3-yl 3-[4-(2-pyridyl)piperazino]propyl sulfide (8i)

White solid. mp 109-101°C.

$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 8.14 (dd, $J=3.8, 1.5$ Hz, 1H, Ar-H); 7.94-7.74 (m, 2H, 2XAr-H); 7.42 (t, $J=7.6$ Hz, 1H, Ar-H); 7.22 (t, $J=7.6$ Hz, 1H, Ar-H); 7.12 (d, $J=6.8$ Hz, 1H, Ar-H); 6.61-6.54 (m, 2H, 2XAr-
5-(3-chlorophenyl)-4H-1,2,4-triazol-3-yl 3-[4-(2-pyrimidinyl)piperazino]propyl sulfide (8j)

White solid. mp 115-117°C.

$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 8.24 (d, $J$= 4.7 Hz, 2H, 2XArkH); 8.06-8.00 (m, 1H, ArH); 7.97-7.89 (m, 1H, Ar-H); 7.42-7.30 (m, 2H, 2XArk-H); 6.45 (t, $J$= 4.7 Hz, 1H, Ar-H); 3.88-3.75 (m, 4H, 2XN-CH$_2$); 3.25 (t, $J$= 7.0 Hz, 2H, CH$_2$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 3448 (N-H), 2924, 2846 (C-H), 1587 (C=C), 1485, 1255 (C-N), 795 (C-H ben).

ESI-MS (m/z): 417 (M+1)$^+$.

Anal. Calcd for C$_{19}$H$_{22}$ClN$_7$S (416): C, 54.87; H, 5.33; N, 23.57. Found: C, 54.89; H, 5.35; N, 23.53.

5-(2-chlorophenyl)-4H-1,2,4-triazol-3-yl [3-(4-ethylpiperazino)propyl] sulfide (8k)

Brown gummy liquid.

$^1$H NMR (200 MHz, CDCl$_3$) $\delta$: 7.99-7.91 (m, 1H, Ar-H); 7.46-7.39 (m, 1H, Ar-H); 7.34-7.24 (m, 2H, 2XArk-H); 3.17 (t, $J$= 6.8 Hz, 2H, S-CH$_2$); 2.68-2.24 (m, 12H, 6XN-CH$_2$); 1.99
$J = 7.6$ Hz, 3H, CH$_3$CH$_2$).

IR (KBr, cm$^{-1}$): 3065 (N-H), 2939, 2818 (-C-H), 1600 (C=C), 1450, 1323, 1266 (C-N), 750 (=C-H ben).

ESI-MS (m/z): 366 (M$^+$).


5-(2-chlorophenyl)-4H-1,2,4-triazol-3-yl 3-[4-(2-pyridyl)piperazino]propyl sulfide (8l)

Yellow solid. mp 88-90$^\circ$C.

$^1$H NMR (200 MHz, CDCl$_3$) δ: 8.15-8.10 (m, 1H, Ar-H); 7.96-7.89 (m, 1H, Ar-H); 7.44-7.35 (m, 2H, 2XAr-H); 7.30-7.23 (m, 2H, 2XAr-H); 3.57-3.46 (m, 4H, 2XN-CH$_2$); 3.16 (t, $J = 6.8$ Hz, 2H, S-CH$_2$); 2.58-2.45 (m, 6H, 3XN-CH$_2$); 1.96 (quintet, $J = 6.8$ Hz, 2H, CH$_2$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 3068 (N-H), 2926, 2831 (-C-H), 1596 (C=C), 1437, 1316, 1246 (C-N), 770, 745 (=C-H ben).

ESI-MS (m/z): 415 (M$^+$).


5-benzyl-4H-1,2,4-triazol-3-yl 3-[4-(2-pyridyl)piperazino]propyl sulfide (8m)

Yellow gummy liquid.

$^1$H NMR (200 MHz, CDCl$_3$) δ: 8.15-8.09 (m, 1H, Ar-H); 7.46-7.37 (m, 1H, Ar-H); 7.33-7.10 (m, 5H, 5XAr-H); 6.61-6.53 (m, 2H, 2XAr-H ); 3.97 (s, 2H, Ph-CH$_2$); 3.58-3.44 (m, 4H, 2XN-CH$_2$); 3.08 (t, $J = 6.8$ Hz, 2H, S-CH$_2$); 2.55-2.41 (m, 6H, 3XN-CH$_2$); 1.91 (quintet, $J = 6.8$ Hz, 2H, CH$_2$CH$_2$CH$_2$).
IR (KBr, cm\(^{-1}\)): 2932, 2825 (-C-H), 1595, 1483 (C=C), 1438, 1313, 1247 (C-N), 757 (=C-H ben).

ESI-MS (m/z): 395 (M+1)\(^+\).

Anal. Calcd for C\(_{21}\)H\(_{26}\)N\(_6\)S (394): C, 63.93; H, 6.64; N, 21.30. Found: C, 63.91; H, 6.65; N, 21.34.

3-[4-(tert-butyl)phenyl]-1-[3-(4-phenylpiperazino)propyl]-4,5-dihydro-1H-1,2,4-triazole-5-thione (11a)

White solid. mp 152-154 °C.

\(^1\)H NMR (200 MHz, CDCl\(_3\)) δ: 7.92 (d, J= 7.3 Hz, 2H, 2XAr-H); 7.41 (d, J= 8.8 Hz, 2H, 2XAr-H); 7.17 (t, J= 7.3 Hz, 2H, 2XAr-H); 4.39-4.28 (m, 2H, NCH\(_2\)); 3.21-3.10 (m, 4H, 2XN-CH\(_2\)); 2.62-2.44 (m, 6H, 3XN-CH\(_2\)); 2.05-1.88 (m, 2H, CH\(_2\)CH\(_2\)CH\(_2\)); 1.34 (s, 9H, C(CH\(_3\))\(_3\)).

IR (KBr, cm\(^{-1}\)): 3422 (N-H), 3060 (=C-H), 2956, 2819 (-C-H), 1599, 1499 (C=C), 1334, 1234 (C-N), 841, 755 (=C-H ben).

ESI-MS (m/z): 435 (M\(^+\)).

Anal. Calcd for C\(_{25}\)H\(_{33}\)N\(_5\)S (435): C, 68.93; H, 7.64; N, 16.08. Found: C, 68.94; H, 7.62; N, 16.11.

3-[4-(tert-butyl)phenyl]-1-3-[4-(3-chlorophenyl)piperazino]propyl]-4,5-dihydro-1H-1,2,4-triazole-5-thione (11b)

White solid. mp 160-162 °C.

\(^1\)H NMR (200 MHz, CDCl\(_3\)) δ: 7.91 (d, J= 8.8 Hz, 2H, 2XAr-H); 7.39 (d, J= 8.0 Hz, 2H, 2XAr-H); 7.11 (t, J= 7.3 Hz, 1H, Ar-H); 6.83-6.67 (m, 3H, 3XAr-H); 3.99-3.79 (m, 2H, N-CH\(_2\)); 3.23-3.11 (m, 4H, 2XN-
CHAPTER III

131

CH$_2$); 2.62-2.45 (m, 6H, 3XN-CH$_2$); 2.06-1.87 (m, 2H, CH$_2$CH$_2$CH$_2$); 1.34 (s, 9H, C(CH$_3$)$_3$).

IR (KBr, cm$^{-1}$): 3083 (N-H), 2957, 2835 (-C-H), 1593 (C=C), 1440, 1330, 1241 (C-N), 838, 773 (=C-H ben).

ESI-MS (m/z): 470 (M$^+$).

Anal. Calcd for C$_{25}$H$_{32}$ClN$_5$S (470): C, 63.88; H, 6.86; N, 14.90. Found: C, 63.85; H, 6.88; N, 14.94.

1-3-[4-(3-chlorophenyl)piperazino]propyl-3-(4-methylphenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (11c)

Yellow solid. mp 73-75 °C.

$^1$H NMR (200 MHz, CDCl$_3$) δ: 7.90 (d, J= 8.3 Hz, 2H, 2XAr-H); 7.20 (d, J= 7.9 Hz, 2H, 2XAr-H); 7.10 (t, J= 7.9 Hz, 1H, Ar-H); 6.81 (t, J= 2.0 Hz, 1H, Ar-H); 6.78-6.69 (m, 2H, 2XAr-H); 4.36 (t, J= 6.4 Hz, 2H, N-CH$_2$); 3.20-3.15 (m, 4H, 2XN-CH$_2$); 2.62-2.56 (m, 4H, 2XN-CH$_2$); 2.53 (t, J= 7.2 Hz, 2H, N-CH$_2$); 2.42 (s, 3H, Ar-CH$_3$); 1.97 (quintet, J= 7.0 Hz, 2H, CH$_2$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 2951, 2822 (-C-H), 1595 (C=C), 1451, 1274 (C-N), 840 (=C-H ben).

ESI-MS (m/z): 428 (M$^+$).


3-(4-chlorophenyl)-1-3-[4-(3-chlorophenyl)piperazino]propyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (11d)

White solid. mp 88-90 °C.

$^1$H NMR (200 MHz, CDCl$_3$) δ: 8.00 (d, J= 8.8 Hz, 2H, 2XAr-H); 7.40 (d, J= 8.8 Hz, 2H, 2XAr-H); 7.13 (t, J= 8.1 Hz, 1H, Ar-H); 6.85-6.70 (m, 3H, 3XAr-H); 4.54-4.37 (m, 2H, N-CH$_2$); 3.30-3.13 (m, 4H, 2XN-
132

CHAPTER III

CH$_2$); 2.64-2.45 (m, 6H, 3XN-CH$_2$); 1.97 (quintet, $J$= 6.6 Hz, 2H, CH$_2$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 3075 (N-H), 2924, 2825 (-C-H), 1594, 1485 (C=C), 1449, 1237 (C-N), 837 (=C-H ben).

ESI-MS (m/z): 448 (M$^+$).


1-[(3-(4-benzylpiperazino)propyl)]-3-(4-fluorophenyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (11e)

Brown gummy liquid.

$^1$H NMR (200 MHz, CDCl$_3$) δ: 8.12-7.98 (m, 2H, 2XArkH); 7.34-7.03 (m, 7H, 7XArk-H); 4.30 (t, $J$= 6.8 Hz, 2H, N-CH$_2$); 3.48 (s, 2H, Ph-CH$_2$); 2.61-2.25 (m, 10H, 5XN-CH$_2$); 1.96 (quintet, $J$= 6.8 Hz, 2H, CH$_2$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 3028 (=C-H), 2936, 2811 (-C-H), 1606 (C=C), 1462, 1326, 1223 (C-N), 844, 746 (=C-H ben).

EI-MS (m/z): 411 (M$^+$).

Anal. Calcd for C$_{22}$H$_{26}$FN$_5$S (411): C, 64.21; H, 6.37; N, 17.02. Found: C, 64.24; H, 6.39; N, 17.00.

3-(3,4-dimethoxyphenyl)-1-(3-morpholinopropyl)-4,5-dihydro-1H-1,2,4-triazole-5-thione (11f)

Brown gummy liquid.

$^1$H NMR (200 MHz, CDCl$_3$) δ: 7.57 (dd, $J$= 6.8, 2.3 Hz, 1H, Ar-H); 7.43 (d, $J$= 1.5 Hz, 1H, Ar-H); 6.88 (dd, $J$= 6.8 & 2.3 Hz, 1H, Ar-H); 4.29 (t, $J$= 6.8 Hz, 2H, N-CH$_2$); 3.87 (s, 6H, 2XOCH$_3$); 3.64-3.56 (m, 4H, 2XO-CH$_2$); 2.49-2.34 (m, 6H, 3XN-CH$_2$); 1.89 (quintet, $J$= 6.8 Hz, 2H, CH$_2$CH$_2$CH$_2$).
IR (KBr, cm\(^{-1}\)): 3085 (N-H), 2956, 2851 (-C-H), 1601, 1514 (C=C), 1345, 1271 (C-N), 866, 764 (=C-H ben).

ESI-MS (m/z): 364 (M\(^+\)).

Anal. Calcd for C\(_{17}\)H\(_{24}\)N\(_4\)O\(_3\)S (364): C, 56.02; H, 6.64; N, 15.37. Found: C, 56.00; H, 6.65; N, 15.40.

3-(3,4-dimethoxyphenyl)-1-[3-(4-ethylpiperazino)propyl]-4,5-dihydro-1H-1,2,4-triazole-5-thione (11g)

Brown gummy liquid.

\(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\): 7.59 (dd, \(J = 6.3, 2.3\) Hz, 1H, Ar-H); 7.45 (d, \(J = 1.6\) Hz, 1H, Ar-H); 6.87 (d, \(J = 8.6\) Hz, 1H, Ar-H); 4.29 (t, \(J = 7.0\) Hz, 2H, N-CH\(_2\)); 3.89 (s, 6H, 2XOCH\(_3\)); 2.57-2.25 (m, 12H, 6XN-CH\(_2\)); 1.91 (quintet, \(J = 7.0\) Hz, 2H, CH\(_2\)CH\(_2\)CH\(_2\)); 1.05 (t, \(J = 7.0\) Hz, 3H, CH\(_3\)CH\(_2\)).

IR (KBr, cm\(^{-1}\)): 3411 (N-H), 2939, 2811 (C-H), 1598, 1514 (C=C), 1345, 1271 (C-N), 765 (=C-H ben).

ESI-MS (m/z): 391 (M\(^+\)).

Anal. Calcd for C\(_{19}\)H\(_{29}\)N\(_5\)O\(_2\)S (391): C, 58.29; H, 7.47; N, 17.89. Found: C, 58.28; H, 7.50; N, 17.86.

3-(4-methoxyphenyl)-1-3-[4-(2-pyridyl)piperazino]propyl-4,5-dihydro-1H-1,2,4-triazole-5-thione (11h)

Yellow solid. mp 110-112\(^\circ\)C.

\(^1\)H NMR (200 MHz, CDCl\(_3\)) \(\delta\): 8.13 (dd, \(J = 3.0, 1.5\) Hz, 1H, Ar-H); 7.96 (d, \(J = 8.3\) Hz, 2H, 2XAr-H); 7.47-7.37 (m, 1H, Ar-H); 6.88 (d, \(J = 8.3\) Hz, 2H, 2XAr-H); 6.60-6.54 (m, 2H, 2XAr-H); 4.35 (t, \(J = 6.8\) Hz,
2H, N-CH$_2$); 3.85 (s, 3H, OCH$_3$); 3.57-3.49 (m, 4H, 2XN-CH$_2$); 2.60-2.50 (m, 6H, 3XN-CH$_2$); 1.98 (quintet, $J=7.6$ Hz, 2H, CH$_2$CH$_2$CH$_2$).

IR (KBr, cm$^{-1}$): 3449 (N-H), 2928, 2842 (-C-H), 1597 (C=C), 1481, 1253 (C-N), 772 (=C-H ben).

ESI-MS (m/z): 411 (M+1)$^+$.  


- **Biology**

Details of the procedures for the anticancer, antibacterial and antifungal activities are already given in experimental part of Section A of the Chapter II.

### 3.3.4 Conclusions

Synthesis of 3-[3-[4-(substituted)-1-cyclicamine]propyl]thio-5-substituted[1,2,4]triazoles (8a-m) and 2-[3-[4-(substituted)-1-cyclicamine]propyl]-5-(substituted)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (11a-h) was achieved with good yields starting from corresponding carboxylic acids using two different methods. The anticancer, antibacterial and antifungal activity of these derivatives was studied. Among the compounds (n = 21) tested for anticancer activity, five compounds had shown good anticancer activity. The test compounds, 11d, 8j and 8i were most potent against both HL-60 and U937 cells. On the structure point of view, the compounds with chloro group at 3$^{rd}$ or 4$^{th}$ position of the phenyl ring and piperizine containing N-3-chlorophenyl, N-2-pyrimidyl and N-2-pyridyl groups had shown good cytotoxic activity. The cytotoxic potency of the compounds varied between the two cell lines suggesting that a structural property of these compounds as possible determinants of their biological activity. The compounds had not shown any remarkable antibacterial activity but most of the compounds had shown moderate antifungal activity especially against *C.rugosa.*
3.4 References