2.0. Introduction.

Triazole refers to either one of a pair of isomeric chemical compounds with molecular formula C$_2$H$_3$N$_3$, having a five membered ring of two carbon atoms and three nitrogen atoms. The two isomers are: 1,2,3-triazole and 1,2,4-triazole.

![1,2,3-triazole](image1.png) ![1,2,4-triazole](image2.png)

1,2,4-Triazole and its derivatives represent one of the most biologically active class of compounds and are associated with diverse pharmacological activities such as antibacterial, antifungal, hypoglycemic, antihypertensive and analgesic properties [1-5]. The substituted 1,2,4-triazole nucleus is particularly common and can be found in marketed drugs such as fluconazole, terconazole, rizatriptan, alperazolame and triazolame [6].

2.1. Recent Advancements

Recent literature survey demonstrates that the 1,2,4-triazoles are becoming of great practical significance. 1,2,4-triazole derivatives possess analgesic, antipyretic and antiphlogistic properties. A group of 1,2,4-triazole-5-ones and their mannich bases have shown antitubercular activity [7] and have been investigated with regard to their mode of action. Few examples of biologically active 1,2,4-triazole derivatives are given in table 1.
Table 1. Biologically active 1,2,4-triazole derivatives

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>IUPAC Name</th>
<th>Structure</th>
<th>Biological Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-{(4-methylpiperazin-1-yl)methyl}-4-phenyl-5-(pyridin-4-yl)-2H-1,2,4-triazole-3(4H)-thione</td>
<td><img src="structure1.png" alt="Structure Image" /></td>
<td>Antibacterial [8]</td>
</tr>
<tr>
<td>2</td>
<td>2-[5-{(1-methyl-1H-pyrrol-2-yl)methyl}-4-phenyl-4H-1,2,4-triazol-3-yliotio]-N-(4-chlorophenyl)acetamide</td>
<td><img src="structure2.png" alt="Structure Image" /></td>
<td>Antimicrobial [9]</td>
</tr>
<tr>
<td>3</td>
<td>7-{4-{(1H-1,2,4-triazol-1-yl)methyl}benzyl oxy}-4-methyl-2H-chromen-2-one</td>
<td><img src="structure3.png" alt="Structure Image" /></td>
<td>Antibacterial [10]</td>
</tr>
<tr>
<td>4</td>
<td>2-[5-{4-(4-bromophenyl sulfonyl)phenyl}-4-p-tolyl-4H-1,2,4-triazol-3-yliotio]-1-phenylethanone</td>
<td><img src="structure4.png" alt="Structure Image" /></td>
<td>Antifungal [11]</td>
</tr>
<tr>
<td>5</td>
<td>5-{[E]-5-(4-bromobenzylideneamino)-4H-1,2,4-triazol-3-yl]methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazole-3-thiol</td>
<td><img src="structure5.png" alt="Structure Image" /></td>
<td>Anticonvulsant [12]</td>
</tr>
</tbody>
</table>
In view of the biological importance of these 1,2,4-triazoles, several methods have been developed for the synthesis of this class of compounds. Some methods are listed below.

### 2.2. Synthesis of 1,2,4-triazole derivatives.

**2.2.1 Thiosemicarbazide (11) on cyclization in alkaline medium afford 1,2,4-triazoles (12, 13) [16,17].**

![Scheme-1](image)

R = Phenyl, 4-Methoxy phenyl, 3-methyl phenyl, 2-Flouro phenyl

**2.2.2 Thiosemicarbazides (14) on heating with triethylamine in ethanol cyclizes to give 1,2,4-triazoles (15) [18].**
2.2.3. Thiosemicarbazide on refluxing with 1,1 cyclopropane dicarboxylic acid (16) and SOCl₂ in alkaline medium cyclizes to give 1,1-bis(3-thio-1,2,4-triazol-5-yl) cyclopropane (17) [19].

2.2.4. 2-Chloro-6-methoxy-4-phenyl-quinoline (18) on refluxing with substituted acylhydrazides in a nitrogen atmosphere yield 7-Methoxy-1-(4-methoxyphenyl)-5-phenyl-1,2,4-triazolo [4,3-a] quinoline (19) [20].

2.2.5. (2E)-4-phenylquinolin-2(1H)-one hydrazone (20) when refluxing with formic acid in ethanol yield 5-phenyl-3,3a-dihydro [1, 2, 4]triazolo[4,3-a]quinoline (21) [21].

2.2.6. 1,2,4-Triazole-3,5-diamine derivatives (23, 24) were synthesized in moderate to high yields in one-pot reaction from the corresponding isothiocyanates (22), mono-
substituted hydrazines, and sodium hydrogen cyanamide in the presence of 1-(3-dimethylaminopropyl)-3 ethylcarbodiimide hydrochloride. Typically, two target compounds were obtained, but high regioselectivity to one isomer was observed when aromatic and sterically bulky hydrazines were used. Examples with a detailed representative procedure are given below [22].

2.2.7. Cyclo condensation reactions of phenyl selanyl propionate, using phenyl selanyl group as the precursor of terminal double bond is critical to the success of the reactions [23].

\[
\begin{align*}
R_1\text{NCS} & \xrightarrow{\text{i. NaHNCN}} \xrightarrow{\text{ii. R}_2\text{NHNH}_2, \text{EDC}} R_1-N\overset{N}{\equiv}N-N-R_2 \\
\text{Scheme-6} & \quad R_1/R_2 = \text{Alkyl, Aryl}
\end{align*}
\]

2.2.8. The N-(4-methylsulfonylphenyl)aryl carbohydrazonamides (27) undergoes a ring closure using 1,1'-thiocarbonylimidazole and subsequent alkylation to afford 5-(4-halophenyl)-4-(4-(methylsulfonyl)phenyl)-2H-1,2,4-triazole-3(4H)-thione (28) [24].

2.2.9. The reaction of N, N-dimethylformamide azine (29) with primary amines mediated by p-toluene sulfonic acid gives the triazole (31) as shown in scheme-9. The
driving force of this reaction is the release of dimethyl amine as well as the stability of the triazole formed (31) [25].

\[
\text{Me}_2\text{N} \quad \text{H} \quad \text{N} \quad \text{N} \\
\text{Me}_2\text{N} \quad \text{H} \quad \text{N} \quad \text{N} \\
\text{Me}_2\text{N} \quad \text{H} \quad \text{N} \quad \text{N} \\
\]

\[
\begin{align*}
\text{Me}_2\text{N} & \quad \text{N} \quad \text{N} \\
\text{H} & \quad \text{N} \quad \text{Me}_2 \\
\text{Me}_2\text{N} & \quad \text{H} \quad \text{N} \quad \text{N} \\
\text{Me}_2\text{N} & \quad \text{H} \quad \text{N} \quad \text{N} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_2\text{N} - \text{R} & \quad \text{p} \quad \text{TosOH} \\
\text{-2} & \quad \text{HNMe}_2 \\
\end{align*}
\]

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

\[
\begin{align*}
\text{R} & = \text{Alkyl/Aryl} \\
\end{align*}
\]

Scheme-9

1,3,4-oxadiazole on reaction with the primary amines gives 3,5-disubstituted 1,2,4-triazole (34) [26].

\[
\begin{align*}
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{R}_1 \\
\text{CH}_3 & \quad \text{H}_2\text{N} - \text{R} \\
\text{32} & \quad \text{33} \\
\end{align*}
\]

\[
\begin{align*}
\text{R}_1/\text{R}_2 & = \text{Alkyl/Aryl} \\
\end{align*}
\]

Scheme-10

2.2.10. (Z)-4-chloro-N’-(ethoxy(p-tolyl)methylene)benzohydrazide (35) undergoes cyclization in presence of hydrazine monohydrate to produce 4-amino-3-(p-chlorophenyl)-5-p-tolyl-4H-1,2,4-triazole (36) [27].

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \quad \text{CH}_3 \\
\text{N} & \quad \text{N} \\
\text{NH} & \quad \text{O} \\
\text{Cl} & \quad \text{35} \\
\end{align*}
\]

\[
\begin{align*}
\text{H}_3\text{C} & \quad \text{O} \quad \text{CH}_3 \\
\text{N} & \quad \text{N} \\
\text{NH} & \quad \text{O} \\
\text{Cl} & \quad \text{36} \\
\end{align*}
\]

\[
\begin{align*}
i. \text{Propanol} \\
ii. \text{NH}_2\text{NH}_2\text{H}_2\text{O} \\
\end{align*}
\]

Scheme-11

2.2.11. Reaction of resin bound S-methyl isothiourea with carboxylic acids yielded resin-bound S-methyl-N-aclylisothiourea (37) which reacted with hydrazines under mild conditions to afford the corresponding resin-bound 3-amino-1,2,4-triazoles (38) with regioselectivity [28].
2.2.12. When substituted phenylthiourea (39) is refluxed with 2-(aryloxy)alkanoic hydrazides (40) in presence of pyridine, it gave 1,2,4-triazole (41). The completion of the reaction was assured by the ceasing of the methylmercaptane evolution [29].

2.3. Reactions of 1,2,4-triazoles

2.3.1. Triazole (43) on condensation with heteroaromatic acids in presence of phosphorus oxychloride produces a series of triazolo thiadiazoles (42, 44, 45) [30].
2.3.2. 1,2,4-triazole-3-thiol (46) on reaction with ethyl bromoacetate in the presence of sodium ethoxide or triethylamine, hydrazine hydrate, CS$_2$ and KOH leads to the formation of 5-[[4-phenyl-5-pyridin-4-yl-4H-1,2,4-triazol-3-yl]thio]methyl]-1,3,4-oxadiazole-2-thiol (47) which on reaction with 2-(4-morpholino) ethylamine in the presence of formaldehyde solution is converted to the corresponding manich base derivatives (48) [31].

![Scheme 15](image)

2.4. Present Work

The 1,2,4-triazole derivatives and their manich bases represent an important class of heterocyclic compounds which are known for their broad spectrum of biological activities including antimicrobial, anti-inflammatory, analgesic and many other uses [32-38]. As resistance to anti-inflammatory and antimicrobial drugs is widespread, there is an increasing demand for the identification of novel structure leads that may be used in designing new potent and less toxic anti-inflammatory and antimicrobial agents. Several 1,2,4-triazole based antimicrobial agents have been synthesized to develop new molecular entities with high potential against various microbial strains. Further several nonsteroidal anti-inflammatory drugs (NSAIDs) have been developed so far to heal the inflammation. There are number of NSAIDs present in the market to heal the inflammation but majority of them cause adverse side effects like gastric ulcer [39], kidney damage [40] and some of the NSAIDs also cause hepatotoxicity [41]. During the last few decades, a considerable attention has been devoted to the synthesis of various 1,2,4-triazole derivatives [42-44]. Several examples of NSAIDs having triazole moieties have been cited in the literature. Among them, 1,2,4-triazole 3-thiol derivatives are of particular interest and have been studied in recent years [45-48].
Most of the NSAIDs having biphenyl derivatives substituted with an aromatic or heteroaromatic aryl nucleus show better anti-inflammatory activity. Some of the biaryl acid derivatives have been evaluated as potential anti-inflammatory agents through the inhibition of 14 kDa human platelet phospholipase A2 (HP-PLA2) [49]. Some of the biphenylic compounds that contain free carboxyl groups cause ulceration [51-52]. Moreover quinoline moiety was also found in several bio-active molecules (Quinine, Ciprofloxacin).

In view of the biological potentials of quinoline, biaryl and 1,2,4-triazole moieties as anti-inflammatory and anti-microbial agents, we considered it of interest to synthesize some small molecular high affinity ligands by conjugating these bioactive pharmacophores. The synthesis and biological activities of these conjugates are presented in the following four sections.

Section 1. Biphenyl based 1,2,4-triazole derivatives and their biological activities

Section 2. Biphenyl based hydrazones of 1,2,4-triazole derivatives and their biological activities

Section 3. Synthesis of novel 3-mercapto 1,2,4-triazole derived mannich bases and their biological activities

Section 4. Quinoline based 1,2,4-triazole derivatives and their biological activities
2.4.1.0. Introduction

As discussed in introductory part of this chapter, triazole derivatives exhibit different biological activities. In view of the importance of 1,2,4-triazoles and biphenyl systems, a focused library of 1,2,4-triazole based conjugates have been synthesized and evaluated for their anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities. The synthetic route is given below.

**Scheme-I**

Reaction of \( p \)-hydroxybiphenyl (50) with ethylchloroacetate in presence of potassium carbonate in anhydrous acetone afforded ethyl 2-(biphenyl-4-yloxy) acetate (51)
which readily yielded 2-(biphenyl-4-yloxy) acetohydrazide (52) by refluxing with hydrazine monohydrate in absolute alcohol. Compound (52) was then converted into corresponding thiosemicarbazide (53-59) by reacting with different substituted aryl isothiocyanates in absolute alcohol. The thiosemicarbazides (53-59) were cyclised into respective substituted 3-mercapto-1,2,4-triazoles (60-66) using triethylamine in absolute alcohol. Finally S-alkylation of 3-mercapto-1,2,4-triazoles with substituted phenacyl bromides and other alkyl halides leads to the formation of target molecules (67-105). All the synthesized compounds have been completely characterized on the basis of their detailed spectral data and this entire series of new compounds was evaluated for different biological activity. The physical data of all the final compounds are given in table 1.1.

**Table 1.1.** Physical data of biphenyl based 1,2,4-triazole derivatives.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Structures</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>67</td>
<td><img src="image" alt="Structure 67" /></td>
<td>72</td>
<td>164-166</td>
</tr>
<tr>
<td>68</td>
<td><img src="image" alt="Structure 68" /></td>
<td>67</td>
<td>170-172</td>
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<td>69</td>
<td><img src="image" alt="Structure 69" /></td>
<td>70</td>
<td>132-134</td>
</tr>
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<td>70</td>
<td><img src="image" alt="Structure 70" /></td>
<td>74</td>
<td>138-140</td>
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<td>71</td>
<td><img src="image" alt="Structure 71" /></td>
<td>74</td>
<td>138-140</td>
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<tr>
<td>72</td>
<td><img src="image" alt="Structure 72" /></td>
<td>68</td>
<td>168-170</td>
</tr>
<tr>
<td>73</td>
<td><img src="image" alt="Structure 73" /></td>
<td>74</td>
<td>174-176</td>
</tr>
<tr>
<td>74</td>
<td><img src="image" alt="Structure 74" /></td>
<td>70</td>
<td>156-158</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
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<td></td>
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<tr>
<td>---</td>
<td>-------------------</td>
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</tr>
<tr>
<td>75</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>79</td>
<td>153-155</td>
</tr>
<tr>
<td>76</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>82</td>
<td>150-152</td>
</tr>
<tr>
<td>77</td>
<td><img src="image3" alt="Chemical Structure" /></td>
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<td>157-159</td>
</tr>
<tr>
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<td><img src="image4" alt="Chemical Structure" /></td>
<td>84</td>
<td>168-170</td>
</tr>
<tr>
<td>79</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>72</td>
<td>173-175</td>
</tr>
<tr>
<td>80</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>78</td>
<td>144-146</td>
</tr>
<tr>
<td>81</td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td>72</td>
<td>160-162</td>
</tr>
<tr>
<td>82</td>
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<td>156-158</td>
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<td>83</td>
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<td>172-174</td>
</tr>
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<td><img src="image10" alt="Chemical Structure" /></td>
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<td>144-146</td>
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<td>68</td>
<td>168-172</td>
</tr>
<tr>
<td>No.</td>
<td>Structure</td>
<td>Page</td>
<td>Range</td>
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<td>-----</td>
<td>-----------</td>
<td>------</td>
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</tr>
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<td>86</td>
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<td>68</td>
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<td>178-180</td>
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<td>68</td>
<td>192-194</td>
</tr>
<tr>
<td>92</td>
<td><img src="image7" alt="Structure 92" /></td>
<td>82</td>
<td>162-164</td>
</tr>
<tr>
<td>93</td>
<td><img src="image8" alt="Structure 93" /></td>
<td>76</td>
<td>143-145</td>
</tr>
<tr>
<td>94</td>
<td><img src="image9" alt="Structure 94" /></td>
<td>80</td>
<td>164-166</td>
</tr>
<tr>
<td>95</td>
<td><img src="image10" alt="Structure 95" /></td>
<td>78</td>
<td>198-200</td>
</tr>
<tr>
<td>96</td>
<td><img src="image11" alt="Structure 96" /></td>
<td>76</td>
<td>158-160</td>
</tr>
<tr>
<td>97</td>
<td><img src="image12" alt="Structure 97" /></td>
<td>66</td>
<td>168-170</td>
</tr>
</tbody>
</table>
2.4.1.1. Results and discussion

2.4.1.1.1. Analytical

A focused library of thirty-nine new compounds 67-105 were synthesized starting from \( p \)-hydroxybibphenyl as outlined in Scheme-I. \( O \)-alkylation of \( p \)-hydroxybibphenyl with \( \alpha \)-chloroethylacetate was confirmed by the \( ^1H \)-NMR spectrum. The appearance of two signals at \( \delta \ 1.85 \) (t, \( J = 5.6 \) Hz, 3H) and 3.92 (q, \( J = 5.8 \) Hz, 2H) suggested the presence of ethyl group and appearance of a strong absorption at \( 1674 \) cm\(^{-1} \) in IR spectrum confirms the formation of ethyl 2-(bibphenyl-4-yloxy) acetate (51). Compound 51 readily yielded 2-(bibphenyl-4-yloxy) acetoxydrazide (52) by its reaction with hydrazine monohydrate. The formation of this hydrazide was confirmed by the presence of signals due to NH-NH\(_2\) group at \( \delta \ 3.2 \) (br, s, NH\(_2\), 2H) and 6.29 (s,
N-H, 1H) and absence of signals due to ethyl group. Compound 52 was then converted into corresponding thiosemicarbazide (53-59) which were confirmed by the presence of extra signals in aromatic region due to phenyl ring of isothiocyanate along with signals at δ 8.81-9.70 (CSNH), 9.21-10.34 (CONH) and 8.02-9.20 ppm (Ar-NH- ) respectively. Cyclization of compounds 53-59 to 60-66 was confirmed from the IR and NMR spectral data. Absorption bands in the region 2680-2690 (S-H, stretching) and 1594-1607 cm\(^{-1}\) (C=N of triazole ring stretching) and the presence of signal as singlet corresponding to thiol proton (-SH) in the range of δ 8.71-13.61 ppm in \(^1\)H NMR spectrum confirmed the formation of 3-mercapto-1,2,4-triazole. Finally S-alkylation of 3-mercapto-1,2,4-triazoles with substituted phenacyl bromides/benzyl bromide/alkyl halides leads to the formation of target molecules 67-105, which were confirmed from IR (absorption bands for C=O, 1655-1687 , phenacyl substituted), \(^1^3\)C NMR spectrum (peaks in the range of δ 190-192 ppm for C=O, Phenacyl substituted) and from \(^1\)H NMR spectrum (absence of S-H signal). Finally all the compounds were confirmed from mass spectrum. All the newly synthesized compounds 67-105 were further screened for their biological activities viz. anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities. These novel triazole derivatives have shown varied therapeutic efficacy in vitro and in vivo experiments.

### 2.4.1.1.2. Biological Activity

#### 2.4.1.1.2.1. Anti-inflammatory activity

The results of anti-inflammatory activity are summarized in table 1.2 and figure 1.1

Among thirty nine compounds from this series, seven compounds viz. 68, 74, 88, 95, 96, 97 and 101 were found to be active and showed significant inhibition (79.84, 64.69, 59.69, 52.12, 52.12, 59.69 % and 83.03, 72.52, 52.55, 60.06, 45.01, 57.50, 67.56 %) in paw oedema at 3h and 5h respectively when compared to the standard drug Ibuprofen (78.93 and 82.58 % at 3h and 5h respectively). Compound 68 has shown better inhibition compared to the standard drug Ibuprofen. From the biological data, the structure activity relationship (SAR) can be shown as follows.

1. Compounds having no substitution on the aromatic ring attached to the 1,2,4-triazolyl ring and the electron withdrawing group on the phenacyl aromatic ring are showing better activity.
2. Substitution of electron donating groups on triazollyl attached aromatic ring decreases the activity but is comparable with the standard.

3. Whereas when the same aromatic ring was substituted with electron withdrawing groups the activity significantly decreases.

4. Compounds having bulkier substitutions like -Br and -NO₂ groups on the phenacyl aromatic ring are showing potential activity.

5. Whereas the simple benzylated 3-mercapto 1,2,4-triazoles viz. 69, 70, 71, 72, 73, 80, 84, 86, 87, 104 and 105 significantly diminishes the activity.

The data suggests that the carbonyl functionality of the phenacyl group may be playing an important role in the enzymatic interactions responsible for anti-inflammatory activity. All data were analyzed by one-way ANOVA test followed by Dunnett’s test in carrageenan induced rat paw oedema model in rats.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Change in paw oedema volume (mL)</th>
<th>Anti-inflammatory activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After drug treatment</td>
<td>% Inhibition</td>
</tr>
<tr>
<td></td>
<td>3h</td>
<td>5h</td>
</tr>
<tr>
<td>67</td>
<td>0.300±0.036***</td>
<td>0.283±0.040***</td>
</tr>
<tr>
<td>68</td>
<td>0.133±0.021***</td>
<td>0.113±0.033***</td>
</tr>
<tr>
<td>69</td>
<td>0.466±0.061m</td>
<td>0.466±0.033m</td>
</tr>
<tr>
<td>70</td>
<td>0.483±0.032m</td>
<td>0.460±0.118m</td>
</tr>
<tr>
<td>71</td>
<td>0.583±0.060m</td>
<td>0.388±0.068*</td>
</tr>
<tr>
<td>72</td>
<td>0.360±0.033**</td>
<td>0.316±0.047**</td>
</tr>
<tr>
<td>73</td>
<td>0.416±0.030*</td>
<td>0.310±0.044**</td>
</tr>
<tr>
<td>74</td>
<td>0.233±0.049***</td>
<td>0.183±0.040***</td>
</tr>
<tr>
<td>75</td>
<td>0.423±0.058**</td>
<td>0.413±0.057m</td>
</tr>
<tr>
<td>76</td>
<td>0.533±0.042m</td>
<td>0.483±0.118m</td>
</tr>
<tr>
<td>77</td>
<td>0.330±0.049***</td>
<td>0.366±0.055*</td>
</tr>
<tr>
<td></td>
<td>0.483±0.030\textsuperscript{**}</td>
<td>0.460±0.117\textsuperscript{**}</td>
</tr>
<tr>
<td>---</td>
<td>---------------------------------</td>
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<tr>
<td>79</td>
<td>0.333±0.033\textsuperscript{***}</td>
<td>0.300±0.057\textsuperscript{***}</td>
</tr>
<tr>
<td>80</td>
<td>0.383±0.030\textsuperscript{**}</td>
<td>0.430±0.034\textsuperscript{*}</td>
</tr>
<tr>
<td>81</td>
<td>0.300±0.051\textsuperscript{***}</td>
<td>0.366±0.076\textsuperscript{**}</td>
</tr>
<tr>
<td>82</td>
<td>0.298±0.057\textsuperscript{***}</td>
<td>0.300±0.057\textsuperscript{***}</td>
</tr>
<tr>
<td>83</td>
<td>0.295±0.046\textsuperscript{***}</td>
<td>0.279±0.036\textsuperscript{***}</td>
</tr>
<tr>
<td>84</td>
<td>0.345±0.036\textsuperscript{**}</td>
<td>0.331±0.039\textsuperscript{**}</td>
</tr>
<tr>
<td>85</td>
<td>0.350±0.042\textsuperscript{***}</td>
<td>0.233±0.091\textsuperscript{***}</td>
</tr>
<tr>
<td>86</td>
<td>0.400±0.044\textsuperscript{*}</td>
<td>0.410±0.091\textsuperscript{*}</td>
</tr>
<tr>
<td>87</td>
<td>0.300±0.033\textsuperscript{***}</td>
<td>0.310±0.090\textsuperscript{***}</td>
</tr>
<tr>
<td>88</td>
<td>0.266±0.071\textsuperscript{***}</td>
<td>0.316±0.090\textsuperscript{***}</td>
</tr>
<tr>
<td>89</td>
<td>0.566±0.049\textsuperscript{**}</td>
<td>0.420±0.125\textsuperscript{**}</td>
</tr>
<tr>
<td>90</td>
<td>0.341±0.056\textsuperscript{***}</td>
<td>0.338±0.068\textsuperscript{***}</td>
</tr>
<tr>
<td>91</td>
<td>0.319±0.067\textsuperscript{***}</td>
<td>0.321±0.075\textsuperscript{***}</td>
</tr>
<tr>
<td>92</td>
<td>0.316±0.060\textsuperscript{**}</td>
<td>0.233±0.033\textsuperscript{***}</td>
</tr>
<tr>
<td>93</td>
<td>0.350±0.042\textsuperscript{**}</td>
<td>0.300±0.057\textsuperscript{**}</td>
</tr>
<tr>
<td>94</td>
<td>0.350±0.042\textsuperscript{**}</td>
<td>0.266±0.042\textsuperscript{**}</td>
</tr>
<tr>
<td>95</td>
<td>0.316±0.060\textsuperscript{***}</td>
<td>0.266±0.049\textsuperscript{***}</td>
</tr>
<tr>
<td>96</td>
<td>0.316±0.047\textsuperscript{***}</td>
<td>0.233±0.033\textsuperscript{***}</td>
</tr>
<tr>
<td>97</td>
<td>0.266±0.042</td>
<td>0.300±0.036</td>
</tr>
<tr>
<td>98</td>
<td>0.583±0.085\textsuperscript{**}</td>
<td>0.600±0.089\textsuperscript{**}</td>
</tr>
<tr>
<td>99</td>
<td>0.266±0.061\textsuperscript{***}</td>
<td>0.250±0.042\textsuperscript{***}</td>
</tr>
<tr>
<td>100</td>
<td>0.300±0.057\textsuperscript{***}</td>
<td>0.316±0.060\textsuperscript{***}</td>
</tr>
<tr>
<td>101</td>
<td>0.266±0.055\textsuperscript{***}</td>
<td>0.216±0.030\textsuperscript{***}</td>
</tr>
<tr>
<td>102</td>
<td>0.383±0.047\textsuperscript{**}</td>
<td>0.350±0.042\textsuperscript{**}</td>
</tr>
<tr>
<td></td>
<td>0.366±0.033**</td>
<td>0.350±0.042**</td>
</tr>
<tr>
<td>-----</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>104</td>
<td>0.383±0.040*</td>
<td>0.300±0.036**</td>
</tr>
<tr>
<td>105</td>
<td>0.316±0.030***</td>
<td>0.283±0.065**</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.130±0.033***</td>
<td>0.110±0.011***</td>
</tr>
<tr>
<td>Control</td>
<td>0.660±0.033</td>
<td>0.666±0.033</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s ‘t’ test (n=6), *p<0.05, **p<0.01 & ***p<0.001 significantly different from standard; ns, not significant.

**Figure 1.1.** Anti-inflammatory activity of biphenyl based 1,2,4 triazole derivatives.
2.4.1.2.2. Analgesic activity

The results of analgesic activity are summarized in table 1.3 and figure 1.2. Among tested compounds, compounds 99 and 101 showed comparable analgesic activity (reaction time) at 30 min., 60 min. and 120 min. respectively with the standard drug Ibuprofen.

Table 1.3. Analgesic activity of biphenyl based 1,2,4-triazole derivatives.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mean value of Tail Flick Latency (sec)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min.</td>
</tr>
<tr>
<td>68</td>
<td>2.16±0.298</td>
</tr>
<tr>
<td>74</td>
<td>1.88±0.324</td>
</tr>
<tr>
<td>88</td>
<td>1.93±0.33</td>
</tr>
<tr>
<td>96</td>
<td>1.98±0.34</td>
</tr>
<tr>
<td>97</td>
<td>2.02±0.310</td>
</tr>
<tr>
<td>99</td>
<td>2.07±0.368</td>
</tr>
<tr>
<td>101</td>
<td>1.98±0.303</td>
</tr>
<tr>
<td>Control</td>
<td>2.03±0.330</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.98±0.307</td>
</tr>
<tr>
<td></td>
<td>30 min.</td>
</tr>
<tr>
<td>68</td>
<td>2.85±0.367&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>74</td>
<td>3.23±0.370&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>88</td>
<td>3.04±0.284&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>96</td>
<td>2.81±0.364&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>97</td>
<td>2.90±0.238&lt;sup&gt;ns&lt;/sup&gt;</td>
</tr>
<tr>
<td>99</td>
<td>3.81±0.257&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>101</td>
<td>3.88±0.342&lt;sup&gt;*&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>1.94±0.236</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>3.71±0.388&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>60 min.</td>
</tr>
<tr>
<td>68</td>
<td>3.91±0.529&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>74</td>
<td>3.60±0.341&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>88</td>
<td>2.84±0.302&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>96</td>
<td>2.86±0.302&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>97</td>
<td>3.22±0.282&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>99</td>
<td>3.96±0.377&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>101</td>
<td>3.98±0.240&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>2.02±0.192</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>3.95±0.473&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>120 min.</td>
</tr>
<tr>
<td>68</td>
<td>2.51±0.323&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>74</td>
<td>2.56±0.227&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>88</td>
<td>2.85±0.372&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>96</td>
<td>2.83±0.371&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>97</td>
<td>2.81±0.230&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>99</td>
<td>3.08±0.499&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>101</td>
<td>3.08±0.499&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
<tr>
<td>Control</td>
<td>2.85±0.237</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>2.76±0.212&lt;sup&gt;°&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s ‘t’ test (n=6), *p<0.05, **p<0.01 & ***p<0.001 significantly different from standard; ns, not significant.

Figure 1.2. Analgesic activity of biphenyl based 1,2,4-triazole derivatives.
2.4.1.2.3. Ulcerogenic study

When compared with ibuprofen, compounds 74 did not cause any gastric ulceration and disruption of gastric epithelial cells at the given oral doses. Hence gastric tolerance to this compound was better than that of Ibuprofen indicating that carboxylic group present in the Ibuprofen is responsible for ulceration, Bhandari et al. [52]. Stomach wall of Ibuprofen treated group at low power (10x) photomicrograph showed damage of the mucosa and the sub mucosa. Stomach wall of the same section at high power (40x) photomicrograph showed desquamated epithelial cells in the lumen whereas in tested compounds 68 and 101 treated animals, surface epithelial damage was significant, slightly submucosal damage was seen however there was lesser damage in comparison to the Ibuprofen. Stomach wall of compound 74 treated animal showed no damage to any layer. The results are shown in table 1.4 and figure 1.3.

Table 1.4. Haematoxylin and Eosin Immunohistochemical staining of gastric ulcers after ulcer induction in rats

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Surface Epithelial Damage</th>
<th>Submucosal Damage</th>
<th>Deep Mucosal Damage</th>
<th>Muscular Layer Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>74</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>88</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>97</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>99</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>101</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

+, ++, +++ (increased ulceration), - (no ulceration)
Continued...
Figure 1.3. Haematoxylin and Eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.
2.4.1.2.4. Antimicrobial Activity

All the newly synthesized compounds were tested against various microbial strains for their antimicrobial activity. The results are summarized in table 1.5. Some of the compounds showed moderate to good activity against certain strains. Compound 96 exhibited moderate activity against all the tested fungal strains. Compounds 71, 75, 79, 80, 93, and 94 were active against A. flavus. Compounds 75, 79, 92 and 102 exhibited moderate activity against A. niger. Compounds 96, 98 and 102 showed moderate activity against C. albicans whereas compounds 70, 74, 75, 78, 80, 96 and 102 were active against C. krusie.

Compound 67, 78, 80 and 95 exhibited moderate activity against bacterial strains K. pneumoniae. Compound 68, 90, 94 and 97 were active against P. aeruginosa and E. coli.

Table 1.5. Antimicrobial activity of biphenyl based 1,2,4-triazole derivatives.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Antifungal Activity (zone of inhibition in mm)</th>
<th>Antibacterial Activity (zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. flavus 200 (100) µg/disc</td>
<td>A. niger 200 (100) µg/disc</td>
</tr>
<tr>
<td>67</td>
<td>18±1.5 (10±0.8)</td>
<td>6±1.5 (6±0.5)</td>
</tr>
<tr>
<td>68</td>
<td>18±1.3 (10±1.2)</td>
<td>10±1 (6±0.5)</td>
</tr>
<tr>
<td>69</td>
<td>14±1.6 (10±0.9)</td>
<td>8±0.6 (6±0.3)</td>
</tr>
<tr>
<td>70</td>
<td>10±0.5 (6±0.3)</td>
<td>10±0.5 (6)</td>
</tr>
<tr>
<td>71</td>
<td>18±1.6 (8±0.6)</td>
<td>10±1.2 (6±0.4)</td>
</tr>
<tr>
<td>72</td>
<td>- - - -</td>
<td>- - -</td>
</tr>
<tr>
<td>73</td>
<td>- - - -</td>
<td>- -</td>
</tr>
<tr>
<td>74</td>
<td>10±0.5 (6±0.3)</td>
<td>12±0.5 (6)</td>
</tr>
<tr>
<td>75</td>
<td>22±1.9 (10±1.2)</td>
<td>14±0.6 (7±0.5)</td>
</tr>
<tr>
<td>76</td>
<td>- - - -</td>
<td>- -</td>
</tr>
<tr>
<td>77</td>
<td>18±1.2 (8±1.6)</td>
<td>10±0.9 (6±0.4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>78</td>
<td>18±1.8</td>
<td>(10±1.1)</td>
</tr>
<tr>
<td>79</td>
<td>24±1.4</td>
<td>(15)</td>
</tr>
<tr>
<td>80</td>
<td>22±1.6</td>
<td>(16±1.2)</td>
</tr>
<tr>
<td>81</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>82</td>
<td>18±1.8</td>
<td>(10±1.2)</td>
</tr>
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<td>14±1.2</td>
<td>(3±0.7)</td>
</tr>
<tr>
<td>84</td>
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<td>-</td>
</tr>
<tr>
<td>85</td>
<td>8±0.6</td>
<td>(6±0.4)</td>
</tr>
<tr>
<td>86</td>
<td>14±1.2</td>
<td>(6±0.3)</td>
</tr>
<tr>
<td>87</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>88</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>89</td>
<td>12±1.6</td>
<td>(6±0.4)</td>
</tr>
<tr>
<td>90</td>
<td>18±1.3</td>
<td>(10±1.2)</td>
</tr>
<tr>
<td>91</td>
<td>12±1.4</td>
<td>(6±0.4)</td>
</tr>
<tr>
<td>92</td>
<td>19±1.6</td>
<td>(8±0.6)</td>
</tr>
<tr>
<td>93</td>
<td>24±1.8</td>
<td>(12±1.2)</td>
</tr>
<tr>
<td>94</td>
<td>22±1.6</td>
<td>(14±0.8)</td>
</tr>
<tr>
<td>95</td>
<td>10±1.3</td>
<td>(8±1.0)</td>
</tr>
<tr>
<td>96</td>
<td>22±1.8</td>
<td>(12±1.6)</td>
</tr>
<tr>
<td>97</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>98</td>
<td>10±0.6</td>
<td>(6±0.5)</td>
</tr>
<tr>
<td>99</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>101</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>No.</td>
<td>Fluconazole</td>
<td>Amoxicillin</td>
</tr>
<tr>
<td>-----</td>
<td>-------------</td>
<td>-------------</td>
</tr>
<tr>
<td>102</td>
<td>16±1.2 (9±0.6)</td>
<td>14±0.9 (6±0.6)</td>
</tr>
<tr>
<td>103</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>104</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>105</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>16±1 (10±.6)</td>
<td>14±1.2 (10±.)</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>16±1 (12±.8)</td>
<td>14±8 (10±.5)</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NT: not tested; -: no zone of inhibition

### 2.5.1.2. Experimental

#### 2.5.1.2.1. Chemistry

All chemicals (reagent grade) used were commercially available. Melting points were measured on a VEEGO-VMP-DS melting point apparatus and are uncorrected. $^1$H NMR was recorded on a Bruker DPX 400, 300 instruments in CDCl$_3$/DMSO-d$_6$ using TMS as internal standard. $^1$H NMR chemical shifts (δ) and coupling constants (J) are given in ppm and Hz respectively. Mass spectra were recorded on either a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system and operated at 70 eV or Maldi-MS (AB-4800). Mass-spectrometric (MS) data are reported in m/z. Elemental analysis was carried out using Elementar Vario EL III elemental analyzer. Elemental analysis data is reported in % standard.

**General procedure for synthesis of Ethyl-2-biphenyl-4-yloxy-acetate (51)**

To a mixture of $p$-hydroxybiphenyl (10 mmol) and ethylchloroacetate (10 mmol) in 50 mL anhydrous acetone was added 15g K$_2$CO$_3$. The suspension was refluxed for 20 h. After completion of reaction, the reaction mixture was filtered in hot condition, the filtrate concentrated under reduced pressure and finally the crude product crystallized from methanol in cold condition in 75% yield as white flakes; m.p. 40-42 °C, $R_f$ = 0.64 (n-hexane : ethyl acetate; 6:4).
**Gene**ral procedure for synthesis of 2-(4-phenylphenoxy)acetohydrazide (52)

The ethanolic solution of (51) (10 mmol) and hydrazine monohydrate (10 mmol) was refluxed for 4-6 h. After the completion of reaction, the reaction mixture was cooled, the white flakes so obtained was filtered and recrystallized from alcohol. **Yield:** 90%; White flakes; m.p.165-167 ºC, Rf = 0.23 (n-hexane: ethyl acetate; 4:6).

**IR (KBr, cm\(^{-1}\))**

\[ : 3350, 3055, 2921, 1674, 1108.\]

**\(^1\)H NMR (300 MHz, CDCl\(_3\))**

\[ \delta 3.20 (s, NH\(_2\), 2H), 5.21 (s, 2H, O-CH\(_2\)), 6.29 (s, NH, 1H), 7.51- 7.52 (m, Ar-H, 4 H), 7.40 (d, J = 7.8 Hz, 2H), 7.03 (d, J = 8.5 Hz, 2H), 7.25-7.32 (m, 1H, Ar-H). \]

**FAB-MS (m/z)**

\[ : 242 (M\(^+\)), 243 (M\(^+\)+1). \]

**Elemental Analysis**

Calculated for molecular formula C\(_{14}\)H\(_{14}\)N\(_2\)O\(_2\):
Calculated: C, 69.41; H, 5.82; N, 11.56
Found: C, 69.13; H, 5.84; N, 11.51%.
General procedure for synthesis of thiosemicarbazides (53-59)
To a solution of hydrazide (52) (1 mmol) in absolute alcohol (50 ml) was added arylisothiocyanate (1 mmol) and the reaction mixture was refluxed for 4-6 h. After the completion of reaction monitored by TLC, the reaction mixture was cooled to room temperature and the white crystals so formed were filtered and crystallized from alcohol to yield the pure thiosemicarbazide (53-59).

1-[(2-(4-Biphenyloxy)acetyl]-4-phenylthiosemicarbazide (53) Yield: 77%; White crystals, m.p. 138-140 °C, Rf = 0.25 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm\(^{-1}\) : 3324, 2945, 1701, 1212, 1109.

\(^1\)H NMR (300 MHz, DMSO-d\(_6\)) : δ 4.68 (s, 2H, O-CH\(_2\)), 7.03 (d, J = 8.6 Hz, 2H), 7.25-7.42 (m, 8H, Ar-H), 7.44-7.54 (m, 4H, Ar-H), 8.92 (brs, 1H, ArNH), 9.21 (brs, 1H, CSNH), 10.14 (brs, 1H, CONH).

FAB-MS (m/z) : 377 (M\(^+\)), 378 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula C\(_{21}\)H\(_{19}\)N\(_3\)O\(_2\)S.
Calculated : C, 66.82; H, 5.07; N, 11.13.
Found : C, 66.48; H, 5.15; N, 11.25%.

1-[(2-(4-Biphenyloxy)acetyl]-4(4-methoxyphenyl)thiosemicarbazide (54) Yield: 72%; White crystals, m.p. 176-178 °C, Rf = 0.24 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm\(^{-1}\) : 3246, 2953, 1702, 1205, 1109.

\(^1\)H NMR (300 MHz, DMSO-d\(_6\)) : δ 3.88 (s, 3H, O-CH\(_3\)), 4.68 (s, 2H, O-CH\(_2\)), 7.01-7.08 (m, 4H, Ar-H), 7.33 (t, J = 7.5 Hz, 1H), 7.46 (t, J = 5.7 Hz, 2H), 7.61-7.47 (m, 6H, Ar-
H), 8.92 (brs, 1H, ArNH), 9.21 (brs, 1H, CONH), 9.12 (brs, 1H, CSNH).

FAB-MS (m/z) : 407 (M⁺), 408 (M⁺+1).

Elemental Analysis : Calculated for molecular formula C₂₂H₂₁N₃O₃S.
Calculated : C, 64.85; H, 5.19; N, 10.31.
Found : C, 65.08; H, 5.22; N, 10.26%.

1-[2-(4-Biphenyloxy)acetyl]-4(4-fluorophenyl)thiosemicarbazide (55) Yield: 76%;
White crystals, m.p. 186-188 ºC, Rf = 0.20 (n-hexane: ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 3244, 3070, 2950, 1683, 1202, 1106. \\
^1\text{H NMR (300 MHz, DMSO-d$_6$)} & : \delta 4.66 (s, 2H, O-CH$_2$), 7.19-7.31 (m, 3H, Ar-H), 7.37-7.42 (m, 4H, Ar-H), 7.46-7.51 (m, 4H, Ar-H), 7.88 (d, J = 6.4 Hz, 2H), 8.67 (brs, 1H, ArNH), 8.96 (brs, 1H, CSNH), 9.56 (brs, 1H, CONH).
\end{align*}
\]

FAB-MS (m/z) : 395 (M⁺), 396 (M⁺+1).

Elemental Analysis : Calculated for molecular formula C₂₁H₁₈FN₃O₂S.
Calculated : C, 63.78; H, 4.59; N, 10.63.
Found : C, 63.98; H, 4.53; N, 10.58%.

1-[2-(4-Biphenyloxy)acetyl]-4(4-chlorophenyl)thiosemicarbazide (56) Yield: 74%;
White crystals, m.p. 183-185 ºC, Rf = 0.23 (n-hexane: ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 3322, 2958, 1714, 1195, 1108.
\end{align*}
\]
\(^1\text{H NMR (300 MHz, DMSO-d}_6\) :} \(\delta 4.69 (s, 2H, O-CH_2)\), \(6.94 (d, J = 6.4 \text{ Hz, } 2H)\), 
\(7.30 (t, J = 5.7 \text{ Hz, } 1H)\), \(7.36 - 7.43 (m, 4H, Ar-H)\), 
\(7.47 - 7.52 (m, 6H, Ar-H)\), \(9.21 (\text{brs, } 1H, \text{ Ar-NH})\), 
\(9.70 (\text{brs, } 1H, \text{ CSNH})\), \(10.34 (\text{brs, } 1H, \text{ CONH})\).

\(\text{FAB-MS (m/z) :} \) 411 (M\(^+\)), 413 (M\(^+\)+2).

\(\text{Elemental Analysis :} \) Calculated for molecular formula \(C_{21}H_{18}ClN_3O_2S\).

\(\text{Calculated} : C, 61.23; H, 4.40; N, 10.20\)

\(\text{Found} : C, 61.41; H, 4.48; N, 10.18\%\).

\(1-[(2-(4-\text{Biphenyloxy})\text{acetyl})-4(3-\text{chlorophenyl})\text{thiosemicarbazide (57) Yield:} 72\%\);

White crystals, \(\text{m.p.} 176-178 \degree C\), \(R_f = 0.20\) (n-hexane : ethyl acetate; 4:6).

\(\text{IR (KBr) cm}^{-1} : 3322, 2958, 1712, 1198, 1108.\)

\(^1\text{H NMR (300 MHz, DMSO-d}_6\) :} \(\delta 4.68 (s, 2H, O-CH_2)\), \(6.94 (d, J = 6.4 \text{ Hz, } 2H)\), 
\(7.30 - 7.43 (m, 5H, Ar-H)\), \(7.48 - 7.50 (m, 6H, Ar-H)\), 
\(9.20 (\text{brs, } 1H, \text{ ArNH})\), \(9.72 (\text{brs, } 1H, \text{ CSNH})\), 
\(10.34 (\text{brs, } 1H, \text{ CONH})\).

\(\text{FAB-MS (m/z) :} \) 411 (M\(^+\)), 413 (M\(^+\)+2).

\(\text{Elemental Analysis :} \) Calculated for molecular formula \(C_{21}H_{18}ClN_3O_2S\).

\(\text{Calculated} : C, 61.23; H, 4.40; N, 10.20\)

\(\text{Found} : C, 61.41; H, 4.48; N, 10.18\%\).
1-[2-(4-Biphenyloxy)acetyl]-4(4-nitrophenyl)thiosemicarbazide (58) Yield: 69%;
Light yellow crystals, m.p. 198-200 °C, R₉ = 0.31 (n-hexane : ethyl acetate; 3:7).

IR (KBr) cm⁻¹: 3246, 2958, 1693, 1208, 1113.
¹H NMR (300 MHz, DMSO-d₆): δ 4.69 (s, 2H, O-CH₂), 6.94 (d, J = 6.6 Hz, 2H),
7.26-7.52 (m, 7H, Ar-H), 7.94 (d, J = 8.6 Hz, 2H), 8.02 (brs, 1H, ArNH), 8.38 (d, J = 8.6 Hz, 2H),
8.81 (brs, 1H, CSNH), 9.58 (brs, 1H, CONH).

FAB-MS (m/z): 422 (M⁺), 423 (M⁺+1).
Elemental Analysis: Calculated for molecular formula C₂₁H₁₈N₄O₄S.
Calculated: C, 59.70; H, 4.29; N, 13.26
Found: C, 59.92; H, 4.32; N, 13.20%.

1-[2-(4-Biphenyloxy)acetyl]-4(2-methylphenyl)thiosemicarbazide (59) Yield: 71%;
White crystals, m.p. 138-140 °C, R₉ = 0.26 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm⁻¹: 3320, 2948, 1704, 1215, 1254, 1204, 1102.
¹H NMR (200 MHz, DMSO-d₆): δ 2.38 (s, 3H, CH₃), 4.67 (s, 2H, O-CH₂), 6.86
(d, J = 6.4 Hz, 2H, Ar-H), 7.30-7.56 (m, 11H, Ar-H), 8.92 (brs, 1H, ArNH), 9.21 (brs, 1H, CSNH
H), 10.12 (brs, 1H, CONH).
Maldi-MS (m/z): 391 (M⁺), 392 (M⁺+1).
Elemental Analysis: Calculated for molecular formula C₂₂H₂₁N₃O₂S.
Calculated: C, 67.50; H, 5.07; N, 10.73.
Found: C, 67.85; H, 5.11; N, 10.68%.
General method for synthesis of 5-[(biphenyl-4-yloxy)methyl]4-aryl-3-mercapto-(4H)-1,2,4-triazole (60-66)

To a solution of thiosemicarbazide (53-59) (5 mmol) in absolute alcohol (50 mL) was added triethyl amine (1 mL) and the reaction mixture refluxed for 4-8 h. After completion of reaction monitored by TLC, the reaction mixture was cooled to room temperature and concentrated under reduced pressure (25 mL), the concentrated solution was poured on crushed ice, the precipitate obtained was filtered off and washed with cold water, dried and crystallized in ethanol to yield the pure 3-mercapto-1,2,4-triazoles (60-66).

5-[(Biphenyl-4-yloxy)methyl]4-(phenyl)-3-mercapto-(4H)-1,2.4-triazole (60) Yield: 78%; White crystals, m.p. 234-236 ºC, \( R_f = 0.64 \) (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm\(^{-1}\): 2912, 2690, 1607, 1110.

\(^1\)H NMR (300 MHz, CDCl\(_3\)): \( \delta \) 4.69 (s, 2H, O-CH\(_2\)), 7.04 (d, \( J = 8.7 \) Hz, 2H, Ar-H), 7.25-7.44 (m, 8H, Ar-H), 7.52-7.57 (m, 4H, Ar-H), 9.57 (brs, 1H, S-H).

Maldi-MS (m/z): 359 (M\(^+\)), 360 (M\(^+\)+1).

Elemental Analysis: Calculated for molecular formula C\(_{21}\)H\(_{17}\)N\(_3\)OS.

Calculated: C, 70.17; H, 4.77; N, 11.69.

Found: C, 70.09; H, 4.64; N, 11.58%.

5-[(Biphenyl-4-yloxy)methyl]4-(4-methoxyphenyl)-3-mercapto-(4H)-1,2,4-triazole (61) Yield: 68%; White crystals, m.p.168-170 ºC, \( R_f = 0.63 \) (n-hexane : ethyl acetate; 4:6).
IR (KBr) cm\(^{-1}\): 3058, 2688, 1603, 1107.

\(^1\)H NMR (300 MHz, CDCl\(_3\)):
\[ \delta \ 3.88 \text{ (s, 3H, O-CH}_3\text{), 4.93 (s, 2H, O-CH}_2\text{), 6.91 (d, } J = 6.6 \text{ Hz, 2H, Ar-H), 7.01 (d, } J = 6.6 \text{ Hz, 2H, Ar-H), 7.30-7.36 \text{ (m, 3H, Ar-H), 7.42 (d, } J = 5.7 \text{ Hz, 2H, Ar-H), 7.49 (d, } J = 6.9 \text{ Hz, 2H, Ar-H), 7.52 (d, } J = 5.4 \text{ Hz, 2H, Ar-H), 8.71 (brs, 1H, SH).}\]

Maldi-MS (m/z): 389 (M\(^+\)), 390 (M\(^+\)+1).

Elemental Analysis:
Calculated for molecular formula C\(_{22}\)H\(_{19}\)N\(_3\)O\(_2\)S.
Calculated: C, 67.84; H, 4.92; N, 10.79
Found: C, 67.76; H, 4.89; N, 10.75%.

5-[(Biphenyl-4-yloxy)methyl]-4-(4-fluorophenyl)-3-mercaptopo-(4H)-1,2,4-triazole (62)
Yield: 76%; White crystals; m.p.186-188 °C, \( R_f = 0.66 \) (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm\(^{-1}\): 3049, 2929, 2680, 1603, 1514, 1105.

\(^1\)H NMR (300 MHz, CDCl\(_3\)):
\[ \delta \ 4.73 \text{ (s, 2H, O-CH}_2\text{), 6.94 (d, } J = 6.3 \text{ Hz, 2H, Ar-H), 7.21 (t, } J = 8.3 \text{ Hz, 2H, Ar-H), 7.28 (t, } J = 5.4 \text{ Hz, 1H, Ar-H), 7.37-7.41 \text{ (m, 4H, Ar-H), 7.51-7.46 \text{ (m, 4H, Ar-H), 8.73 (brs, 1H, SH).}} \]

Maldi-MS (m/z): 377 (M\(^+\)), 378 (M\(^+\)+1).

Elemental Analysis:
Calculated for molecular formula C\(_{21}\)H\(_{16}\)FN\(_3\)OS.
Calculated: C, 66.83; H, 4.27; N, 11.13
Found: C, 66.92; H, 4.25; N, 11.06%.
5-[(Biphenyl-4-yloxy)methyl]4-(4-chlorophenyl)-3-mercapto-(4H)-1,2,4-triazole

(63) Yield: 85%; White crystals, m.p. 228-230 ºC, Rf = 0.67 (n-hexane : ethyl acetate; 4:6).

![Chemical Structure](image)

**IR (KBr) cm⁻¹**: 3056, 2922, 2690, 1606.

**¹H NMR (300 MHz, CDCl₃)**: δ 4.52 (s, 2H, O-CH₂), 7.03-7.06 (d, J = 8.7 Hz, 2H, Ar-H), 7.29-7.32 (t, J = 8.1 Hz, 1H, Ar-H), 7.39-7.44 (m, 6H, Ar-H), 7.52-7.54 (d, J = 6.9 Hz, 2H, Ar-H), 7.54-7.57 (t, J = 8.4 Hz, 2H, Ar-H), 8.71 (brs, 1H, SH).

**Maldi-MS (m/z)**: 393 (M⁺), 394 (M⁺+1).

**Elemental Analysis**: Calculated for molecular formula C₂₁H₁₆ClN₃OS.

Calculated: C, 64.03; H, 4.09; N, 10.67.

Found: C, 64.08; H, 4.12; N, 10.63%.

5-[(Biphenyl-4-yloxy)methyl]4-(3-chlorophenyl)-3-mercapto-(4H)-1,2,4-triazole

(64) Yield: 78%; White crystals, m.p. 220-222 ºC, Rf = 0.68 (n-hexane : ethyl acetate; 4:6).

![Chemical Structure](image)

**IR (KBr) cm⁻¹**: 3056, 2922, 2690, 1606.

**¹H NMR (300 MHz, CDCl₃)**: δ 4.74 (s, 2H, O-CH₂), 6.89 (d, J = 8.4 Hz, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 7.29-7.43 (m, 4H, Ar-H), 7.47-7.52 (m, 6H, Ar-H).

**Maldi-MS (m/z)**: 393 (M⁺), 394 (M⁺+1).

**Elemental Analysis**: Calculated for molecular formula C₂₁H₁₆ClN₃OS.
Calculated: C, 64.03; H, 4.09; N, 10.67.

Found: C, 64.09; H, 4.10; N, 10.63%.

5-[(Biphenyl-4-yloxy)methyl]4-(4-nitrophenyl)-3-mercapto-(4H)-1,2,4-triazole (65)

Yield: 89%; Light yellow crystals, m.p. 221-222 °C, R_f = 0.64 (n-hexane : ethyl acetate; 4:6).

[Chemical structure image]

IR (KBr) cm\(^{-1}\): 2912, 2690, 1604, 1102.

\(^1\)H NMR (300 MHz, CDCl\(_3\)):
\(\delta\) 4.81 (s, 2H, O-CH\(_2\)), 6.96 (d, \(J = 6.6\) Hz, 2H, Ar-H), 7.28 (t, \(J = 6.9\) Hz, 1H, Ar-H), 7.32-7.52 (m, 6H, Ar-H), 7.65 (d, \(J = 8.8\) Hz, 2H, Ar-H) 8.39 (d, \(J = 8.6\) Hz, 2H, Ar-H), 8.93(brs, 1H, S-H).

Maldi-MS (m/z): 393 (M\(^+\)), 394 (M\(^+\)+1).

Elemental Analysis: Calculated for molecular formula C_{21}H_{16}N_{4}O_{3}S.
Calculated: C, 62.60; H, 3.99; N, 13.85.
Found: C, 62.60; H, 4.01; N, 12.79%.

5-[(Biphenyl-4-yloxy)methyl]4-(2-methylphenyl)-3-mercapto-(4H)-1,2,4-triazole (66)

Yield: 72%; White crystals, m.p. 186-188 °C, R_f = 0.68 (n-hexane : ethyl acetate; 4:6).

[Chemical structure image]

IR (KBr) cm\(^{-1}\): 2934, 2686, 1604, 1594, 1108.

\(^1\)H NMR (300 MHz, CDCl\(_3\)):
\(\delta\) 2.54 (3H, s, CH\(_3\)), 4.68 (s, 2H, O-CH\(_2\)), 6.89 (2H, d, \(J = 6.6\)Hz, Ar-H), 7.43 (m, 7H, Ar-H), 7.51-7.54 (m, 4H, Ar-H), 8.31(brs, 1H, S-H).
Maldi-MS (m/z) : 373 (M⁺), 374 (M⁺+1).

Elemental Analysis: Calculated for molecular formula C_{22}H_{19}N_{3}O_{5}.
Calculated: C 70.75; H 5.13; N 11.25.
Found: C 70.82; H 5.10; N 11.30%.

General method for synthesis of 1(Aryl)-2-[5-{(biphenyl-4-yloxy)methyl}4-aryl-3-mercapto-(4H)-1,2,4-triazol-3-ylthio)] ethanone/ethane (67-105)

To a solution of 3-mercapto-1,2,4-triazole (62-66, 0.5 mmole) in absolute ethanol (50 ml) was added sodium metal (0.5 mmole) and different substituted phenacyl bromide (0.5 mmole) and the reaction mixture was refluxed for 1-6 h. After completion of reaction monitored by TLC, the reaction mixture was concentrated under reduced pressure (25 ml), the concentrated solution cooled and the crystals obtained were filtered, washed with water, dried and recrystallized from dichloromethane (DCM) and methanol to yield the pure compound (67-105).

2-{5-[4-(Biphenyloxy)methyl]-4-(phenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-bromophenyl) ethanone (67) Yield: 72%; White crystals, m.p:164-166 ºC, R_f = 0.43 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm⁻¹ : 2907, 1686, 1570, 1093.

¹H NMR (400 MHz, CDCl₃) δ 4.97 (s, 2H, S-CH₂), 5.02 (s, 2H, O-CH₂), 6.87 (d, J = 6.3 Hz, 2H, Ar-H), 7.23 (t, J = 5.4 Hz, 1H, Ar-H), 7.34-7.41 (m, 11H, Ar-H), 7.56 (d, J = 6.3 Hz, 2H, Ar-H), 7.83 (d, J = 6.0 Hz, 2H, Ar-H).

¹³C NMR (100 MHz, CDCl₃) δ 41.01, 63.35, 115.18, 126.79, 126.81, 126.91, 126.95, 128.26, 128.78, 129.98, 130.07, 132.24, 132.52, 134.91, 135.14, 147.37, 150.17, 156.99, 192.19.

Maldi-MS (m/z) : 556 (M⁺), 557 (M⁺+1).
Elemental Analysis: Calculated for molecular formula \( \text{C}_{29}\text{H}_{22}\text{BrN}_{3}\text{O}_{2}\text{S} \).

Calculated: C, 62.59; H, 3.98; N, 7.55.

Found: C, 62.64; H, 4.01; N, 7.52%.

2-\{5-[\text{(4-Bihenyoxy)methyl]-4-(phenyl)-4H-1,2,4-triazol-3-ylthio]}-1-(4-nitrophenyl)\} ethanone (68) Yield: 67%; Light yellow crystals, m.p. 170-172 °C, \( R_f = 0.37 \) (n-hexane : ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 2924, 1689, 1525, 1192. \\
{}^{1}\text{H NMR (400 MHz, CDCl}_{3}\text{)} & : \delta 4.92 (s, 2H, S-\text{CH}_{2}), 5.10 (s, 2H, O-\text{CH}_{2}), 6.95 (d, J = 6.3 \text{ Hz}, 2H \text{ Ar-H}), 7.30 (t, J = 5.4 \text{ Hz}, 1H, \text{ Ar-H}), 7.39-7.42 (m,4H, \text{ Ar-H}), 7.47 (d, J = 6.6 \text{ Hz}, 2H, \text{ Ar-H}), 7.51 (d, J = 6.3Hz, 2H, \text{ Ar-H}), 7.54-7.56 (m, 3H, \text{ Ar-H}), 8.22 (d, J = 6.6 \text{ Hz}, 2H, \text{ Ar-H}), 8.34 (d, J = 6.6Hz, 2H, \text{ Ar-H}).
\end{align*}
\]

\[
\begin{align*}
{}^{13}\text{C NMR (100 MHz, CDCl}_{3}\text{)} & : \delta 39.86, 59.92, 115.17, 124.09, 126.78, 126.92, 128.27, 128.78, 129.72, 130.03, 130.53, 132.43, 134.97, 139.75, 140.46, 150.73, 152.22, 156.95, 191.88.
\end{align*}
\]

TOF-MS (m/z): 522 (M^+), 523 (M^+1).

Elemental Analysis: Calculated for molecular formula \( \text{C}_{29}\text{H}_{22}\text{N}_{3}\text{O}_{2}\text{S} \).

Calculated: C, 66.65; H, 4.24; N, 10.72.

Found: C, 66.69; H, 4.23; N, 10.6%.
3-(Phenylethylthio)-5-[(4-biphenyloxy)methyl]-4-phenyl-4H-1,2,4-triazole (69)

Yield: 70%; White crystals, m.p. 132-134 °C, Rf = 0.52 (n-hexane : ethyl acetate; 4:6).

![Chemical Structure](image)

IR (KBr) cm⁻¹: 2907, 1541, 1523, 1107.

¹H NMR (400 MHz, CDCl₃): δ 3.08 (t, J = 7.2 Hz, 2H, S-CH₂), 3.50 (t, J = 7.8 Hz, 2H, S-CH₂), 5.10 (s, 2H, O-CH₂), 6.96 (d, J = 9.0 Hz, 2H, Ar-H), 7.49-7.52 (m, 6H, Ar-H), 7.18-7.25 (m, 6H, Ar-H), 7.31-7.34 (m, 3H, Ar-H), 7.40 (t, J = 7.2 Hz, 2H, Ar-H).

¹³C NMR (100 MHz, CDCl₃): δ 35.86, 36.74, 59.84, 115.16, 123.89, 124.24, 126.88, 128.77, 128.76, 128.79, 129.98, 130.04, 130.34, 130.43, 130.45, 134.91, 142.43, 145.56, 148.34, 148.79, 156.88.

Maldi-MS (m/z): 463 (M⁺), 464 (M⁺+1).

Elemental Analysis: Calculated for molecular formula C₂₉H₂₅N₃OS.
Calculated: C, 75.13; H, 5.44; N, 9.06.
Found: C, 75.17; H, 5.42; N, 9.09%.

3-(4-Nitrophenylethylthio)-5-[(4-biphenyloxy)methyl]-4-phenyl-4H-1,2,4-triazole(70) Yield: 74%; Light yellow crystals, m.p. 138-140 °C, Rf = 0.48 (n-hexane : ethyl acetate; 4:6).

![Chemical Structure](image)

IR (KBr) cm⁻¹: 2907, 1525, 1128.

¹H NMR (400 MHz, CDCl₃): δ 3.12 (t, J = 8.0 Hz, 2H, Ar-CH₂), 3.74 (t, J = 8.4 Hz, 2H, S-CH₂), 5.09 (s, 2H, O-CH₂),
6.94 (d, \( J = 5.7 \) Hz, 2H, Ar-H), 7.23 (d, \( J = 6.6 \) Hz, 2H, Ar-H), 7.30 (t, \( J = 5.4 \) Hz, 1H, Ar-H), 7.41 (d, \( J = 7.2 \) Hz, 2H, Ar-H), 7.47-7.56 (m, 7H, Ar-H), 8.12 (d, \( J = 6.0 \) Hz, 2H, Ar-H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\))

\( \delta \) 35.87, 37.92, 59.87, 115.17, 123.82, 126.88, 128.77, 129.45, 130.45, 130.11, 130.43, 135.92, 140.45, 144.24, 147.43, 148.45, 156.90.

Maldi-MS (m/z): 508 (M\(^+\)), 509 (M\(^{+}+1\)).

Elemental Analysis: Calculated for molecular formula C\(_{29}\)H\(_{24}\)N\(_4\)O\(_3\)S.

Calculated: C, 68.49; H, 4.76; N, 11.02.

Found: C, 68.55; H, 4.74; N, 11.05%.

3-(4-Nitrophenylmethylthio)-5-[(4-biphenyloxy)methyl]-4-phenyl-1,2,4-triazole (71) Yield: 72%; Light yellow crystals, m.p. 136-138 °C, \( R_f = 0.49 \) (n-hexane : ethyl acetate; 4:6).

\( \begin{align*}
\text{IR (KBr) cm}^{-1} & : 2907, 1516, 1108. \\
\text{\(^1\)H NMR (400 MHz, CDCl\(_3\))} & : \delta 4.44 (s, 2H, S-CH\(_2\)), 5.04 (s, 2H, O-CH\(_2\)), 6.86 (d, \( J = 8.4 \) Hz, 2H, Ar-H), 7.06-7.14 (m, 5H, Ar-H), 7.22 (t, \( J = 5.8 \) Hz, 1H, Ar-H), 7.33 (t, \( J = 7.6 \) Hz, 2H, Ar-H), 7.38-7.46 (m, 6H, Ar-H), 8.04 (2H, d, \( J = 8.4 \) Hz, Ar-H). \\
\text{\(^{13}\)C NMR (100 MHz, CDCl\(_3\))} & : \delta 36.88, 59.91, 114.98, 115.10, 115.12, 123.31, 126.29, 126.88, 128.80, 129.91, 130.18, 130.29, 130.88, 130.46, 131.26, 134.90, 137.75, 146.26, 146.56, 155.74, 156.86. \\
\text{Maldi-MS (m/z)} & : 494 (M\(^+\)), 495 (M\(^{+}+1\)). \\
\text{Elemental Analysis} & : Calculated for Molecular formula C\(_{28}\)H\(_{22}\)N\(_4\)O\(_3\)S: \\
\text{Calculated} & : C, 68.00; H, 4.48; N, 11.33. \\
\text{Found} & : C, 68.10; H, 4.51; N, 11.36%.
\end{align*} \)
3-((Methylthio)-5-[(4-biphenyloxy)methyl]-4-phenyl-1,2,4-triazole (72) Yield: 68%; White crystals, m.p. 168-170 °C, Rf = 0.28 (n-hexane : ethyl acetate; 4:6).

\[
\text{IR (KBr) cm}^{-1} : 2912, 1541, 1524, 1109.
\]

\[
\text{\(^1\)H NMR (300 MHz, CDCl}^3\) : \delta 2.70 (s, 3H, S-CH}_3\), 5.10 (2H, s, O-CH}_2\), 6.94 (d, \(J = 8.4\) Hz, 2H), 7.30-7.52 (m, 12H, Ar-H).
\]

\[
\text{\(^{13}\)C NMR (75 MHz, CDCl}^3\) : \delta 14.59, 59.99, 115.20, 126.77, 126.86, 126.97, 128.22, 128.75, 129.83, 130.23, 132.83, 134.85, 140.53, 151.86, 154.26, 157.05.
\]

\[
\text{Maldi-MS (m/z) : 373 (M}\text{\(^+\)}, 374 (M\text{\(^+\)}+1).
\]

\[
\text{Elemental Analysis : Calculated for molecular formula C}_{22}\text{H}_{19}\text{N}_3\text{O}_1\text{S}.
\]

Calculated : C, 70.75; H, 5.13; N, 11.25.
Found : C, 70.69; H, 5.16; N, 11.29%.

3-((Ethylthio)-5-[(4-biphenyloxy)methyl]-4-phenyl-1,2,4-triazole (73) Yield: 74%; White crystals, m.p. 174-176 °C, Rf = 0.26 (n-hexane : ethyl acetate; 4:6).

\[
\text{IR (KBr) cm}^{-1} : 2907, 1541, 1523, 1108.
\]

\[
\text{\(^1\)H NMR (300 MHz, CDCl}^3\) : \delta 1.42 (t, \(J = 5.6\) Hz, 3H, -CH}_3\), 3.29 (q, \(J = 5.4\) Hz, 2H, S-CH}_2\), 5.14 (s, 2H, O-CH}_2\), 6.88 (d, \(J = 8.3\) Hz, 2H), 7.21 (t, \(J = 7.8\) Hz, 2H), 7.44-7.54 (m, 5H, Ar-H), 7.26-7.42 (m, 5 H, Ar-H).
\[ ^{13}C \text{NMR (75 MHz, CDCl}_3 \]: \[ \delta 14.60, 29.68, 59.92, 115.06, 116.72, 117.04, 126.74, 126.89, 128.25, 128.68, 129.02, 129.19, 135.06, 140.44, 156.92. \]

\[ \text{Maldi-MS (m/z) : 387 (M}^+\text{), 388 (M}^+\text{+1).} \]

\[ \text{Elemental Analysis : Calculated for molecular formula C}_{23}\text{H}_{21}\text{N}_{3}\text{OS.} \]

\[ \text{Calculated : C, 71.29; H, 5.46; N, 10.84.} \]

\[ \text{Found : C, 71.27; H, 5.45; N, 10.32%.} \]

\[ 2-\text{[5-\{(4-Biphenyloxy)methyl\}-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-bromophenyl)ethanone (74) Yield: 70 %; White crystals, m.p. 156-158 °C, R}_f = 0.18 \text{ (n-hexane : ethyl acetate; 4:6).} \]

\[ \begin{align*}
\text{IR (KBr) cm}^{-1} & : 2907, 1686, 1605, 1536, 1105. \\
\text{\textsuperscript{1}H NMR (CDCl}_3 \): & \delta 3.88 (s, 3H, O-CH}_3\text{), 4.85 (s, 2H, S-CH}_2\text{), 5.15 (s, 2H, O-CH}_2\text{), 6.90 (d, J = 6.6 Hz, 2H, Ar-H), 7.01 (d, J = 6.7 Hz, 2H, Ar-H), 7.18-7.24 (m, 2H, Ar-H), 7.28 (t, J = 5.5 Hz, 1H, Ar-H), 7.37-7.51 (m, 6H, Ar-H), 7.48-7.53 (m, 4H, Ar-H).} \\
\text{\textsuperscript{13}C NMR (CDCl}_3 \): & \delta 37.58, 56.32, 60.09, 114.94, 122.48, 126.80, 126.98, 127.22, 128.56, 128.74, 128.84, 128.96, 129.08, 130.65, 132.24, 134.81, 135.89, 140.34, 147.34, 150.25, 156.84, 160.65, 191.45. \\
\text{Maldi-MS (m/z) : 586 (M}^+\text{), 587 (M}^+\text{+1).} \]
\end{align*} \]

\[ \text{Elemental Analysis : Calculated for molecular formula} \]
\[ \text{\textsuperscript{C}_{30}\text{H}_{24}\text{BrN}_{3}\text{O}_{3}\text{S.}} \]

\[ \text{Calculated : C, 61.44; H, 4.12; N, 7.16.} \]

\[ \text{Found : C, 61.48; H, 4.09; N, 7.13%.} \]
2-[5-{(4-Biphenyloxy)methyl}-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone (75) Yield: 79%; White crystals, m.p. 153-155 °C, Rf = 0.21 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm\(^{-1}\) : 2907, 1655, 1605, 1239, 1093.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) : \(\delta\) 3.85 (s, 3H, O-CH\(_3\)), 4.91 (s, 2H, S-CH\(_2\)), 5.12 (s, 2H, O-CH\(_2\)), 6.96 (d, \(J = 8.4\) Hz, 2H, Ar-H), 7.00 (d, \(J = 9\) Hz, 2H, Ar-H), 7.25-7.32 (m, 5H, Ar-H), 7.40 (t, \(J = 7.5\) Hz, 2H, Ar-H), 7.90 (d, \(J = 8.4\) Hz, 2H, Ar-H), 7.46-7.52 (m, 4H, Ar-H).

\(^{13}\)C NMR (100MHz, CDCl\(_3\)) : \(\delta\) 38.03, 55.35, 60.12, 114.94, 115.27, 126.81, 127.12, 128.08, 128.57, 128.99, 129.03, 130.73, 132.24, 134.81, 135.87, 137.87, 140.21, 147.35, 150.15, 156.84, 160.69, 190.24.

Maldi-MS (m/z) : 542 (M\(^+\)), 543 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula C\(_{30}\)H\(_{24}\)ClN\(_3\)O\(_3\)S.

Calculated : C, 66.47; H, 4.46; N, 7.75.

Found : C, 66.53; H, 4.44; N, 7.69%.

2-[5-{(4-Biphenyloxy)methyl}-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio]-1-(phenyl)ethanone (76) Yield: 82%; White crystals, m.p. 150-152 °C, Rf = 0.22 (n-hexane : ethyl acetate; 4:6).
IR (KBr) cm$^{-1}$: 2907, 1686, 1541, 1102.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.85 (s, 3H, O-CH$_3$), 4.92 (s, 2H, S-CH$_2$), 5.10 (s, 2H, O-CH$_2$), 6.96 (d, $J = 8.4$ Hz, 2H, Ar-H), 7.25-7.32 (m, 6H, Ar-H), 7.40 (t, $J = 7.5$ Hz, 2H, Ar-H), 7.40-7.46 (m, 6H, Ar-H), 7.91 (d, $J = 8.4$ Hz, 2H, Ar-H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 36.13, 56.14, 60.24, 114.94, 115.12, 126.79, 127.12, 128.09, 128.56, 128.92, 129.05, 130.76, 132.27, 133.20, 134.83, 135.87, 137.26, 147.37, 150.17, 156.81, 160.69, 190.20.

Maldi-MS (m/z): 507 (M$^+$), 508 (M$^+$+1).

Elemental Analysis: Calculated for molecular formula C$_{30}$H$_{25}$N$_3$O$_3$S.

Calculated: C, 70.98; H, 4.96; N, 8.28

Found: C, 71.02; H, 4.95; N, 8.25%.

2-[5-{(4-Biphenyloxy)methyl}-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-methoxyphenyl)ethanone (77) Yield: 78% ; White crystals, m.p. 157-159 ºC, R$_f$ = 0.21 (n-hexane : ethyl acetate; 4:6).

![Structure Image]

IR (KBr) cm$^{-1}$: 2907, 1687, 1586, 1087.

$^1$H NMR (400 MHz, CDCl$_3$): $\delta$ 3.87 (s, 6H, O-CH$_3$), 4.92 (s, 2H, S-CH$_2$), 5.15 (s, 2H, O-CH$_2$), 6.88-7.08 (m, 6H, Ar-H), 7.31 (t, $J = 5.8$ Hz, 1H, Ar-H), 7.41 (t, $J = 7.5$ Hz, 2H, Ar-H), 7.46-7.52 (m, 4H, Ar-H), 7.67 (d, $J = 8.9$ Hz, 2H, Ar-H), 7.99 (d, $J = 9.0$ Hz, 2H, Ar-H).

$^{13}$C NMR (100 MHz, CDCl$_3$): $\delta$ 37.10, 55.63, 60.25, 114.92, 114.94, 115.13, 126.80, 127.10, 127.13, 128.56, 128.92, 129.07,
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129.96, 130.96, 134.54, 135.14, 147.38, 150.19, 160.68, 156.82, 164.56, 191.94,

Maldi-MS (m/z) : 537 (M⁺), 538 (M⁺+1).

Elemental Analysis : Calculated for molecular formula C₃₁H₂₇N₃O₄S.
Calculated : C, 69.25; H, 5.06; N, 7.82.
Found : C, 69.30; H, 5.02; N, 7.79%.

2-[5-{(4-Biphenyloxymethyl)-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-phenylphenyl)ethanone (78) Yield: 84 %; White crystals, m.p. 168-170 ºC, Rᵣ = 0.20 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm⁻¹ : 2907, 1688, 1586, 1109.

¹H NMR (400 MHz, CDCl₃) : δ 3.87 (s, 3H, O-CH₃), 4.92 (s, 2H, S-CH₂), 5.15 (s, 2H, O-CH₂), 6.88-7.10 (m, 4H, Ar-H), 7.31 (m, 2H, Ar-H), 7.41 (t, J = 7.5 Hz, 2H, Ar-H), 7.48-7.50 (m, 10H, Ar-H), 7.68 (d, J = 8.8 Hz, 2H, Ar-H), 8.09 (d, J = 9.0 Hz, 2H, Ar-H).

¹³C NMR (100 MHz, CDCl₃) : δ 37.10, 56.64, 61.35, 114.92, 114.94, 115.13, 126.80, 127.10, 127.13, 128.56, 128.92, 129.07, 129.96, 130.96, 134.54, 135.14, 147.38, 150.19, 156.88, 160.68, 164.59, 192.14.

ES-MS (m/z) : 584 (M⁺+1), 585 (M⁺+2).

Elemental Analysis : Calculated for molecular formula C₃₆H₂₉N₃O₃S.
Calculated : C, 74.08; H, 5.01; N, 7.20.
Found : C, 74.12; H, 5.02; N, 7.22%.
3-(4-Nitrophenylmethylthio)-5-[(4-biphenyloxy)methyl]-4-(4-methoxyphenyl)-4H-1,2,4-triazole (79) Yield: 72 %; White crystals, m.p. 173-175 °C, \( R_f = 0.25 \) (n-hexane : ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 2914, 1687, 1556, 1091. \\
\text{\textsuperscript{1}H NMR (400 MHz, CDCl} & : \delta 3.87 (s, 3H, O-CH}_3, 4.42 (s, 2H, S-CH}_2, 5.12 (s, 2H, O-CH}_2, 6.86-6.88 (m, 4H, Ar-H), 7.06-7.14 (m, 2H, Ar-H), 7.22 (t, \( J = 5.8 \) Hz, 1H, Ar-H), 7.33 (t, \( J = 7.6 \) Hz, 2H, Ar-H), 7.40-7.44 (m, 6H, Ar-H), 8.04 (d, \( J = 8.4 \) Hz, 2H, Ar-H).
\end{align*}
\]

\[
\begin{align*}
\text{\textsuperscript{13}C NMR (100 MHz, CDCl} & : \delta 36.88, 59.91, 64.34, 164.72, 114.98, 115.16, 114.82, 123.34, 126.31, 126.88, 128.82, 129.94, 130.21, 130.29, 130.84, 131.36, 135.12, 137.75, 146.16, 146.58, 155.74, 156.88. \\
\text{ES-MS (m/z) } & : 525 (M^+1), 526 (M^+2) \\
\text{Elemental Analysis} & : \text{Calculated for molecular formula } C_{29}H_{24}N_4O_4S. \\
& \text{Calculated: } C, 66.40; H, 4.61; N, 10.68 \\
& \text{Found: } C, 66.42; H, 4.62; N, 10.67\%.
\end{align*}
\]

3-(phenylethylthio)-5-[(4-biphenyloxy)methyl]-4-(4-methoxyphenyl)-4H-1,2,4-triazole (80) Yield: 78 %; White crystals, m.p.144-146 °C, \( R_f = 0.32 \) (n-hexane : ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 2916, 1689, 1562, 1096. \\
\text{\textsuperscript{1}H NMR (400 MHz, CDCl} & : \delta 3.08 (2H, t, \( J = 7.2 \) Hz), 3.50 (t, \( J = 7.8 \) Hz, 2H, S-CH}_2), 3.86 (s, 3H, O-CH}_3, 5.10 ( 2H, s,}
O-CH$_2$), 6.88-6.96 (m, 4H, Ar-H), 7.16-7.24 (m, 4H, Ar-H), 7.32-7.34 (m, 2H, Ar-H), 7.40 (t, $J =$ 7.2 Hz, 2H, Ar-H), 7.48-7.54 (m, 6H, Ar-H).

$^{13}$C NMR (100 MHz, CDCl$_3$) : $\delta$ 35.86, 36.74, 56.62, 59.84, 115.18, 123.89, 124.22, 126.88, 128.70, 128.74, 128.89, 129.96, 130.04, 130.24, 130.40, 130.48, 134.92, 142.42, 145.46, 148.24, 148.74, 156.88, 164.12.

Maldi-MS ($m/z$) : 493 (M$^+$), 494 (M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{30}$H$_{27}$N$_3$O$_2$S.

Calculated : C, 73.00; H, 5.51; N, 8.51.

Found : C, 73.08; H, 5.52; N, 8.49%.

2-[5-{(4-Biphenyloxy)methyl}-4-(4-flourophenyl)-4H-1,2,4-triazol-3-ylthio]-1-4bromophenyl ethanone (81) Yield: 72%; White crystals; m.p. 160-162°C, $R_f$ = 0.39 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm$^{-1}$ : 2907, 1684, 1605, 1108.

$^1$H NMR (400 MHz, CDCl$_3$) : $\delta$ 4.92 (s, 2H, S-CH$_2$), 5.08 (s, 2H, O-CH$_2$), 6.92-7.90 (m, 17 H, Ar-H).

$^{13}$C NMR (100 MHz, CDCl$_3$) : $\delta$ 36.79, 59.85, 114.97, 115.15, 126.23, 126.79, 126.87, 128.78, 130.09, 130.41, 130.46, 130.76, 130.87, 131.29, 134.89, 137.24, 145.58, 145.69, 156.87, 162.98, 192.34.

Maldi-MS ($m/z$) : 574 (M$^+$), 575 (M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{29}$H$_{21}$BrFN$_3$O$_2$S.

Calculated : C, 60.63; H, 3.68; N, 7.31

Found : C, 60.67; H, 3.69; N, 7.32%. 
2-[5-[(4-Biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(phenyl) ethanone (82) Yield: 74%; White crystals, m.p. 156-158°C, Rf = 0.29 (n-hexane : ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 2933, 1698, 1515, 1105. \\
^1\text{H NMR (300 MHz, CDCl}_3\text{)} & : \delta 4.92 (s, 2H, S-CH\text{2}), 5.08 (s, 2H, O-CH\text{2}), 6.93 \\
& \quad \text{(d, } J = 6.3\text{Hz, 2H, Ar-H), 7.29 (t, } J = 5.4\text{ Hz, 1H), 7.41 (t, } J = 8.4\text{Hz, 2H, Ar-H), 7.37-7.41} \\
& \quad \text{(m, 4H, Ar-H), 7.46-7.51 (m, 5H, Ar-H), 7.63} \\
& \quad \text{(d, } J = 6\text{Hz, 2H), 7.89 (d, } J = 6.3\text{ Hz, 2H).} \\
^{13}\text{C NMR (100 MHz, CDCl}_3\text{)} & : \delta 37.03, 58.97, 114.79, 115.13, 126.25, 128.77, \\
& \quad 129.89, 130.10, 130.12, 130.45, 130.67, 130.87, \\
& \quad 130.88, 133.89, 134.34, 136.15, 137.15, 145.27, \\
& \quad 145.63, 156.78, 162.97, 191.36. \\
\text{Maldi-MS (m/z)} & : 495 (M^+), 496 (M^+1). \\
\text{Elemental Analysis} & : \text{Calculated for molecular formula} \\
& \quad \text{C}_{29}\text{H}_{22}\text{ClF}_{3}\text{N}_3\text{O}_2\text{S.} \\
\text{Calculated} & : \text{C, 70.29; H, 4.47; N, 8.48.} \\
\text{Found} & : \text{C, 70.28; H, 4.43; N, 8.50%}. \\
\end{align*}
\]

2-[5-[(4-biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-methoxyphenyl) ethanone (83) Yield: 85%; White crystals, m.p. 172-174 °C, Rf = 0.30 (n-hexane : ethyl acetate; 4:6).
IR (KBr) cm\(^{-1}\): 2907, 1686, 1605, 1093.

\(^1\)H NMR (400 MHz, CDCl\(_3\)): \(\delta\) 3.87 (s, 3H, O-CH\(_3\)), 4.91 (s, 2H, S-CH\(_2\)), 5.14 (s, 2H O-CH\(_2\)), 6.87-7.89 (m, 17 H, Ar-H).

\(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 37.47, 55.87, 58.98, 114.15, 114.78, 114.96, 115.14, 126.26, 128.00, 129.87, 130.11, 130.12, 130.15, 130.26, 130.89, 133.91, 145.27, 146.00, 156.87, 161.67, 162.97, 192.05.

Maldi-MS (m/z): 525 (M\(^+\)), 526 (M\(^+\)+1).

Elemental Analysis: Calculated for molecular formula C\(_{30}\)H\(_{24}\)FN\(_3\)O\(_3\)S.

Calculated: C, 68.56; H, 4.60; N, 7.99.

Found: C, 68.60; H, 4.58; N, 8.01%.

\(3\)-\((\text{Phenylethylthio})\)-5-\([\text{(4-biphenyloxy)methyl}]\)-4-(\text{4-fluorophenyl})-4H-1,2,4-triazole (84) Yield: 62%; White crystals, m.p. 144-146 °C, \(R_f = 0.41\) (\(n\)-hexane : ethyl acetate; 4:6).

IR (KBr) cm\(^{-1}\): 2907, 1520, 1517, 1108.

\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 3.08 (t, \(J = 8.1\) Hz, 2H, -CH\(_2\)-), 3.50 (t, \(J = 7.5\) Hz, 2H, S-CH\(_2\)), 5.08 (s, 2H, O-CH\(_2\)), 6.96 (d, \(J = 9.0\) Hz, 2H, Ar-H), 7.18-7.34 (m, 8H, Ar-H), 7.40 (t, \(J = 7.8\) Hz, 2H, Ar-H), 7.49-7.52 (m, 6H, Ar-H).

\(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 37.49, 55.58, 59.12, 114.56, 114.78, 123.07, 127.99, 129.17, 129.85, 130.03, 130.08, 130.25, 130.45, 130.98, 134.79, 135.14, 145.14, 147.18, 149.00, 156.98, 161.69.

Maldi-MS (m/z): 481 (M\(^+\)), 482 (M\(^+\)+1).
Elemental Analysis: Calculated for molecular formula C₂₉H₂₄FN₃OS.

Calculated: C, 72.33; H, 5.02; N, 8.73.

Found: C, 72.29; H, 5.04; N, 8.69%.

3-(4-Nitrophenylmethylthio)-5[(4-biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazole (85) Yield: 68%; Light yellow crystals; m.p. 168-172 °C, Rₓ = 0.29 (n-hexane : ethyl acetate; 4:6).

\[ \text{IR (KBr) cm}^{-1} : 2916, 1524, 1106. \]

\[ \text{¹H NMR (400 MHz, CDCl₃)} : \delta 4.45 (s, 2H, S-CH₂), 5.01 (s, 2H, O-CH₂), 6.87 (d, J = 8.4 Hz, 2H, Ar-H), 7.08-7.16 (m, 4H, Ar-H), 7.23 (t, J = 5.7 Hz, 1H, Ar-H), 7.33 (t, J = 7.6 Hz, 2H, Ar-H), 7.40-7.49 (m, 6H, Ar-H), 8.04 (d, J = 8.4 Hz, 2H, Ar-H). \]

\[ \text{¹³C NMR (100 MHz, CDCl₃)} : \delta 36.86, 59.89, 114.97, 115.14, 123.27, 126.27, 126.89, 128.79, 129.89, 130.12, 130.27, 130.48, 130.89, 131.27, 134.91, 137.73, 146.23, 146.57, 155.76, 156.89, 162.97. \]

Maldi-MS (m/z): 512 (M⁺), 513 (M⁺+1).

Elemental Analysis: Calculated for molecular formula C₂₈H₂₁FN₄O₃S.

Calculated: C, 65.61; H, 4.13; N, 10.93.

Found: C, 65.67; H, 4.09; N, 10.90%.
3-(Ethylthio)-5[(4-biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazole  (86)

Yield: 68%; White crystals; m.p. 142-144 °C, Rf = 0.25 (n-hexane : ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 2920, 1526, 1108. \\
\text{\textsuperscript{1}H NMR (300 MHz, CDCl} & \text{3}) : \delta 1.42 (t, J = 5.6 \text{ Hz}, 3 \text{H, -CH} & \text{3}), 3.29 (q, J = 6.4 \text{ Hz}, 2 \text{H, S-CH} & \text{2-}), 5.11 (s, 2 \text{H, O-CH} & \text{2}), 6.95 (d, J = 8.1 \text{ Hz}, 2 \text{H}), 7.20 (t, J = 7.8 \text{ Hz}, 2 \text{H}), 7.30-7.43 (m, 5 \text{ H, Ar-H}), 7.46-7.52 (m, 4 \text{H, Ar-H}). \\
\text{\textsuperscript{13}C NMR (75 MHz, CDCl} & \text{3}) : \delta 14.66, 29.68, 59.90, 115.07, 116.76, 117.07, 126.75, 126.89, 128.73, 129.07, 129.19, 135.01, 140.42, 156.86, 164.94. \\
\text{Maldi-MS (m/z)} & : 405 (M\text{+}), 406 (M\text{+}+1). \\
\text{Elemental Analysis} & : \text{Calculated for molecular formula C}_{23}\text{H}_{20}\text{FN}_{3}\text{OS.} \\
\text{Calculated} & : \text{C, 68.13; H, 4.97; N, 10.36.} \\
\text{Found} & : \text{C, 68.27; H, 4.95; N, 10.32%}. \\
\end{align*}
\]

3-(Methylthio)-5[(4-biphenyloxy)methyl]-4-(4-fluorophenyl)-4H-1,2,4-triazole  (87)

Yield: 69%; Light yellow crystals; m.p. 140-142 °C, Rf = 0.24 (n-hexane : ethyl acetate; 4:6)

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 2916, 1524, 1106. \\
\text{\textsuperscript{1}H NMR (300 MHz, CDCl} & \text{3}) : \delta 2.70 (s, 3 \text{H, S-CH} & \text{3}), 5.09 (s, 2 \text{H, O-CH} & \text{2}), 6.95-7.49 (m, 13 \text{H}). \\
\end{align*}
\]
$^{13}$C NMR (75 MHz, CDCl$_3$) : δ 14.54, 59.95, 115.11, 116.81, 117.11, 126.78, 126.92, 128.29, 128.71, 128.77, 129.01, 129.14, 134.96, 140.47, 151.92, 154.43, 156.93, 161.59, 164.92.

Maldi-MS (m/z) : 391 (M$^+$), 392 (M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{22}$H$_{18}$FN$_3$OS.
Calculated : C, 67.50; H, 4.63; N, 10.73.
Found : C, 67.57; H, 4.66; N, 10.76%.

2-[5-{(4-Biphenyloxy)methyl}-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-bromophenyl)ethanone (88) Yield: 68%; white crystals, m.p. 154-156 °C, $R_f$ = 0.37 (n-hexane : ethyl acetate; 4:6)

IR (KBr) cm$^{-1}$ : 2912, 1683, 1607, 1584, 1093.

$^1$H NMR (400 MHz, CDCl$_3$) : δ 4.92 (s, 2H, S-CH$_2$), 5.12 (s, 2H, O-CH$_2$), 6.94 (d, $J = 6.3$ Hz, 2H), 7.29 (t, $J = 5.7$ Hz, 1H), 7.34 (d, $J = 6.3$ Hz, 2H), 7.39 (t, $J = 5.7$ Hz, 2H, Ar-H), 7.46-7.51 (m, 6H, Ar-H), 7.62 (d, $J = 6.0$ Hz, 2H, Ar-H), 7.88 (d, $J = 6$ Hz, 2H, Ar-H).

$^{13}$C NMR (100 MHz, CDCl$_3$) : δ 35.87, 59.96, 115.18, 126.88, 126.92, 127.65, 128.76, 128.82, 128.87, 129.27, 129.35, 130.06, 130.26, 130.85, 134.76, 135.17, 142.00, 145.25, 148.24, 156.95, 192.64.

Maldi-MS (m/z) : 590 (M$^+$), 591(M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{29}$H$_{21}$ClBrN$_3$O$_2$S.
Calculated : C, 58.94; H, 3.58; N, 7.11.
Found : C, 59.02 H, 3.59; N, 7.08%.
2-[5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-nitrophenyl)ethanone (89) Yield: 72%; light yellow crystals, m.p. 183-184 °C, Rf = 0.35 (n-hexane : ethyl acetate; 4:6).

![Chemical Structure of 2-[5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-nitrophenyl)ethanone](image)

**IR (KBr) cm⁻¹**: 2905, 1684, 1605, 1525, 1320, 1093.

**¹H NMR (400 MHz, CDCl₃)**: δ 4.91 (s, 2H, S-CH₂), 5.10 (s, 2H, O-CH₂), 6.95 (d, J = 6.3 Hz, 2H, Ar-H), 7.31 (t, J = 5.7 Hz, 1H, Ar-H), 7.37 (d, J = 6.6 Hz, 2H, Ar-H), 7.41 (t, J = 5.7 Hz, 2H), 7.47-7.53 (m, 6H, Ar-H), 8.20 (d, J = 6.6 Hz, 2H, Ar-H).

**¹³C NMR (100 MHz, DMSO-d₆)**: δ 36.75, 59.87, 115.10, 124.09, 126.79, 126.97, 128.31, 128.78, 130.28, 130.92, 131.12, 135.17, 136.00, 139.72, 140.41, 147.00, 150.00, 156.81, 191.65.

**Maldi-MS (m/z)**: 557 (M⁺), 558 (M⁺+1).

**Elemental Analysis**

Calculated for molecular formula C₂₉H₂₁ClN₄O₄S:

- C: 62.53; H: 3.80; N: 10.06.

Found:

- C: 62.62; H: 3.82; N: 9.96%.

2-[5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-methoxyphenyl)ethanone (90) Yield: 75%; White crystals, m.p. 178-180 °C, Rf = 0.34 (n-hexane : ethyl acetate; 4:6).

![Chemical Structure of 2-[5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-methoxyphenyl)ethanone](image)

**IR (KBr) cm⁻¹**: 2912, 1681, 1607, 1093.

**¹H NMR (400 MHz, CDCl₃)**: δ 3.83 (s, 3H, O-CH₃), 4.53 (s, 2H, S-CH₂), 5.01 (s, 2H, O-CH₂), 6.91-6.96 (m, 4H, Ar-H),
65

7.27-7.33 (m, 3H, Ar-H), 7.46-7.51 (m, 6H, Ar-H), 7.61 (d, J = 6.4 Hz, 2H, Ar-H), 7.85 (d, J = 6.4 Hz, 2H, Ar-H).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) : $\delta$ 36.96, 56.24, 60.07, 114.98, 117.56, 126.88, 127.24, 127.85, 128.95, 128.96, 129.57, 129.86, 130.16, 130.26, 131.05, 134.78, 135.16, 145.75, 148.47, 156.34, 156.78, 192.94.

Maldi-MS (m/z) : 542 (M$^+$), 543 (M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{30}$H$_{24}$ClN$_3$O$_3$S.

Calculated : C, 66.47; H, 4.46; N, 7.75.

Found : C, 66.50; H, 4.47; N, 7.72%.

2-[5-{(4-Biphenyloxy)methyl}-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(phenyl) ethanone (91) Yield: 68 %; White crystals, m.p. 192-194 ºC, R$_f$ = 0.36 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm$^{-1}$ : 2924, 1680, 1584, 1107.

$^1$H NMR (400 MHz, DMSO-d$_6$) : $\delta$ 4.58 (s, 2H, S-CH$_2$), 5.42 (s, 2H, O-CH$_2$), 6.98-7.06 (m, 4H, Ar-H), 7.22-7.35 (m, 6H, Ar-H), 7.52-7.54 (m, 8H, Ar-H).

$^{13}$C NMR (100 MHz, DMSO-d$_6$) : $\delta$ 35.84, 59.89, 115.05, 123.84, 126.39, 126.98, 128.88, 128.94, 129.04, 130.12, 139.09, 140.39, 143.98, 147.41, 152.34, 156.80, 162.06, 164.56, 192.56.

Maldi-MS (m/z) : 511 (M$^+$), 513 (M$^+$+2).

Elemental Analysis : Calculated for molecular formula C$_{29}$H$_{22}$ClN$_3$O$_2$S.

Calculated : C, 68.03; H, 4.33; N, 8.21.

Found : C, 68.09; H, 4.30; N, 8.19%.
2-[[5-((4-Biphenyloxy)methyl)-4-(3-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-methoxyphenyl) ethanone (92) Yield: 82%; White Crystals, m.p. 162-164 °C, Rf = 0.20 (n-hexane : ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 2917, 1526, 1109. \\
\text{\textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3})} & : \delta 3.88 (s, 3H, O-CH\textsubscript{3}), 4.93 (s, 2H, S-CH\textsubscript{2}), 5.11 (s, 2H, O-CH\textsubscript{2}), 6.94-6.97 (m, 4H), 7.31 (d, J = 6.6 Hz, 1H), 7.38-7.53 (m, 10H, Ar-H), 8.01 (d, J = 8.7 Hz, 2H). \\
\text{\textsuperscript{13}C NMR (100 MHz, DMSO-d\textsubscript{6})} & : \delta 36.96, 55.89, 60.07, 114.98, 117.56, 126.88, 127.24, 127.85, 128.95, 129.86, 128.96, 129.57, 130.16, 130.26, 131.05, 134.78, 135.16, 146.15, 148.42, 156.38, 156.72, 191.92. \\
\text{ES-MS (m/z)} & : 542 (M\textsuperscript{+}+1), 543 (M\textsuperscript{+}+2).
\end{align*}
\]

Elemental Analysis : Calculated for molecular formula C\textsubscript{30}H\textsubscript{24}ClN\textsubscript{3}O\textsubscript{3}S.

Calculated : C, 66.47; H, 4.46; N, 7.75.

Found : C, 66.58; H, 4.49; N, 7.75%.

2-[[5-((4-Biphenyloxy)methyl)-4-(3-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1(phenyl) ethanone (93) Yield: 76%; White Crystals, m.p. 143-145 °C, Rf = 0.31 (n-hexane : ethyl acetate; 4:6).

\[
\begin{align*}
\text{ES-MS (m/z)} & : 542 (M\textsuperscript{+}+1), 543 (M\textsuperscript{+}+2).
\end{align*}
\]
Chapter II, Section 1

IR (KBr) cm\(^{-1}\) : 2914, 1520, 1108.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) : \(\delta \) 4.98 (s, 2H, S-CH\(_2\)), 5.12 (s, 2H, O-CH\(_2\)), 6.96 (d, \(J = 7.9\) Hz, 2H), 7.32 (d, \(J = 7.2\) Hz, 2H), 7.39-7.43 (m, 3H, Ar-H), 7.51-7.59 (m, 8H, Ar-H), 7.62 (t, \(J = 7.2\) Hz, 1H, Ar-H), 8.03 (d, \(J = 8.1\) Hz, 2H).

\(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)) : \(\delta \) 38.12, 60.12, 114.89, 126.83, 127.26, 127.84, 128.66, 128.96, 128.97, 129.19, 129.72, 130.27, 132.75, 131.04, 135.16, 136.45, 145.73, 148.45, 156.42, 191.82.

Maldi-MS (m/z) : 512 (M\(^+\)), 513 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula
\[ \text{C}_{29}\text{H}_{22}\text{ClN}_3\text{O}_2\text{S}. \]

Calculated : C, 68.03; H, 4.33; N, 8.21.

Found : C, 68.58; H, 4.36; N, 8.25%.

2-[5-{(4-Biphenyloxy)methyl}-4-(3-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-phenyl-phenyl)ethanone (94) Yield: 80%; White Crystals, m.p. 164-166 °C, \(R_f = 0.41\) (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm\(^{-1}\) : 2916, 1556, 1108.

\(^1\)H NMR (400 MHz, CDCl\(_3\)) : \(\delta \) 5.0 (s, 2H, S-CH\(_2\)), 5.12 (s, 2H, O-CH\(_2\)), 6.96 (d, \(J = 8.7\) Hz, 2H), 7.28-7.50 (m, 14H), 7.58 (d, \(J = 6.9\) Hz, 2H), 7.72 (d, \(J = 8.1\) Hz, 2H), 8.10 (d, \(J = 8.4\) Hz, 2H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) : \(\delta \) 37.10, 56.64, 61.33, 114.90, 114.92, 115.10, 126.80, 127.06, 127.10, 128.54, 128.90, 129.17,
129.94, 130.94, 134.51, 135.14, 146.36, 150.19, 156.84, 160.68, 163.88, 192.14.

**Maldi-MS (m/z)**: 588 (M⁺), 589 (M⁺+2).

**Elemental Analysis**: Calculated for molecular formula C₃₅H₂₆ClN₃O₂S.

Calculated: C, 71.48; H, 4.46; N, 7.14.

Found: C, 71.62; H, 4.51; N, 7.19%.

2-[5-{(4-Biphenyloxy)methyl}-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-methoxyphenyl)ethanone (95) **Yield**: 78%; Light yellow crystals; **m.p.** 198-200 °C, **Rf** = 0.19 (n-hexane : ethyl acetate; 4:6).

![Chemical structure](image)

**IR (KBr) cm⁻¹**: 2912, 1686, 1518, 1528, 1105.

**¹H NMR (400 MHz, CDCl₃)**: δ 3.89 (s, 3H, O-CH₃), 4.92 (s, 2H, S-CH₂), 5.12 (s, 2H, O-CH₂), 6.98 (d, J = 6.6 Hz, 2H, Ar-H), 7.28-7.52 (m, 9H, Ar-H), 7.66 (d, J = 8.9 Hz, 2H, Ar-H), 7.98 (d, J = 9.0 Hz, 2H, Ar-H), 8.42 (d, J = 8.7 Hz, 2H, Ar-H).

**¹³C NMR (100 MHz, CDCl₃)**: δ 35.79, 56.82, 59.84, 115.15, 122.36, 124.06, 126.74, 128.30, 128.65, 130.25, 130.64, 135.74, 135.84, 138.54, 141.44, 147.32, 150.10, 156.86, 192.22.

**Maldi-MS (m/z)**: 552 (M⁺), 553 (M⁺+1).

**Elemental Analysis**: Calculated for molecular formula C₃₀H₂₄N₄O₅S.

Calculated: C, 65.20; H, 4.38; N, 10.14.

Found: C, 65.22; H, 4.37; N, 10.12%.
2-[5-[(4-Biphenyloxy)methyl]-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-chlorophenyl)ethanone (96) Yield: 76%; Light yellow crystals, m.p. 158-160 °C, Rf = 0.33 (n-hexane : ethyl acetate; 4:6).

![Chemical Structure]

IR (KBr) cm⁻¹: 2912, 1686, 1604, 1556, 1105.

¹H NMR (300 MHz, CDCl₃): δ 4.91 (s, 2H, S-CH₂), 5.14 (s, 2H, O-CH₂), 6.92 (d, J = 8.7 Hz, 2H, Ar-H), 7.32 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.5 Hz, 1H, Ar-H), 7.47-7.68 (m, 8H, Ar-H), 7.87 (d, J = 8.4 Hz, 2H, Ar-H), 8.42 (d, J = 8.7 Hz, 2H, Ar-H).

¹³C NMR (100 MHz, CDCl₃): δ 34.56, 59.96, 115.23, 122.54, 124.19, 126.83, 128.36, 128.42, 128.59, 130.32, 130.71, 135.81, 135.86, 134.59, 141.52, 148.14, 150.24, 156.92, 191.26.

Maldi-MS (m/z): 557 (M⁺) 559 (M⁺+1).

Elemental Analysis: Calculated for molecular formula C₂₉H₂₁ClN₄O₄S.

Calculated: C, 62.53; H, 3.80; N, 10.06.

Found: C, 62.50; H, 3.81; N, 10.08%.

2-[5-[(4-Biphenyloxy)methyl]-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(phenyl)ethanone (97) Yield: 66%; Light yellow crystals, m.p. 168-170 °C, Rf = 0.27 (n-hexane : ethyl acetate; 4:6).

![Chemical Structure]

IR (KBr) cm⁻¹: 2907, 1686, 1515, 1524, 1093.

¹H NMR (400 MHz, CDCl₃): δ 4.91 (s, 2H, S-CH₂), 5.14 (s, 2H, O-CH₂), 6.96 (d, J = 6.6 Hz, 2H, Ar-H), 7.28-7.52 (m, 10H,
Ar-H), 7.66 (d, J = 8.9 Hz, 2H, Ar-H), 7.98 (d, J = 9.0 Hz, 2H, Ar-H), 8.41(d, J = 8.7 Hz, 2H, Ar-H).

**\(^{13}\)C NMR (100 MHz, CDCl\(_3\))**

δ 35.00, 59.86, 79.72, 115.17, 122.34, 124.09, 126.79, 128.31, 128.32, 128.65, 130.27, 130.68, 135.76, 135.86, 138.56, 141.46, 147.34, 150.12, 156.88, 191.24.

**Maldi-MS (m/z)**

: 522 (M\(^+\)), 523 (M\(^+\)+1).

**Elemental Analysis**

: Calculated for molecular formula C\(_{29}\)H\(_{22}\)N\(_4\)O\(_4\)S.

Calculated

: C, 66.65; H, 4.24; N, 10.72

Found

: C, 66.72; H, 4.26; N, 10.68%.

2-[5-{(4-Biphenyloxy)methyl}-4-(4-nitrophenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-phenylphenyl)ethanone (98) **Yield:** 70%; Light yellow crystals, m.p.175-177 °C, R\(_f\) = 0.35 (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm\(^{-1}\)

: 2907, 1671, 1601, 1558, 1093.

**\(^{1}\)H NMR (300 MHz, CDCl\(_3\))**

δ 5.00 (s, 2H, S-CH\(_2\)), 5.15 (s, 2H, O-CH\(_2\)), 7.31 (t, J = 5.7 Hz, 1H, Ar-H), 6.93 (d, J = 7.8 Hz, 2H, Ar-H), 7.63-7.73 (m, 7H, Ar-H), 7.39-7.52 (m, 8H, Ar-H), 8.07-8.10 (d, J = 7.8 Hz, 2H, Ar-H), 8.41 (d, J = 8.4 Hz, 2H, Ar-H).

**\(^{13}\)C NMR (100 MHz, CDCl\(_3\))**

δ 37.06, 61.09, 115.16, 122.36, 124.09, 126.80, 126.87, 127.34, 128.32, 128.37, 130.29, 130.69, 130.70, 130.73, 135.12,135.38, 135.84, 138.57, 138.86, 141.47, 147.33, 150.14, 156.98, 191.28.

**Maldi-MS (m/z)**

: 598 (M\(^+\)) 599 (M\(^+\)+1).

**Elemental Analysis**

: Calculated for molecular formula C\(_{35}\)H\(_{26}\)N\(_4\)O\(_4\)S.

Calculated

: C, 70.22; H, 4.38; N, 9.36.

Found

: C, 70.14; H, 4.35; N, 9.34%.
3-(4-Nitrophenvmethylthio)-5[(4-biphenyloxy)methyl]-4-(4-nitrophenyl)-4H-1,2,4-
triazole (99) Yield: 78%; Light yellow crystals, m.p. 160-162 °C, Rf = 0.28 (n-
hexane : ethyl acetate; 4:6).

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

**IR (KBr) cm**\(^{-1}\): 2912, 1546, 1106.

**\(^1\)H NMR (300 MHz, CDCl\(_3\))**: \(\delta\) 4.56 (s, 2H, S-CH\(_2\)), 5.13 (s, 2H, O-CH\(_2\)), 6.92 (d, \(J = 8.7\) Hz, 2H, Ar-H), 7.31 (t, \(J = 7.2\) Hz, 1H), 7.39-7.58 (m, 10H, Ar-H), 8.13 (d, \(J = 8.7\) Hz, 2H, Ar-H), 8.37 (d, \(J = 9.0\) Hz, 2H).

**\(^{13}\)C NMR (100 MHz, CDCl\(_3\))**: \(\delta\) 36.86, 59.78, 114.97, 115.14, 123.25, 126.26, 126.84, 128.74, 129.82, 130.15, 130.26, 130.46, 130.86, 131.24, 134.92, 137.83, 146.53, 146.58, 155.86, 156.

**Maldi-MS (m/z)**: 539 (M\(^+\)), 540 (M\(^+\)+1).

**Elemental Analysis**: Calculated for molecular formula C\(_{28}\)H\(_{21}\)N\(_5\)O\(_5\)S.

*Calculated*: C, 62.33; H, 3.92; N, 12.98.

*Found*: C, 62.48; H, 3.89; N, 12.96 %.

2-[5-{(4-Biphenyloxy)methyl]-4-(2-methylphenyl)-4H-1,2,4-triazol-3-ythio]-1-(4-
bromophenyl)ethanone (100) Yield: 82%; White Crystals, m.p. 197-199 °C, Rf = 0.40 (n-hexane : ethyl acetate; 4:6)

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

**IR (KBr) cm**\(^{-1}\): 2916, 1534, 1106.

**\(^1\)H NMR (300 MHz, CDCl\(_3\))**: \(\delta\) 2.10 (s, 3H, Ar-CH\(_3\)), 4.90-4.93 (m, 2H, S-CH\(_2\)), 5.05 (s, 2H, O-CH\(_2\)), 7.90 (d, \(J = 8.4\) Hz, 2H), 7.63 (d, \(J = 7.5\)Hz, 2H), 7.30-7.50 (m,10H,
Ar-H), 7.19 (d, $J = 8.4$ Hz, 1H), 6.88 (d, $J = 8.7$ Hz, 2H).

$^{13}$C NMR (75 MHz, CDCl$_3$) : δ 17.32, 38.41, 60.02, 115.05, 126.76, 126.87, 127.41, 128.20, 128.75, 129.35, 130.05, 130.91, 131.39, 131.64, 132.21, 133.94, 134.83, 136.40, 140.50, 152.11, 152.71, 157.11, 192.13.

Maldi-MS (m/z) : 570 (M$^+$), 571 (M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{30}$H$_{24}$BrN$_3$O$_2$S.

Calculated : C, 63.16; H, 4.24; N, 7.37.

Found : C, 63.21; H, 4.21; N, 7.40%.

2-[(5-(4-Biphenyloxy)methyl)-4-(2-methylphenyl)-4H-1,2,4-triazol-3-ylthio]-1-(4-methoxyphenyl)ethanone (101) Yield: 80%; White Crystals, m.p. 194-196 °C, $R_f = 0.41$ (n-hexane : ethyl acetate; 4:6).

IR (KBr) cm$^{-1}$ : 2926, 1686, 1534, 1107.

$^1$H NMR (300 MHz, CDCl$_3$) : δ 2.10 (s, 3H, Ar-CH$_3$), 3.87 (s, 3H, O-CH$_3$), 5.01-5.03 (m, 2H, S-CH$_2$), 5.06 (s, 2H, O-CH$_2$), 6.88 (d, $J = 8.1$ Hz, 2H), 6.95 (d, $J = 8.4$ Hz, 1H), 7.21 (d, $J = 6.3$ Hz, 2H), 7.29 (t, $J = 6.3$ Hz, 1H), 7.37-7.50 (m, 9H, Ar-H), 8.04 (d, $J = 6.0$ Hz, 2H).

$^{13}$C NMR (75 MHz, CDCl$_3$) : δ 17.39, 41.31, 55.55, 59.89, 114.02, 115.02, 126.72, 126.84, 127.42, 127.79, 128.18, 128.71, 130.95, 131.65, 134.86, 136.35, 140.46, 157.02, 164.25, 191.29.

ES-MS (m/z) : 522 (M$^+$+1), 523 (M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{31}$H$_{27}$N$_3$O$_3$S.

Calculated : C, 71.38; H, 5.22; N, 8.06.

Found : C, 71.31; H, 5.18; N, 8.04%.
2-[(4-Biphenyloxy)methyl]-4-(2-methylphenyl)-4H-1,2,4-triazol-3-ylthio]-I-(4-phenyl)ethanone (102) Yield: 68%; White Crystals, m.p. 158-156 °C, R_f = 0.43 (n-hexane : ethyl acetate; 4:6).

\[
\text{IR (KBr) cm}^{-1}: 2926, 1520, 1108.
\]

\[
\text{^1H NMR (300 MHz, CDCl}_3\text{)}: \delta 2.11 (s, 3H, Ar-CH}_3\text{), 4.96-5.00 (m, 4H, O-CH}_2\text{, S-CH}_2\text{), 6.89 (d, J = 8.7 Hz, 2H), 7.21 (d, J = 7.8 Hz, 1H), 7.22 (t, J = 6.9 Hz, 1H), 7.32-7.52 (m, 8H), 7.62 (d, J = 6.9 Hz, 2H), 7.71 (d, J = 8.1 Hz, 2H), 8.11 (d, J = 8.1 Hz, 2H).
\]

\[
\text{^{13}C NMR (100 MHz, CDCl}_3\text{)}: \delta 16.46, 36.86, 59.89, 114.97, 115.14, 123.17, 126.28, 126.78, 128.72, 129.82, 130.10, 130.22, 130.40, 130.84, 131.20, 137.72, 134.82, 146.20, 146.55, 155.73, 191.62.
\]

Maldi-MS (m/z): 491 (M^+), 492 (M^+1).

Elemental Analysis

Calculated: C, 73.30; H, 5.13; N, 8.55.

Found: C, 73.34; H, 5.10; N, 8.53%.

2-[(4-Biphenyloxy)methyl]-4-(2-methylphenyl)-4H-1,2,4-triazol-3-ylthio]-I-(4-phenyl-phenyl)ethanone (103) Yield: 74%; White Crystals, m.p. 168-170 °C, R_f = 0.45 (n-hexane : ethyl acetate; 4:6).

\[
\text{IR (KBr) cm}^{-1}: 2926, 1524, 1109.
\]

\[
\text{^1H NMR (300 MHz, CDCl}_3\text{)}: \delta 2.11 (s, 3H, Ar-CH}_3\text{), 4.96-5.00 (m, 4H, O-CH}_2\text{, S-CH}_2\text{), 6.89 (d, J = 8.7 Hz, 2H), 7.21 (d, J = 7.8 Hz, 1H), 7.22 (t, J = 6.9 Hz, 2H),
\]

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7.37-7.51 (m, 11H), 7.63 (d, \( J = 6.9 \) Hz, 2H),
7.71 (d, \( J = 8.1 \) Hz, 2H), 8.11 (d, \( J = 8.1 \) Hz, 2H).

\[^{13}\text{C NMR (100 MHz, CDCl}_3\)]: \( \delta \) 16.46, 36.86, 59.89, 114.97, 115.14, 123.17,
126.28, 126.78, 128.72, 129.82, 130.10, 130.22,
130.40, 130.84, 131.20, 134.82, 146.20,
146.55, 155.73, 156.84, 162.94, 191.62.

Maldi-MS (m/z): 567 (M\(^+\)), 568 (M\(^{+1}\)).

Elemental Analysis: Calculated for molecular formula C\(_{36}\)H\(_{29}\)N\(_3\)O\(_2\)S.
Calculated: C, 76.16; H, 5.15; N, 7.40.
Found: C, 76.22; H, 5.16; N, 7.41%.

5-[((4-Biphenyloxy)methyl)-3-(ethylthio)-4-o-toly]-1,2,4-triazole (104) Yield:
76%; White Crystals, m.p. 174-176 \(^\circ\)C, \( R_f = 0.40 \) (n-hexane : ethyl acetate; 4:6)

IR (KBr) cm\(^{-1}\): 2926, 1524, 1109.

\[^{1}\text{H NMR (300 MHz, CDCl}_3\)]: \( \delta \) 2.08 (s, 3H, Ar-CH\(_3\)), 2.74 (t, \( J = 3.8 \) Hz, 3H,
S-CH\(_2\)), 3.29 (q, \( J = 4.6 \) Hz, 2H, S-CH\(_2\)-), 5.05
(s, 2H, O-CH\(_2\)), 6.88 (d, \( J = 8.4 \) Hz, 2H), 7.17
(d, \( J = 8.4 \) Hz, 1H), 7.25-7.51 (m, 10H).

\[^{13}\text{C NMR (100 MHz, CDCl}_3\)]: \( \delta \) 15.24, 16.36, 29.12, 59.89, 114.97, 115.14,
123.27, 126.27, 126.89, 128.79, 129.89, 130.12,
130.27, 130.48, 130.89, 131.27, 134.91, 137.73,
146.23, 146.57, 155.72, 155.84, 162.92.

Maldi-MS (m/z): 401 (M\(^+\)), 402 (M\(^{+1}\)).

Elemental Analysis: Calculated for molecular formula C\(_{24}\)H\(_{23}\)N\(_3\)OS:
Calculated: C, 71.79; H, 5.77; N, 10.47.
Found: C, 71.69; H, 5.75; N, 10.46%.
5’-{(4-Biphenyloxy)methyl}-3-(methylthio)-4-o-tolyl-4H-1,2,4-triazole (105) Yield: 72%; White Crystals, m.p. 110-112 °C, Rf = 0.41 (n-hexane : ethyl acetate; 4:6)

![Chemical Structure](image)

**IR (KBr) cm\(^{-1}\):** 2916, 1534, 1106.

**\(^1\)H NMR (300 MHz, CDCl\(_3\)):** \(\delta\) 2.08 (s, 3H, Ar-CH\(_3\)), 2.70 (s, 3H, S-CH\(_3\)), 5.05 (s, 2H, O-CH\(_2\)), 6.88 (d, \(J = 8.4\) Hz, 2H), 7.17 (d, \(J = 8.4\) Hz, 1H), 7.25-7.51 (m, 10H).

**\(^13\)C NMR (100 MHz, CDCl\(_3\)):** \(\delta\) 14.54, 15.38, 59.89, 114.97, 115.14, 123.27, 126.27, 126.89, 162.97, 128.79, 129.89, 130.12, 130.27, 130.48, 130.89, 131.27, 134.91, 137.73, 146.23, 146.57, 155.76, 156.89.

**Maldi-MS (m/z):** 387 (M\(^+\)), 388 (M\(^{+1}\)).

**Elemental Analysis:** Calculated for molecular formula C\(_{23}\)H\(_{21}\)N\(_3\)OS.

Calculated: C, 71.29; H, 5.46; N, 10.84.

Found: C, 71.62; H, 4.51; N, 10.69%.

**2.5.1.2. Biological activity**

All the novel synthesized compounds were screened for their biological activities. Albino Wistar rats of either sex (150-200 g) were obtained from Central Animal House, Hamdard University, New Delhi. Fourteen hours before the start of the experiment, the animals were sent to lab and fed only with water ad libitum. The experiments were performed in accordance with the rules of Institutional Animals Ethics Committee (registration number 173-CPCSEA). The animals were divided into groups of six animals each. Bacterial and fungal cultures were obtained from Microbiology laboratory, Batra Hospital, New Delhi, India and the screening tests were carried out at the Microbiology laboratory, Majeedia hospital, Jamia Hamdard, New Delhi.
2.5.1.2.2.1. **Anti-inflammatory activity**

All the newly synthesized compounds were evaluated for their anti-inflammatory activity [53] against carrageenan-induced acute paw oedema. The first group of rats was treated with 0.1 mL of 0.5% CMC suspension orally (control), second group was administered with a dose of 10 mg/kg of the suspension of Ibuprofen (standard) and the test group was treated with equimolar dose of the suspension of test compounds relative to standard drug. After 30 min the animals were injected with 0.1 mL of 1% carrageenan in normal saline subcutaneously to the sub-plantar region of right hind paw. The paw volume was measured at 0 h, 1 h, 3 h and 5 h, by using plethysmometer. The amount of oedema in the drug-treated groups was compared in relation to the control group with the corresponding time intervals.

The percent oedema inhibition was calculated from the mean effect in the control and treated animals according to the following equation.

Percentage inhibition = 100 \(\left(1 - \frac{V_t}{V_c}\right)\).

\(V_t\) = mean increase in paw volume of test

\(V_c\) = mean increase in paw volume of control group of rats.

The results of anti-inflammatory activity are presented in table 1.2 and figure 1.1.

2.5.1.2.2.2. **Analgesic activity**

The analgesic activity was determined using tail flick method [54]. The animals were weighed and divided into control, standard, and test groups and each group contained six rats. The first group of rats was treated with 0.1 mL of 0.5% CMC suspension orally (control), second group was administered with a dose of 10 mg/kg of the suspension of Ibuprofen (standard) and the test group was treated with equimolar dose of the suspension of test compounds relative to standard drug. After administration of drug, reaction time was measured at 0 min., 30 min., 60 min., and 120 min., by immersing the tail in hot water \((55 \pm 0.5 \, ^\circ C)\). Analgesic activity was expressed as reaction time of tail flicking, compounds having analgesic activity will show more reaction time than the control. The results are presented in table 1.3 and figure 1.2.

2.5.1.2.2.3. **Histopathological study**

For the histopathological study, rats were sacrificed under light anesthesia after 4 h of the doses (3 times to the dose used for anti-inflammatory) and their stomach
specimens were removed and kept into 10% formalin solution. A longitudinal section of stomach along the greater curvature, which included the ulcer base and both sides of the ulcer margin, was taken and fixed in 10% formalin for 24 h at 4 ºC and embedded in white solid paraffin. Morphological examination was performed with Haematoxylin and eosin staining to analyze histological changes and examined under microscope. The results are given in table 1.4 and figure 1.3.

2.5.1.2.4. **In vitro Antimicrobial activity**

The antimicrobial studies of the synthesized compounds were carried out against different bacterial and fungal strains. All bacterial and fungal strains were obtained from the Batra Hospital, New Delhi, India and Department of Biochemistry, Jamia Hamdard, New Delhi, India as follows

**Bacterial Strains**
1. *Pseudomonas aeruginosa* (ATCC 27853)
2. *Klebsiella pneumoniae* (ATCC 700603)
3. *Escherichia coli* (ATCC 25922)
4. *Staphylococcus aureus* (ATCC 25923)

**Fungal Strains**
1. *Candida albicans* (ATCC 10231)
2. *Candida krusie* (ATCC 6258)
3. *Aspergilus niger* (MTCC 8189)
4. *Aspergilus flavus* (MTCC 277)

All the newly synthesized compounds were dissolved in dimethyl sulfoxide (DMSO) to prepare chemicals of stock solution of 2 mg/mL.

2.5.1.2.4.1. **Agar-well diffusion method**

Simple susceptibility screening test using agar-well diffusion method [55] was used. Each microorganism was suspended in Mueller Hinton (MH) (Difco, Detroit, MI) broth and diluted approximately to $10^6$ colony forming unit (cfu)/mL. They were “flood-inoculated” onto the surface of MH agar and Sabouraud Dextrose Agar (SDA) and then dried. For *Candida albicans, Candida krusie, Aspergilus niger* and *Aspergilus flavus*, SDA were used. For *Pseudomonas aeruginosa, Klebsiella*
*pneumoniae*, Maccony agar was used and for *Escherichia coli* and *Staphylococcus aureus* Muller Hinton agar were used. Six-millimeter diameter wells were cut from the agar using a sterile cork-borer and 200 µg and 100 µg of the test compounds were delivered into the wells. The plates were incubated for 18 h at 35 ºC for bacteria and 48 h for fungal strains. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organism. Ampicillin (200, 100 µg) and Fluconazole (200, 100 µg) were used as standard drugs. Dimethyl sulfoxide was used as solvent (controls). The antimicrobial activity results are summarized in table 1.5.
2.5.1.3. Conclusion

In conclusion a focused library of biphenyl based 1,2,4-triazole derivatives has been successfully synthesized and evaluated for their anti-inflammatory, analgesic and antimicrobial activities. From the results, it is concluded that seven compounds 68, 74, 88, 96, 97, 99, and 101, out of thirty-nine compounds showed good anti-inflammatory activity against carrageenan induced rat paw oedema method. Compounds 68 showed better anti-inflammatory activity (79.84, 83.03%) than standard drug Ibuprofen (78.89 and 80.10%) at 3 h and 5 h respectively. Compounds 99 and 101 showed better analgesic activities (3.81, 3.96 and 3.08) and (3.88, 3.98 and 2.95 sec) respectively compared to the standard drug Ibuprofen (3.71, 3.95 and 2.76 sec) at 30, 60 and 120 min. All the tested compounds showed less ulceration than standard drug Ibuprofen whereas compound 74 did not caused any ulceration. The antimicrobial activity revealed that most of the compounds showed moderate to significant activity. Compounds having nitro, chloro, bromo and fluoro group showed better activity.
2.4.1.0. Introduction

As discussed in introductory part of this chapter, hydrazone, triazole and biphenyl derivatives exhibit different biological activities. In view of the biological importance of hydrazones, 1,2,4-triazoles and biphenyl systems, a focused library of conjugates have been synthesized and evaluated for their anti-inflammatory, analgesic and ulcerogenic studies. The synthetic route is given below.

SCHEME II
Reaction of triazoles (63, 65) with ethylbromoacetate in presence of sodium metal in absolute alcohol afforded ethyl esters (106) and (107), which readily yielded hydrazides (108) and (109) by refluxing with hydrazine monohydrate in absolute alcohol. The hydrazides when further reacted with different aromatic aldehydes in absolute alcohol yielded hydrazones (110-123). All the synthesized compounds have been completely characterized on the basis of their detailed spectral data and all the novel compounds of this series were evaluated for their biological activities. The physical data of all the novel synthesized compounds are given in table 2.1.

Table 2.1 Physical data of biphenyl based hydrazones of 1,2,4-triazole derivatives

<table>
<thead>
<tr>
<th>Comp. no.</th>
<th>Structure</th>
<th>% Yield</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>110</td>
<td>![Structure Image]</td>
<td>68</td>
<td>146-148</td>
</tr>
<tr>
<td>111</td>
<td>![Structure Image]</td>
<td>72</td>
<td>187-189</td>
</tr>
<tr>
<td>112</td>
<td>![Structure Image]</td>
<td>71</td>
<td>168-170</td>
</tr>
<tr>
<td>113</td>
<td>![Structure Image]</td>
<td>68</td>
<td>144-146</td>
</tr>
<tr>
<td>114</td>
<td>![Structure Image]</td>
<td>78</td>
<td>173-175</td>
</tr>
<tr>
<td>115</td>
<td>![Structure Image]</td>
<td>72</td>
<td>171-173</td>
</tr>
<tr>
<td>116</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>66</td>
<td>193-195</td>
</tr>
<tr>
<td>117</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>69</td>
<td>179-181</td>
</tr>
<tr>
<td>118</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>68</td>
<td>176-178</td>
</tr>
<tr>
<td>119</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>72</td>
<td>184-186</td>
</tr>
<tr>
<td>120</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>68</td>
<td>181-183</td>
</tr>
<tr>
<td>121</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>75</td>
<td>180-182</td>
</tr>
<tr>
<td>122</td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td>80</td>
<td>168-170</td>
</tr>
<tr>
<td>123</td>
<td><img src="image8" alt="Chemical Structure" /></td>
<td>78</td>
<td>165-167</td>
</tr>
</tbody>
</table>
2.4.2.1. Results and discussion

2.4.2.1.1. Analytical

A focused library of fourteen compounds 110-123 were synthesized starting from 3-mercapto-1,2,4-triazole as outlined in scheme-II. S-alkylation of 3-mercapto-1,2,4-triazole 63 and 65 to 106 and 107 were confirmed from the $^1$H NMR spectrum. The appearance of two signals at $\delta$ 1.25 and 1.26 each (t, $J$ = 7.2 Hz, 3H, -CH$_3$) and at $\delta$ 4.20 (q, $J$ = 7.2 Hz, 2H, O-CH$_2$) integration for five protons suggested the presence of ethoxy group. The presence of strong absorption band at 1673-1675 cm$^{-1}$ and absence of bands for S-H, at 2680-2690 cm$^{-1}$ confirms the S-alkylation of thiol group. The compounds 106 and 107 readily yielded their hydrazides 108 and 109 by their reaction with hydrazine monohydrate. The formation of these hydrazides were confirmed from the appearance of signals at $\delta$ 8.91 (s, 1H, N-H) and $\delta$ 1.62 (s, 2H, NH$_2$) and disappearance of ethyl group signals. These hydrazides were then converted into the target molecules 110-123 which was confirmed from IR (absorption bands for N=C, 1655-1687 cm$^{-1}$), extra signals in aromatic region of $^1$H NMR spectrum due to introduction of phenyl ring of aldehydes to the target molecules (hydrazones) and finally confirmed from their mass spectra.

All the newly synthesized compounds 110-123 were screened for their biological activities (anti-inflammatory, analgesic and ulcerogenic activities). These novel hydrazones showed therapeutic efficacy.

2.4.2.1.2. Biological activity

2.4.2.1.2.1. Anti-inflammatory activity

The results of anti-inflammatory activity are summarized in table 2.2 and figure 2.1. Among the tested compounds, two compounds 115 and 120 were found significantly active (83.76, 75.64 % and 82.84, 81.13 % inhibition) when compared to the standard drug Ibuprofen (78.89 and 80.10 %) in paw oedema at 3 h and 5 h respectively whereas compounds 116 and 121 showed moderate activity. All the data were analyzed by one-way ANOVA test followed by Dunnett’s test in carrageenan induced rat paw oedema model in rats.
Table 2.2. Anti-inflammatory activity of biphenyl based hydrazones of 1,2,4-triazole derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Change in paw oedema volume (mL) After drug treatment</th>
<th>Anti-inflammatory activity % Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
<td>5h</td>
</tr>
<tr>
<td>110</td>
<td>0.35±0.022</td>
<td>0.32±0.033</td>
</tr>
<tr>
<td>111</td>
<td>0.40±0.044**</td>
<td>0.45±0.022**</td>
</tr>
<tr>
<td>112</td>
<td>0.48±0.022**</td>
<td>0.45±0.022**</td>
</tr>
<tr>
<td>113</td>
<td>0.40±0.039**</td>
<td>0.43±0.042**</td>
</tr>
<tr>
<td>114</td>
<td>0.35±0.022</td>
<td>0.41±0.030**</td>
</tr>
<tr>
<td>115</td>
<td>0.10±0.036***</td>
<td>0.10±0.025***</td>
</tr>
<tr>
<td>116</td>
<td>0.33±0.033**</td>
<td>0.18±0.030***</td>
</tr>
<tr>
<td>117</td>
<td>0.35±0.042</td>
<td>0.25±0.021*</td>
</tr>
<tr>
<td>118</td>
<td>0.46±0.036**</td>
<td>0.43±0.033**</td>
</tr>
<tr>
<td>119</td>
<td>0.40±0.036**</td>
<td>0.43±0.021**</td>
</tr>
<tr>
<td>120</td>
<td>0.15±0.022***</td>
<td>0.11±0.026***</td>
</tr>
<tr>
<td>121</td>
<td>0.25±0.036***</td>
<td>0.18±0.030***</td>
</tr>
<tr>
<td>122</td>
<td>0.33±0.05*</td>
<td>0.30±0.033*</td>
</tr>
<tr>
<td>123</td>
<td>0.36±0.033</td>
<td>0.30±0.036*</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>0.13±0.033</td>
<td>0.11±0.011</td>
</tr>
<tr>
<td>Control</td>
<td>0.61±0.047</td>
<td>0.58±0.030</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s ‘t’ test (n=6), *p<0.05, **p<0.01 & ***p<0.001 significantly different from standard; ns, not significant

Figure 2.1. Anti-inflammatory activity of biphenyl based hydrazones of 1,2,4- triazole derivatives
2.4.2.1.2.2. **Analgesic activity**

The results of analgesic activity are summarized in **table 2.3** and **figure 2.2**. The tested compounds 115 and 120 showed comparable analgesic activity (reaction time) with the standard drug Ibuprofen. All data were analyzed by one-way ANOVA test followed by Dunnett’s test.

**Table 2.3.** Analgesic activity of biphenyl based hydrazones of 1,2,4 triazole derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Tail flick latency in second.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
</tr>
<tr>
<td>115</td>
<td>1.93±0.136</td>
</tr>
<tr>
<td>120</td>
<td>2.08±0.320</td>
</tr>
<tr>
<td>121</td>
<td>2.03±0.320</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.98±0.307</td>
</tr>
<tr>
<td>Control</td>
<td>2.03±0.330</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s ‘t’ test (n=6), *p<0.05, **p<0.01 & ***p<0.001 significantly different from standard; ns, not significant

**Figure 2.2.** Analgesic activity of biphenyl based hydrazones of 1,2,4-triazole derivatives
2.4.2.3. Histopathological Study

When compared with Ibuprofen, compound 115 did not cause any gastric ulceration and disruption of gastric epithelial cells at the given doses. Hence gastric tolerance to this compound was better than that of Ibuprofen indicating that carboxylic group present in the Ibuprofen is responsible for ulceration, Bhandari et al. [52]. Stomach wall of Ibuprofen treated group at low power (10x) photomicrograph showed damage of the mucosa and the sub mucosa. Stomach wall of the same section at high power (40x) photomicrograph showed desquamated epithelial cells in the lumen whereas tested compounds 115, 120 and 121 treated animals showed significant surface epithelial damage and slightly submucosal damage however there was lesser damage in comparison to the Ibuprofen. Stomach wall of compound 115 treated animal showed no damage of any layer. The results are shown in table 2.4 and figure 2.3.

Table 2.4. Haematoxylin and Eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Surface Epithelial Damage</th>
<th>Submucosal Damage</th>
<th>Deep Mucosal Damage</th>
<th>Muscular Layer Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>115</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>120</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>121</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(++; ++; ++++) Increase ulceration; (-) no ulceration
Figure 2.3 Haematoxylin and Eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.
2.4.2.2. Experimental

2.4.2.2.1. Chemistry

All chemicals (reagent grade) used were commercially available. Melting points were measured on a VEEGO-VMP-DS melting point apparatus and are uncorrected. \(^1\)H NMR was recorded on a Bruker DPX 400, 300 instruments respectively in CDCl\(_3\)/DMSO-d\(_6\) using TMS as internal standard. \(^1\)H NMR chemical shifts (\(\delta\)) and coupling constants (\(J\)) are given in ppm and Hz respectively. Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system and operated at 70 eV and Maldi-MS (AB-4800). Mass-spectrometric (MS) data are reported in \(m/z\). Elemental analysis was carried out using Elementar Vario EL III elemental analyzer. Elemental analysis data are reported in % standard.

General Procedure for synthesis of ethyl ester of 3-mercapto-1,2,4-triazole (106, 107)

To a solution of 3-mercapt0-1,2,4-triazole (61, 63, 10 mmol each) in absolute alcohol (50 mL) was added sodium metal and ethyl bromoacetate (equimolar) and refluxed for 4-6 hr. The resulting solution was concentrated under reduced pressure and poured on crushed ice; the precipitate so obtained was filtered, washed with cold water, dried and recrystallized from alcohol.

**Ethyl2-[5{(4-biphenyloxy)methyl]-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio] acetate (106)** Yield: 78%; White Crystals, \(m.p.\) 138-140 °C; \(R_f = 0.56\) (CHCl\(_3\): MeOH; 9:1).

![Chemical Structure](image)

**IR (KBr) cm\(^{-1}\)**

: 3062, 2989, 1673, 1611, 1241, 1093.

\(^1\)H NMR (300 MHz, CDCl\(_3\))

: \(\delta\) 1.26 (t, \(J = 7.2\) Hz, 3H, CH\(_2\)-CH\(_3\)), 3.85 (s, 3H, O-CH\(_3\)), 4.08 (s, 2H, S-CH\(_2\)), 4.20 (q, \(J = 7.2\) Hz, 2H, O-CH\(_2\)), 5.09 (s, 2H, O-CH\(_2\)), 6.95-
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7.01 (m, 4H), 7.26-7.31 (m, 3H), 7.40 (t, J = 7.5 Hz, 2H), 7.50-7.53 (m, 4H, Ar-H).

Maldi-MS (m/z) : 475 (M⁺), 476 (M⁺+1)

Elemental Analysis : Calculated for molecular formula C₂₆H₂₅N₅O₄S:
Calculated : C, 65.67; H, 5.30; N, 8.84
Found : C, 65.57; H, 5.29; N 8.83%

Ethyl-2-[5[(4-biphenyloxy)methyl]-4-(4-chlorophenyl)-1,2,4-triazol-3-ylthio]acetate (107) Yield: 72%; White Crystals, m.p. 128-130⁰C; Rf = 0.55 (CHCl₃: MeOH: 9:1).

IR (KBr) cm⁻¹ : 3058, 3025, 1675, 1603, 1241, 1107.

¹H NMR (300 MHz, CDCl₃) : δ 1.26 (t, J = 7.2 Hz, 3H, CH₂-CH₃), 4.09 (s, 2H, S-CH₂), 4.20 (q, J = 7.2 Hz, 2H), 5.09 (s, 2H, O-CH₂), 6.94 (d, J = 8.7 Hz, 2H), 7.25-7.43 (m, 5H), 7.46-7.52 (m, 6H, Ar-H).

Maldi-MS (m/z) : 480 (M⁺), 482 (M⁺+2)

Elemental Analysis : Calculated for molecular formula C₂₆H₂₅N₅O₄S:
Calculated : C, 65.67; H, 5.30; N, 8.84
Found : C, 65.57; H, 5.29; N 8.83%

General procedure for synthesis of hydrazide (108, 109)
The ethanolic solution of compounds 106, 107 (10 mmol) and hydrazine monohydrate (10 mmol) was refluxed for 4-5 h. After the completion of reaction, the reaction mixture was cooled, poured on crushed ice, the precipitate so obtained was filtered, washed with cold water, dried and recrystallized from alcohol.
2-[5[(4-biphenyloxy)methyl]-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio] acetohydrazide (108) Yield: 78%; White Crystals, m.p. 141-143 °C; Rf = 0.51 (CHCl₃: MeOH; 9:1).

IR (KBr) cm⁻¹: 3503, 1683, 1603, 1507.

¹H NMR (300 MHz, CDCl₃): δ 1.62 (brs, 2H, -NH₂), 3.85 (s, 3H, O-CH₃), 4.08 (s, 2H, S-CH₂), 5.07 (s, 2H, O-CH₂), 6.95-7.01 (m, 4H), 7.25-7.32 (m, 3H), 7.40 (t, J = 7.5 Hz, 2H), 7.46-7.52 (m, 4H, Ar-H), 8.91 (s, 1H, N-H).

Maldi-MS (m/z): 461 (M⁺), 462 (M⁺+1)

Elemental Analysis: Calculated for molecular formula C₂₄H₂₃N₅O₃S
Calculated: C, 62.46; H, 5.02; N, 15.17
Found: C 62.39%; H 5.01; N 15.15%

2-[5[(4-biphenyloxy)methyl]-4-(4-methoxyphenyl)-4H-1,2,4-triazol-3-ylthio] acetohydrazide (109) Yield: 88%; White crystals, m.p. 137-139 °C; Rf = 0.53 (CHCl₃: MeOH; 9:1).

IR (KBr) cm⁻¹: 3246, 3058, 2690, 1668, 1241, 1107.

¹H NMR (300 MHz, CDCl₃): δ 2.95 (brs, 2H, -NH-NH₂), 3.94 (s, 2H, S-CH₂), 5.11 (s, 2H, O-CH₂), 6.96 (d, J = 8.4 Hz, 2H), 7.30-7.32 (m, 3H), 7.43 (t, J = 7.8 Hz, 2H), 7.49-7.54 (m, 6H, Ar-H), 8.14 (s, 1H, N-H).

Maldi-MS (m/z): 465 (M⁺), 467 (M⁺+2)
Elemental Analysis: Calculated for molecular formula C_{31}H_{26}ClN_{5}O_{2}S
Calculated: C, 59.29; H, 4.33; N, 15.03
Found: C 59.21; H 4.31; N 15.05%

General procedure for synthesis of hydrazones of 1,2,4-triazole (110-123)
To a solution of hydrazide 108, 109 (1 mmol) in absolute alcohol (50 mL) was added different aromatic aldehydes (equimolar) with few drops of glacial acetic acid and the reaction mixture was refluxed for 4-6 hr. After the completion of reaction monitored by TLC, the reaction mixture was cooled to RT and concentrated under reduced pressure, the concentrated solution was poured on crushed ice, and the precipitate so obtained was filtered off and washed with cold water, dried and crystallized in ethanol to yield the pure hydrazone 110-123.

N’-(3-Methylbenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]acetohydrazide (110) Yield: 68%; White crystals, m.p. 146-148 ºC; R_{f} = 0.50 (CHCl_{3}: MeOH; 9:1).

IR (KBr) cm⁻¹: 3254, 3058, 2912, 1608, 1241, 1107.

¹H NMR (300 MHz, CDCl₃): δ 2.29 (s, 3H, Ar-CH₃), 3.96 (s, 2H, S-CH₂), 5.04 (s, 2H, O-CH₂), 6.86 (d, J = 7.8 Hz, 2H), 7.15 (d, J = 7.5 Hz, 2H), 7.22 (t, J = 7.2 Hz, 1H), 7.33 (t, J = 7.2 Hz, 2H), 7.43-7.40 (m, 9 H), 9.41 (s, 1H, N-H), 7.56 (d, 2H, J = 7.5 Hz).

¹³C NMR (75 MHz, CDCl₃): δ 21.47, 39.35, 59.12, 115.01, 115.05, 134.92, 135.23, 136.38, 136.95, 140.28, 140.41, 140.73, 140.83, 145.52, 148.98, 154.10, 156.67, 156.85, 164.48, 169.14.

Maldi-MS (m/z): 568 (M⁺), 569 (M⁺+1)

Elemental Analysis: Calculated for molecular formula C_{31}H_{26}ClN_{5}O_{2}S
Chapter II, Section 2

Calculated: C, 65.54; H, 4.61; N, 12.33
Found: C 65.57; H 4.62; N 12.34%

*N′-(4-Chlorobenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]acetohydrazide* (111) Yield: 68%; White crystals; m.p. 187-189 ºC; Rf = 0.53 (CHCl₃: MeOH; 9:1).

![Chemical structure image]

**IR (KBr) cm⁻¹**: 3089, 2977, 1683, 1608, 1491, 1396, 1233, 1092, 835, 763.

**¹H NMR (300 MHz, CDCl₃)**: δ 4.09 (s, 2H, S-CH₂), 5.06 (s, 2H, O-CH₂), 6.87(d, J = 5.7 Hz, 2H), 7.21-7.35(m, 6H), 7.41-7.42 (m, 8H), 8.06 (d, J = 6.6 Hz, 1H), 8.26 (s, 1H, N-H), 8.52 (s, 1H).

**¹³C NMR (75 MHz, CDCl₃)**: δ 39.16, 59.64, 114.70, 126.25, 126.47, 127.77, 128.09, 129.80, 127.93, 128.34, 128.46, 129.61, 130.79, 131.89, 132.13, 134.37, 135.85, 139.91, 143.09, 146.76, 151.74, 151.39, 156.36, 156.48, 164.05, 168.59.

**Maldi-MS (m/z)**: 587 (M⁺), 588 (M⁺+1)

**Elemental Analysis**: Calculated for molecular formula C₃₀H₂₃N₅Cl₂N₉O₂S

Calculated: C, 61.23; H, 3.94; N, 11.90
Found: C 61.25; H 3.95; N 11.92%
**Chapter II, Section 2**

*N'-(3-Chlorobenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]acetohydrazide (112)** Yield: 71%; White flakes; m.p. 168-170 °C; \( R_f = 0.54 \) (CHCl\(_3\): MeOH; 9:1).

![Structure of N'-(3-Chlorobenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]acetohydrazide (112)](image)

**IR (KBr) cm\(^{-1}\)**: 3066, 2940, 1684, 1605, 1491, 1234, 1093.

**\(^1\)H NMR (300 MHz, CDCl\(_3\))**: \( \delta \) 4.02 (s, 2H, S-CH\(_2\)), 5.06 (s, 2H, O-CH\(_2\)), 6.86 (d, \( J = 7.2 \) Hz, 2H), 7.25-7.37 (m, 7H), 7.41-7.47 (m, 7H), 7.63 (d, \( J = 7.2 \) Hz, 2H), 8.11 (s, 1H, N-H).

**\(^{13}\)C NMR (100 MHz, CDCl\(_3\))**: \( \delta \) 34.28, 59.74, 115.10, 126.79, 127.00, 127.18, 128.04, 128.26, 128.29, 128.35, 128.48, 128.75, 129.66, 130.39, 131.28, 131.36, 134.42, 135.00, 135.26, 136.54, 137.00, 140.37, 140.49, 141.97, 145.12, 156.72, 156.86, 164.80, 169.28.

**Maldi-MS (m/z)**: 588 (M\(^+\)), 589 (M\(^+\)+1).

**Elemental Analysis**: Calculated for molecular formula \( \text{C}_{30}\text{H}_{23}\text{Cl}_2\text{N}_5\text{O}_2\text{S} \).

Calculated: C, 61.23; H, 3.94; N, 11.90

Found: C 61.26; H 3.95; N 11.89%.

**N'-(2-Chlorobenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]acetohydrazide (113)** Yield: 68%; White crystals; m.p. 144-146 °C; \( R_f = 0.52 \) (CHCl\(_3\): MeOH; 9:1).

![Structure of N'-(2-Chlorobenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]acetohydrazide (113)](image)
IR (KBr) cm\(^{-1}\): 3204, 3066, 2940, 1684, 1605, 1491, 1233, 1093.

\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 4.09 (s, 2H, S-CH\(_2\)), 5.06 (s, 2H, O-CH\(_2\)), 6.87 (d, \(J = 5.7\) Hz, 2H), 7.21-7.35 (m, 7H), 7.4-7.42 (m, 7H), 8.52 (s, 1H), 8.26 (s, 1H, N-H), 8.06 (d, \(J = 6.6\) Hz, 1H).

\(^13\)C NMR (100 MHz, CDCl\(_3\)): \(\delta\) 34.28, 59.74, 115.04, 115.12, 126.79, 126.99, 127.16, 128.03, 128.24, 128.29, 128.35, 128.46, 128.77, 129.68, 130.37, 131.26, 131.36, 134.41, 135.00, 135.25, 136.50, 137.00, 140.35, 140.47, 141.97, 145.24, 156.72, 156.89, 164.82, 169.20.

Maldi-MS (m/z): 587 (M\(^+\)), 588 (M\(^+\)+1).

Elemental Analysis: Calculated for molecular formula C\(_{30}\)H\(_{23}\)N\(_3\)Cl\(_2\)N\(_5\)O\(_2\)S

Calculated: C, 61.23; H, 3.94; N, 11.90

Found: C 61.25; H 3.95; N 11.92%.

**N’-(4-methoxybenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthi]acetohydrazide (114) Yield:** 78%; white crystals; m.p. 173-175 °C; \(R_f\) = 0.28 (CHCl\(_3\): MeOH; 9:1).

![Chemical Structure](image)

IR (KBr) cm\(^{-1}\): 3216, 3066, 1675, 1603, 1515, 1492, 1398, 1276, 1097, 1005, 835, 764.

\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 3.84 (s, 3H, O-CH\(_3\)), 4.08 (s, 2H, S-CH\(_2\)), 5.13 (s, 2H, O-CH\(_2\)), 6.89 (d, \(J = 8.4\) Hz, 2H), 6.96 (d, \(J = 8.4\) Hz, 2H), 7.31-7.52 (m, 5H), 7.50-7.55 (m, 6H, Ar-H), 7.86 (d, \(J = 8.4\) Hz, 2H), 8.14 (s, 1H, N-H), 11.52 (s, 1H, OH).
\( ^{13} \text{C NMR (100 MHz, CDCl}_3 \text{): } \delta 35.01, 55.37, 59.28, 114.51, 114.67, 122.64, \\
124.91, 125.21, 126.28, 127.50, 127.95, 128.16, \\
129.55, 130.66, 134.11, 135.56, 139.69, 144.69, \\
147.26, 147.33, 148.21, 148.63, 151.10, 152.54, \\
156.21, 156.31, 163.37, 168.02. \)

Maldi-MS \((m/z)\) : 584 \((M^+), 585 \((M^+)+1)\)

Elemental Analysis : Calculated for molecular formula 
\( \text{C}_{31}\text{H}_{26}\text{ClN}_5\text{O}_3\text{S} \)

Calculated : C, 63.75; H, 4.49; N, 11.49

Found : C, 63.78; H, 4.50; N, 11.51%.

\(-N'-(3,4\text{-dimethoxybenzylidene})-2-[5-\{(4\text{-phenylphenoxy)methyl}\}-4-(4\text{-chlorophenyl})
-4\text{H-1,2,4-triazol-3-thio}jacetohydrazide\) \( (115) \) Yield: 68%; White crystals; 
m.p.183-185 \(^\circ\)C; \(R_f = 0.46 \) (CHCl\(_3\); MeOH; 9:1).

IR \((\text{KBr}) \) cm\(^{-1}\) : 3216, 3054, 2936, 1684, 1603, 1515, 1492, 1377, 
1268, 1134, 1093, 1024, 835, 764, 699.

\(^1\text{H NMR (300 MHz, CDCl}_3 \text{): } \delta 3.91\text{-}3.99 \text{ (m, 6H, 2 O-CH}_3), 4.15 \text{ (s, 2H, S-CH}_2\text{),} \\
5.14 \text{ (s, 2H, O-CH}_2\text{),} 6.82\text{-}7.30 \text{ (m, 6H),} \\
7.33\text{-}7.62 \text{ (m, 11H),} 8.13 \text{ (s, 1H, N-H).} \)

\(^{13} \text{C NMR (100 MHz, CDCl}_3 \text{): } \delta 34.17, 55.91, 59.89, 108.46, 110.47, 115.05, \\
123.01, 126.46, 127.02, 128.28, 128.36, 128.48, \\
130.10, 130.39, 135.28, 137.04, 140.29, 145.59, \\
149.14, 151.92, 152.23, 152.72, 154.16, 156.86, \\
164.51, 169.01. \)

Maldi-MS \((m/z)\) : 614 \((M^+), 616 \((M^+)+1)\).

Elemental Analysis : Calculated for molecular formula 
\( \text{C}_{32}\text{H}_{28}\text{ClN}_5\text{O}_4\text{S}. \)
Calculated: C, 62.58; H, 4.60; N, 11.40
Found: C 62.56; H 4.61; N, 11.38%

\[ N'(3,5\text{-di-methoxy-4-hydroxybenzylidene})-2-[5-(4\text{-phenylphenoxy})methyl]-4-(4\text{-chlorophenyl})-4H-1,2,4-\text{triazol-3-ylthio}]acetohydrazide \] (116) Yield: 62%; White crystals; m.p. 190-192 °C; \( R_f = 0.40 \) (n-hexane : ethyl acetate; 4:6).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 3180, 3054, 2932, 1662, 1592, 1519, 1494, 1378, 1129, 1005, 836, 760. \\
^1\text{H NMR (300 MHz, CDCl}_3) & : \delta 3.89-3.84 \text{ (s, O-CH}_3, 6\text{H}), 3.90 \text{ (s, 2H, S-CH}_2\text{), 5.09 \text{ (s, 2H, O-CH}_2\text{), 6.89 \text{ (s, 1H), 7.03-6.95 \text{ (m, 5H), 7.31-7.27 \text{ (m, 4H), 7.40 \text{ (t, } J = 7.2 \text{ Hz, 2H), 7.51-7.48 \text{ (m, 4H), 8.07 \text{ (s, 1H, N-H), 11.51 \text{ (s, 1H, OH).}}}}\
^1\text{C NMR (75 MHz, CDCl}_3) & : \delta 38.56, 55.71, 59.15, 114.44, 124.10, 125.93, 126.20, 127.43, 127.78, 127.90, 128.09, 129.29, 129.45, 134.25, 135.51, 139.65, 144.77, 147.21, 152.29, 156.23, 163.09, 167.90. \\
\text{Maldi-MS (m/z)} & : 630 (M^+), 632 (M^+2) \\
\text{Elemental Analysis} & : \text{Calculated for molecular formula } C_{32}H_{28}ClN_{5}O_{5}S. \\
\text{Calculated} & : C, 61.00; H, 4.48; N, 11.11 \\
\text{Found} & : C 61.04; H 4.47; N 11.12\%.
\( N'(3\text{-ethoxy,4-hydroxybenzylidene})\text{-2-[5\text{-}(4\text{-phenylphenoxy)methyl}]\text{-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ythio}\text{acetohydrazide (117)} \) Yield: 68%; White crystals; m.p. 174-176 °C; \( R_f = 0.53 \) (CHCl\(_3\); MeOH; 9:1).

\[
\begin{align*}
&\text{IR (KBr) cm}\text{\(^{-1}\) }\quad \text{:} 3204, 3066, 1678, 1603, 1492, 1378, 1276, 1089, 834, 764. \\
&\text{\(^{1}\text{H NMR (300 MHz, CDCl}\text{\text{3}) }\quad \text{: } \delta 1.31 \text{ (t, } J = 6.9 \text{ Hz, 3H, -CH}_3\text{), 3.93 \text{ (q, } J = 6.8 \text{ Hz, 2H, O-CH}_2\text{), 4.05 \text{ (s, 2H, S-CH}_2\text{), 5.14 \text{ (s, 2H, O-CH}_2\text{), 6.80-6.88 \text{ (m, 4H, 6.94 \text{ (d, } J = 7.8 \text{ Hz, 1H), 7.23 \text{ (d, } J = 7.2 \text{ Hz, 2H), 7.31-7.43 \text{ (m, 10H, 8.00 \text{ (s, 1H, N-H), 11.37 \text{ (s, 1H, OH).}}}}}}}
\end{align*}
\]

\[
\begin{align*}
&\text{\(^{13}\text{C NMR (100 MHz, CDCl}\text{\text{3}) }\quad \text{: } \delta 14.61, 35.33, 59.67, 64.26, 114.91, 121.60, 125.55, 126.36, 126.66, 127.86, 128.29, 128.38, 128.55, 129.73, 129.85, 131.07, 134.38, 135.83, 139.97, 144.99, 146.83, 146.92, 148.84, 149.21, 151.47, 152.53, 156.64, 156.70, 163.51, 168.00. \\
&\text{Maldi-MS (m/z) }\quad \text{: 614 (M\text{\text{+}}), 615 (M\text{\text{+}}+1).} \\
&\text{Elemental Analysis }\quad \text{: } \text{Calculated for molecular formula } C_{32}H_{28}ClN_5O_4S. \\
&\text{Calculated }\quad \text{: C, 62.58; H, 4.60; N, 11.40} \\
&\text{Found }\quad \text{: C 62.59; H 4.61; N 11.41\%} \\
\end{align*}
\]

\( (E)\text{-N'}(1\text{H-indol-3-yl)methylene-2-[5\text{-}(4\text{-phenylphenoxy)methyl}]\text{-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ythio}\text{acetohydrazide (118)} \) Yield: 68%; light yellow flakes, m.p. 176-178 °C; \( R_f = 0.37 \) (CHCl\(_3\); MeOH; 9:1).

\[
\begin{align*}
&\text{\text{\( \text{(E)-N'}(1\text{H-indol-3-yl)methylene-2-[5\text{-}(4\text{-phenylphenoxy)methyl}]\text{-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ythio}\text{acetohydrazide (118)} \) Yield: 68%; light yellow flakes, m.p. 176-178 °C; \( R_f = 0.37 \) (CHCl\(_3\); MeOH; 9:1).} \\
\end{align*}
\]

98
IR (KBr) cm\(^{-1}\) : 3474, 1671, 1613, 1248, 1092.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) : \(\delta\) 4.54 (s, 2H, S-CH\(_2\)), 4.98 (s, 2H, O-CH\(_2\)), 6.82 (d, \(J = 6\) Hz, 2H), 7.20-7.05 (m, 3H), 7.40-7.29 (m, 14H), 8.00 (s, 1H, N-H), 9.88 (1H, s, OH).

\(^13\)C NMR (100 MHz, CDCl\(_3\)) : \(\delta\) 35.30, 59.90, 111.28, 111.93, 115.28, 120.61, 121.59, 122.67, 124.03, 126.26, 126.86, 127.75, 128.85, 129.08, 129.83, 130.66, 131.61, 133.57, 134.83, 137.11, 139.61, 141.45, 144.38, 151.65, 151.98, 156.91, 162.26, 167.38.

Maldi-MS (m/z) : 593 (M\(^+\)), 595 (M\(^+\)+2).

Elemental Analysis : Calculated for molecular formula C\(_{32}\)H\(_{25}\)ClN\(_6\)O\(_2\)S.

Calculated : C, 64.80; H, 4.25; N, 14.17

Found : C 64.83; H 4.26; N 14.19%.

\(N'\)-(3-nitrobenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-4H-1,2,4-triazol-3-ylthio]acetohydrazide (119) Yield: 68%; White crystals, m.p. 184-186 °C; \(R_f = 0.31\) (CHCl\(_3\); MeOH; 9:1).

IR (KBr) cm\(^{-1}\) : 3305, 1678, 1607, 1516, 1450, 1239, 1092.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) : \(\delta\) 4.06 (s, 2H, S-CH\(_2\)), 5.14 (s, 2H, O-CH\(_2\)), 6.96 (d, \(J = 8.4\) Hz, 2H), 7.32-7.56 (m, 11H), 8.01-8.08 (m, 2H), 8.14 (d, \(J = 6.9\) Hz, 1H), 8.19 (t, \(J = 7.5\) Hz, 1H), 8.31 (s, 1H, N-H), 8.49 (s, 1H, Ar-H).

\(^13\)C NMR (100 MHz, CDCl\(_3\)) : \(\delta\) 34.94, 59.47, 114.71, 121.50, 123.80, 126.25, 126.52, 127.79, 127.94, 128.11, 128.35, 129.38, 129.64, 129.82, 130.80, 132.04, 134.44, 135.50,
Maldi-MS (m/z) : 599 (M^+), 600 (M^+1)
Elemental Analysis : Calculated for molecular formula C_{30}H_{23}ClN_{6}O_{4}S.

Calculated : C, 60.15; H, 3.87; N, 14.03
Found : C 60.17; H 3.88; N 14.04%.

N’-(2-Nitrobenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-chlorophenyl)-1H-1,2,4-triazol-3-ylthio]acetohydrazide (120) Yield: 68%; White crystals, m.p. 181-183 Â°C; R_f = 0.37 (CHCl_3: MeOH; 9:1).

IR (KBr) cm^{-1} : 3058, 2932, 1682, 1607, 1522, 1492, 1378, 1352, 1235, 1093.

^1H NMR (300 MHz, CDCl_3) : δ 4.06 (s, 2H, S-CH_2), 5.14 (s, 2H, O-CH_2), 6.96 (d, J = 8.4 Hz, 2H), 7.32-7.56 (m, 12H), 8.01 (d, J = 6.8 Hz, 1H), 8.14 (d, J = 6.9 Hz, 1H), 8.19 (t, J = 7.5 Hz, 1H), 8.31 (s, 1H, N-H), 8.49 (s, 1H, Ar-H).

^13C NMR (100 MHz, CDCl_3) : δ 34.36, 59.87, 115.03, 124.63, 126.73, 126.90, 128.25, 128.29, 128.46, 128.71, 129.41, 129.82, 130.10, 130.37, 133.49, 134.96, 135.14, 136.95, 140.33, 140.66, 144.17, 148.05, 151.95, 152.30, 153.82, 156.70, 164.93, 169.35.

ES-MS (m/z) : 599 (M^+), 601(M^+2).
Elemental Analysis : Calculated for molecular formula C_{30}H_{23}ClN_{6}O_{4}S.

Calculated : C, 60.15; H, 3.87; N, 14.03
Found : C 60.19; H 3.85; N 14.04%.
Chapter II, Section 2

\[N'(3,4,5\text{-trimethoxybenzylidene})-2-[5-\{(4\text{-phenylphenoxy})methyl\}-4-(4\text{-chlorophenyl})-4H-1,2,4\text{-triazol-3-ylthio}]\text{acetohydrazide} (121) \text{ Yield: 75\%; White crystals, m.p. 180-182}^\circ \text{C; } R_f = 0.44 \text{ (n-hexane : ethylacetate; 4:6).} \]

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 3054, 2932, 1662, 1519, 1494, 1378, 1239, 1129, 1005, 836. \\
^1\text{H NMR (300 MHz, CDCl}_3) & : \delta 3.93 \text{ (s, 6H, O-CH}_3\text{)}, 3.99 \text{ (s, 3H, O-CH}_3\text{)}, 4.09 \text{ (s, 2H, S-CH}_2\text{)}, 5.14 \text{ (s, 2H, O-CH}_2\text{)}, 6.95 \text{ (d, J = 7.2 Hz, 2H)}, 7.02 \text{ (s, 2H)}, 7.17 \text{ (s, 1H)}, 7.35 \text{ (d, J = 7.2 Hz, 1H)}, 7.42 \text{ (t, J = 7.5 Hz, 2H)}, 7.57-7.50 \text{ (m, 8H), 8.13 \text{ (s, 1H, N-H).}} \\
^{13}\text{C NMR (100 MHz, CDCl}_3) & : \delta 38.58, 55.76, 59.21, 108.12, 114.48, 124.16, 125.93, 126.20, 127.43, 127.68, 127.90, 128.13, 129.39, 128.84, 134.23, 135.54, 139.61, 144.74, 146.81, 152.23, 156.20, 163.06, 167.94.
\end{align*}
\]

Maldi-MS \((m/z)\) : 644 (M\(^{+}\)), 645 (M\(^{+}\)+1).

**Elemental Analysis** : Calculated for molecular formula \(C_{33}H_{30}N_3ClN_5O_5S\).
Calculated : C, 61.53; H, 4.69; N, 10.87
Found : C 61.57; H 4.68; N 10.86%.

\[(E)-N'(4\text{-nitrobenzylidene})-2-[5-\{(4\text{-phenylphenoxy})methyl\}-4-(4\text{-methoxyphenyl})-4H-1,2,4\text{-triazol-3-ylthio}]\text{acetohydrazide} (122) \text{ Yield: 80\%; light yellow flakes, m.p. 168-170}^\circ \text{C; } R_f = 0.32 \text{ (CHCl}_3: \text{MeOH; 9:1).} \]

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 3054, 2932, 1662, 1519, 1494, 1378, 1239, 1129, 1005, 836. \\
^1\text{H NMR (300 MHz, CDCl}_3) & : \delta 3.93 \text{ (s, 6H, O-CH}_3\text{)}, 3.99 \text{ (s, 3H, O-CH}_3\text{)}, 4.09 \text{ (s, 2H, S-CH}_2\text{)}, 5.14 \text{ (s, 2H, O-CH}_2\text{)}, 6.95 \text{ (d, J = 7.2 Hz, 2H)}, 7.02 \text{ (s, 2H)}, 7.17 \text{ (s, 1H)}, 7.35 \text{ (d, J = 7.2 Hz, 1H)}, 7.42 \text{ (t, J = 7.5 Hz, 2H)}, 7.57-7.50 \text{ (m, 8H), 8.13 \text{ (s, 1H, N-H).}} \\
^{13}\text{C NMR (100 MHz, CDCl}_3) & : \delta 38.58, 55.76, 59.21, 108.12, 114.48, 124.16, 125.93, 126.20, 127.43, 127.68, 127.90, 128.13, 129.39, 128.84, 134.23, 135.54, 139.61, 144.74, 146.81, 152.23, 156.20, 163.06, 167.94.
\end{align*}
\]

Maldi-MS \((m/z)\) : 644 (M\(^{+}\)), 645 (M\(^{+}\)+1).

**Elemental Analysis** : Calculated for molecular formula \(C_{33}H_{30}N_3ClN_5O_5S\).
Calculated : C, 61.53; H, 4.69; N, 10.87
Found : C 61.57; H 4.68; N 10.86%.

\[(E)-N'(4\text{-nitrobenzylidene})-2-[5-\{(4\text{-phenylphenoxy})methyl\}-4-(4\text{-methoxyphenyl})-4H-1,2,4\text{-triazol-3-ylthio}]\text{acetohydrazide} (122) \text{ Yield: 80\%; light yellow flakes, m.p. 168-170}^\circ \text{C; } R_f = 0.32 \text{ (CHCl}_3: \text{MeOH; 9:1).} \]
IR (KBr) cm\(^{-1}\): 3056, 1679, 1603, 1454, 1252, 1105.

\(^1\)H NMR (300 MHz, CDCl\(_3\)): \(\delta\) 3.91 (s, 3H, O-CH\(_3\)), 4.62 (s, 2H, S-CH\(_2\)), 5.08 (s, 2H, O-CH\(_2\)), 6.96 (d, \(J = 7.2\) Hz, 2H), 6.99 (d, \(J = 7.2\) Hz, 2H), 7.38-7.6 (m, 5H), 7.43 (t, \(J = 6.9\) Hz, 2H), 7.51-7.55 (m, 4H), 7.68 (d, \(J = 7.5\) Hz, 2H), 7.81 (s, 1H, NH), 8.10 (s, 1H, CH).

\(^13\)C NMR (75 MHz, CDCl\(_3\)): \(\delta\) 36.56, 56.54, 59.86, 114.70, 115.12, 126.25, 126.47, 127.77, 128.09, 129.80, 127.93, 128.34, 128.46, 129.61, 130.79, 131.89, 132.13, 134.37. (E)-N\(^{\prime}\)-(4-Chlorobenzylidene)-2-[5-[(4-phenylphenoxy)methyl]-4-(4-ethoxyphenyl)-4H-1,2,4-triazol-3-ylthio]acetohydrazide (123)

Yield: 78%; White crystals, m.p. 166-168 ºC; R\(_f\) = 0.51 (CHCl\(_3\); MeOH; 9:1).
135.85, 139.91, 143.09, 146.76, 151.74, 151.39, 156.36, 156.48, 164.05, 168.50.

**Maldi-MS (m/z)**

- 584 (M$^+$), 585 (M$^+$+1).

**Elemental Analysis**

- Calculated for molecular formula $C_{31}H_{26}ClN_5O_3S$.
- Calculated: C, 63.75; H, 4.49; N, 11.99
- Found: C 63.78; H 4.50; N 11.91%

### 2.4.2.2.2. Biological activity

#### 2.4.2.2.2.1. Anti-inflammatory activity

All the targeted synthesized compounds were evaluated for their anti-inflammatory activity against carrageenan-induced paw oedema in Albino Wistar rats weighing 150-200 g. Same method was used as discussed in section 1 of this chapter. The results of anti-inflammatory activity are presented in table 2.2 and figure 2.1.

#### 2.4.2.2.2.2. Analgesic activity

The same method was used as given in section 1 of this chapter. The results are presented in table 2.3 and figure 2.2.

#### 2.4.2.2.2.3. Histopathological Study

For the histopathological study, same methods was used as given in section 1 of this chapter, the results are presented in table 2.4 and figure 2.3.
2.4.2.3. Conclusion

From the above results, it was concluded that two compounds 115 and 120 out of fourteen compounds from this series were found to possess better anti-inflammatory activity (83.76, 75.64 % and 82.84, 81.13 % inhibition) compared to the standard drug Ibuprofen (78.89 and 80.10 % ) at 3 h and 5 h respectively. Compounds 115 and 120 showed comparable analgesic activity {(3.86, 3.88 and 2.85 sec.)} and {(3.80, 3.22 and 2.94 sec.)} with standard drug Ibuprofen (3.71, 3.95 and 2.76 sec.) at 30, 60 and 120 min. respectively. Compound 115 was found to be more potent causing no ulceration than the standard drug Ibuprofen which showed ulceration.
2.4.3.0. Introduction

As discussed in introductory part of this chapter, manniach bases, biphenyl and triazole derivatives exhibit different biological activities. In view of this manniach bases of 1,2,4-triazoles and biphenyl systems, a focused library of conjugates have been synthesized and evaluated for their anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities. The synthetic route is given below.

**SCHEME III**

![Scheme III](image)

The above synthesized 3-mercapto-1,2,4-triazoles (63, 65, 68) were treated with formaldehyde and secondary amines under manniach reaction conditions to form the novel manniach bases (124-128). Various secondary amines including morpholine, pyrrolidine, N-methylpiperazine were used to check the generality of the reaction and also to bring the functional group variations. All the synthesized compounds have been completely characterized on the basis of their detailed spectral data and the entire novel compounds of this series were evaluated for their biological activities. The physical data are given in table 3.1.
**Table 3.1** Physical data of 3-mercapto 1,2,4-triazole derived mannnich bases

<table>
<thead>
<tr>
<th>Compound no.</th>
<th>Structure</th>
<th>% Yield</th>
<th>m.p (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>124</td>
<td><img src="image1" alt="Structure" /></td>
<td>58</td>
<td>180-182</td>
</tr>
<tr>
<td>125</td>
<td><img src="image2" alt="Structure" /></td>
<td>54</td>
<td>184-186</td>
</tr>
<tr>
<td>126</td>
<td><img src="image3" alt="Structure" /></td>
<td>80</td>
<td>174-176</td>
</tr>
<tr>
<td>127</td>
<td><img src="image4" alt="Structure" /></td>
<td>64</td>
<td>132-134</td>
</tr>
<tr>
<td>128</td>
<td><img src="image5" alt="Structure" /></td>
<td>52</td>
<td>126-128</td>
</tr>
</tbody>
</table>

### 2.4.3.1. Results and Discussion

#### 2.4.3.1. Analytical

Five novel compounds 124-128 were synthesized starting from triazole 63, 65 and 68 as outlined in scheme-III. Reaction of triazole 63, 65 and 68 with formaldehyde and secondary amines in absolute alcohol afforded mannnich bases (124-128). The 1,2,4-triazole ring exists in two tautomeric forms, under the mannnich reaction conditions, the -NH proton of the triazole ring will participate in the reaction and will form the final mannnich bases (124-128). Formation of mannnich bases was confirmed from $^1H$
NMR spectrum exhibiting a singlet at δ 4.47-4.93 ppm due to methylene proton (N-CH$_2$-N) integrating for two protons. The $^{13}$C NMR spectrum of compounds 124-128 displayed peaks in the range δ 68.76-68.82 for the carbon bonded with two nitrogen (N-CH$_2$-N) and the peaks in the range of δ 170-172 ppm for the carbon attached with sulphur (C=S) in the triazole ring and the spectral data are in line with Barbuceanu et al. [9] which confirms the formation of the compounds 124-128. Finally the structure was confirmed from mass spectrum. All the newly synthesized compounds 124-128 were screened for their biological activities (anti-inflammatory, analgesic, ulcerogenic and antimicrobial).

2.4.3.1.2. Biological Activity

2.4.3.1.2.1. Anti-inflammatory activity

The results of anti-inflammatory activity are summarized in table 3.2 and figure 3.1. Among five compounds from this series, two compounds 125 and 128 were found to be active and exhibited significant activity (54.54, 79.69 % and 60.06, 84.08 % inhibition) compared to the standard drug Ibuprofen (78.93 and 82.58 %) at 3 h and 5 h respectively. Compound 128 was found to be more potent than the standard drug Ibuprofen. All data were analyzed by one-way ANOVA test followed by Dunnett’s test in carrageenan induced rat paw oedema.

Table 3.2. Anti-inflammatory activity of 3-mercapto 1,2,4-triazole derived manich bases

<table>
<thead>
<tr>
<th>Compds.</th>
<th>Change in paw oedema volume (mL)</th>
<th>Anti-inflammatory activity % Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After drug treatment</td>
<td>3h</td>
</tr>
<tr>
<td>124</td>
<td></td>
<td>0.387±0.043**</td>
</tr>
<tr>
<td>125</td>
<td></td>
<td>0.300±0.066***</td>
</tr>
<tr>
<td>126</td>
<td></td>
<td>0.410±0.072***</td>
</tr>
<tr>
<td>127</td>
<td></td>
<td>0.566±0.042***</td>
</tr>
<tr>
<td>128</td>
<td></td>
<td>0.134±0.055***</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td></td>
<td>0.139±0.021***</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>0.660±0.042</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s t test (n=6), *p<0.05, **p<0.01 & ***p<0.001 significantly different from standard; ns, not significant.
2.4.3.1.2.2. **Analgesic Activity**

The results of analgesic activity are summarized in **Table 3.3** and **Figure 3.2**. Both the tested compounds, **125** and **128** showed comparable analgesic activity (reaction time) with the standard drug (Ibuprofen). All data were analyzed by one-way ANOVA test followed by Dunnett’s test.

**Table 3.3** Analgesic activity of 3-mercapto 1,2,4-triazole derived mannich bases

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mean value of Tail Flick Latency (sec)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min.</td>
</tr>
<tr>
<td>125</td>
<td>2.08±0.32</td>
</tr>
<tr>
<td>128</td>
<td>1.93±0.303</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.98±0.307</td>
</tr>
<tr>
<td>Control</td>
<td>2.03±0.330</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s ‘t’ test (n=6). *p<0.05, **p<0.01 & ***p<0.001 significantly different from standard; ns, not significant.
2.4.3.1.2.3. Histopathological Study

Histopathological results revealed that compounds 125 and 128 did not cause any gastric ulceration and disruption of gastric epithelial cells at the given oral doses when compared to standard Ibuprofen. Hence gastric tolerance of these compounds was better than that of Ibuprofen indicating that carboxylic group present in Ibuprofen is responsible for ulceration. Stomach wall of Ibuprofen treated group at low power (10x) photomicrograph showed damage of the mucosa and the sub mucosa. Stomach wall of the same section at high power (40x) photomicrograph showed desquamated epithelial cells in the lumen. The results are shown in table 3.4 and figure 3.3.

Table 3.4. Haematoxylin and Eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.

<table>
<thead>
<tr>
<th>Compds.</th>
<th>Surface Epithelial Damage</th>
<th>Submucosal Damage</th>
<th>Deep Mucosal Damage</th>
<th>Muscular Layer Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>128</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

- indicates no ulceration; +, ++, +++ indicates increased ulceration.
Figure 3.3 Haematoxylin and Eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.
2.4.3.1.2.4. **Antimicrobial Activity**

All the newly synthesized compounds from this series 124-128 were tested against various microbial strains at the concentration of 200 µg/disc, 100 µg/disc for their antimicrobial activity. The results are summarized in **table 3.5**. Some of the compounds from this series showed moderate to good activity against bacterial stains. Compounds 124, 125 and 128 showed good activity against *S. aureus*.

**Table 3.5.** Antimicrobial activity of 3-mercapto 1,2,4-triazole derived mannich bases

<table>
<thead>
<tr>
<th>Compds</th>
<th>Antifungal Activity (zone of inhibition in mm)</th>
<th>Antibacterial Activity (zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. flavus</em> 200 (100) µg/disc</td>
<td><em>A. niger</em> 200 (100) µg/disc</td>
</tr>
<tr>
<td>124</td>
<td>8±0.6 (6±0.4)</td>
<td>8±0.6 (-)</td>
</tr>
<tr>
<td>125</td>
<td>6±0.6 (-)</td>
<td>8±0.7 (8±0.6)</td>
</tr>
<tr>
<td>126</td>
<td>6±0.4 (-)</td>
<td>8±0.8 (-)</td>
</tr>
<tr>
<td>127</td>
<td>12±1.6 (6±0.4)</td>
<td>10±1.6 (8±0.5)</td>
</tr>
<tr>
<td>128</td>
<td>10±1.2 (8±0.6)</td>
<td>11±1 (6±0.3)</td>
</tr>
<tr>
<td>Flucon.</td>
<td>22±1.8 (12±0.9)</td>
<td>16±1 (10±0.6)</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amoxicil.</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

Flucon: Fluconazole; Amoxicil.: Amoxicillin; NT: not tested; -: no zone of inhibition.
2.4.3.2. Experimental

2.4.3.2.1. Chemistry

*General procedure for synthesis of 4-(substituted phenyl)-3-(amine-4-ylmethylthio)-5-(4-biphenyloxy methyl)-4H-1,2,4-triazole (124-128)*

To 50 mL absolute alcohol was added appropriate triazole 63, 65 and 68 (5 mmol) formaldehyde (5 mmol) and appropriate amine (5 mmol), the reaction mixture was stirred for 24 h, and allowed to stand over night in refrigerator. The precipitate so obtained was filtered, washed with cold alcohol and recrystallized from alcohol.

5-[(4-Biphenyloxy)methyl]-4-(4-chlorophenyl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (124) **Yield:** 58%; White crystals, m.p. 180-182 ºC; **R**

\[\text{IR (KBr) cm}^{-1}: 2909, 1608, 1381, 1115.\]

\[\text{\textsuperscript{1}H NMR (300 MHz, CDCl}_3): \delta 2.86 [t, J = 4.5 Hz, 4H, N-(CH}_2)_2] 3.72 [(t, J = 3.9 Hz, 4H, O-(CH}_2)_2], 4.93 (s, 2H, N-CH}_2-N), 5.18 (s, 2H, O-CH}_2), 6.89 (d, J = 8.7 Hz, 2H), 7.29-7.86 (m, 11H).\]

\[\text{\textsuperscript{13}C NMR (100 MHz, CDCl}_3): \delta 50.74, 59.99, 66.81, 69.86, 115.11, 126.76, 127.03, 128.34, 129.26, 129.95, 132.08, 135.51, 136.29, 140.22, 146.12, 156.41, 170.72.\]

\[\text{ES-MS (m/z): 493 (M}^+)\]

**Elemental Analysis:** Calculated for molecular formula C\textsubscript{26}H\textsubscript{25}N\textsubscript{4}O\textsubscript{2}S. Calculated: C, 63.34; H, 5.11; N, 11.36. Found: C, 63.52; H, 5.18; N, 11.41%.
5-[(4-Biphenyloxy)methyl]-(4-methoxyphenyl)-3-(N-methyl-piperazine-4-ylmethylthio)-4H-1,2,4-triazole (125) Yield: 54%; White crystals, m.p. 184-186 °C; \( R_f = 0.59 \) (CHCl₃: MeOH; 9:1).

IR (KBr) cm⁻¹: 2907, 1557, 1326, 1109.

\(^1\)H NMR (200 MHz, CDCl₃): δ 2.25 (s, 3H, N-CH₃), 2.51 (t, \( J = 9 \) Hz, 4H), 2.71 (t, \( J = 7.8 \) Hz, 4H), 3.81 (s, 3H, O-CH₃), 4.49 (s, 2H, N-CH₂-N), 5.07 (s, 2H O-CH₂), 6.96-7.11 (m, 4H), 7.16-7.60 (m, 9H).

\(^13\)C NMR (100 MHz, CDCl₃): δ 44.14, 49.26, 56.24, 57.15, 68.45, 68.812, 15.45, 126.92, 127.62, 127.89, 128.62, 129.61, 129.64, 130.29, 135.69, 149.66, 156.68, 155.64, 163.49, 171.82.

Maldi-MS (m/z): 501 (M⁺), 502 (M⁺+1).

Elemental Analysis: Calculated for molecular formula C₂₈H₃₁N₅O₂S.
Calculated: C, 67.04; H, 6.23; N, 13.96.
Found: C, 67.01; H, 6.21; N, 13.93%.

5-[(4-Biphenyloxy)methyl]-4-(4-methoxyphenyl)-2-[(pyrrolidin-1-yl)methyl]-2H-1,2,4-triazole-3(4H)-thione (126) Yield: 80%; White crystals, m.p. 174-176°C; \( R_f = 0.50 \) (CHCl₃: MeOH; 9:1).

IR (KBr) cm⁻¹: 2907, 1607, 1516, 1316, 1104.
\(^{1}\)H NMR (200 MHz, CDCl\(_3\)) : \(\delta 1.53 (t, J = 7.5 \text{ Hz}, 4\text{H}, \text{CH}_2-\text{CH}_2), 2.24 (t, J = 8.0 \text{ Hz}, 4\text{H}, \text{N-CH}_2), 3.82 (s, 3\text{H}, \text{O-CH}_3), 4.47 (s, 2\text{H}, \text{N-CH}_2-\text{N}), 5.22 (s, 2\text{H}, \text{O-CH}_2), 7.54 - 6.91 (m, 13\text{H}, \text{Ar-H}).\)

\(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)) : \(\delta 26.41, 52.42, 56.24, 68.45, 68.76, 15.42, 126.75, 126.89, 127.79, 128.69, 128.89, 129.58, 129.67, 130.27, 133.89, 135.65, 149.68, 155.76, 163.54, 172.86.\)

Maldi-MS (m/z) : 472 (M\(^+\)), 473 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula C\(_{27}\)H\(_{28}\)N\(_4\)O\(_2\)S.

Calculated : C, 68.62; H, 5.97; N, 11.85.

Found : C, 68.68; H, 5.99; N, 11.87%.

### 5-[(4-Biphenyloxy)methyl]-4-(phenyl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (127)

Yield: 64 %; White crystals, m.p. 132-134 °C; \(R_f = 0.53\) (CHCl\(_3\); MeOH; 9:1).

![Chemical Structure](image)

IR (KBr) cm\(^{-1}\) : 2912, 1607, 1324, 1103.

\(^{1}\)H NMR (200 MHz, DMSO-d\(_6\)) : \(\delta 2.90 (t, J = 7.0 \text{ Hz}, 4\text{H}), 4.76 (t, J = 6.7 \text{ Hz}, 4\text{H}), 4.97 (s, 2\text{H}, \text{N-CH}_2-\text{N}), 5.22 (s, 2\text{H}, \text{O-CH}_2), 6.93 (d, J = 8.8 \text{ Hz}, 2\text{H}), 7.40-7.54 (m,12\text{H}).\)

\(^{13}\)C NMR (100 MHz, DMSO-d\(_6\)) : \(\delta 49.23, 64.86, 68.45, 68.76, 115.42, 126.89, 127.79, 128.69, 128.89, 129.58, 129.67, 130.27, 133.89, 135.65, 149.68, 155.76, 163.49, 170.56.\)

Maldi-MS (m/z) : 458 (M\(^+\)), 459(M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula C\(_{26}\)H\(_{26}\)N\(_4\)O\(_2\)S.

Calculated : C, 68.10; H, 5.71; N, 12.22.

Found : C, 68.07; H, 5.69; N, 12.20%.
5-[(4-Biphenyloxy)methyl]-4-(2-methylphenyl)-2-(morpholinomethyl)-2H-1,2,4-triazole-3(4H)-thione (128) Yield: 52%; White crystals, m.p. 126-128 °C; \( R_f = 0.51 \) (CHCl₃: MeOH: 9:1).

![Chemical structure](image)

**IR (KBr) cm⁻¹**

\[
\begin{align*}
&: 2917, 1516, 1324, 1108. \\
\end{align*}
\]

**¹H NMR (300 MHz, CDCl₃)**

\[
\begin{align*}
&: \delta 2.50 \text{ (s, 3H, Ar-CH₃)}, 2.72 \text{ (t, } J = 6.9 \text{ Hz, 4H),} \\
&: 3.57 \text{ (t, } J = 6.7 \text{ Hz, 4H),} 4.93 \text{ (s, 2H, N-CH₂-N),} \\
&: 5.23 \text{ (s, 2H, O-CH₂),} 6.90 \text{ (d, } J = 8.7 \text{ Hz, 2H),} \\
&: 7.30-7.58 \text{ (m, 11H).} \\
\end{align*}
\]

**¹³C NMR (100 MHz, DMSO-d₆)**

\[
\begin{align*}
&: \delta 14.42, 49.24, 64.87, 68.56, 68.79, 115.43, \\
&: 26.88, 126.97, 126.98, 128.81, 128.86, 129.57, \\
&: 129.63, 130.26, 134.43, 135.66, 136.84, 149.66, \\
&: 155.86, 163.51, 170.58. \\
\end{align*}
\]

**Maldi-MS (m/z)**

\[
\begin{align*}
&: 472 \text{ (M⁺),} 473 \text{(M⁺+1)} \\
\end{align*}
\]

**Elemental Analysis**

Calculated: C, 68.62; H, 5.97; N, 11.85

Found: C, 68.65; H, 5.95; N, 11.83%.

2.4.3.2.2. **Biological activity**

2.4.3.2.2.1. **Anti-inflammatory activity**

All the synthesized compounds were evaluated for their anti-inflammatory activity against carrageenan-induced rat paw oedema in albino Wistar rats weighing 150-200 g. Same method was used as discussed in section 1 of this chapter. The results of anti-inflammatory activity are presented in table 3.2 and figure 3.1.

2.4.3.2.2.2. **Analgesic activity**

The analgesic activity was determined using tail flick method in Albino Wistar rats weighing 150-200 g. The same method was used as given in section-1 of this Chapter. The results are presented in table 3.3 and figure 3.2.
2.4.3.2.3. Histopathological study

For the histopathological study, same method was used as given in section 1 of this chapter. The results are presented in table 3.4 and figure 3.3.

2.4.3.2.4. Antimicrobial activity

All the synthesized compounds were evaluated for their antimicrobial activity against the various bacterial and fungal strains. Same methods were used as given in section 1 of this chapter. The results are presented in table 3.5.
2.4.3.3. Conclusion

The designed molecules were successfully synthesized using manich reaction. All the novel manich bases synthesized were tested for their anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities. From the results, it was concluded that two compounds 125 and 128 showed good anti-inflammatory activity (54.54, 79.69 at 3h and 60.00, 84.08 % at 5 h respectively) compared to the standard drug ibuprofen. Among the tested compounds, compound 128 showed moderate analgesic activity without any gastric ulceration whereas the standard drug ibuprofen caused ulceration. From antimicrobial studies it was concluded that the compounds from this series showed moderate activity against the tested bacterial strains.
2.4.4.0. Introduction

In view of the biological importance 1,2,4-triazoles and quinoline as discussed in introductory part of this chapter, we intended to conjugate two moieties in order to develop novel antiinflammatory and antimicrobial agents. A focused library of conjugates have been synthesized and evaluated for their anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities. The synthetic route is given below.

**SCHEME-IV**

Reaction of 8-hydroxyquinoline (129) with ethyl chloroacetate in presence of potassium carbonate in anhydrous acetone afforded Ethyl 2-(quinolin-8-yloxy)acetate (130) which readily yielded 2-(quinolin-8-yloxy)acetohydrazide (131) by refluxing with hydrazine monohydrate in absolute alcohol. Compound 131 was converted into corresponding thiosemicarbazide (132-135) by reacting with different substituted aryl isothiocyanate in absolute alcohol. The thiosemicarbazides (132-135) were cyclised.
into respective 4-substituted phenyl-5-[(quinolin-8-yloxy)methyl]-4H-1,2,4-triazole-3-thiol (136-139) using triethyl amine in absolute alcohol. Finally S-alkylation of 3-mercaptop-1,2,4-triazoles with substituted phenacyl bromides leads to the formation of target molecules 140-149. All the synthesized compounds have been completely characterized on the basis of their detailed spectral data and the entire novel compounds of this series were evaluated for their biological activities. The physical data of all the final compounds are given in Table 4.1.

**Table 4.1** Physical data of quinoline based 1,2,4-triazole derivatives.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Structure</th>
<th>% Yield</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td><img src="image140" alt="Structure 140" /></td>
<td>54</td>
<td>170-172</td>
</tr>
<tr>
<td>141</td>
<td><img src="image141" alt="Structure 141" /></td>
<td>62</td>
<td>228-230</td>
</tr>
<tr>
<td>142</td>
<td><img src="image142" alt="Structure 142" /></td>
<td>72</td>
<td>218-220</td>
</tr>
<tr>
<td>143</td>
<td><img src="image143" alt="Structure 143" /></td>
<td>63</td>
<td>188-190</td>
</tr>
<tr>
<td>144</td>
<td><img src="image144" alt="Structure 144" /></td>
<td>72</td>
<td>180-182</td>
</tr>
<tr>
<td>145</td>
<td><img src="image145" alt="Structure 145" /></td>
<td>68</td>
<td>168-170</td>
</tr>
<tr>
<td>146</td>
<td><img src="image146" alt="Structure 146" /></td>
<td>49</td>
<td>172-174</td>
</tr>
<tr>
<td>147</td>
<td><img src="image147" alt="Structure 147" /></td>
<td>62</td>
<td>160-162</td>
</tr>
</tbody>
</table>
2.4.4.1. Results and discussion

2.4.4.1. Analytical

A focused library of new compounds 140-149 was synthesized starting from 8-hydroxy quinoline as outlined in scheme-IV. O-alkylation of 8-hydroxy quinoline with α-chloro ethylacetate was confirmed by the $^1$H NMR spectrum. The appearance of two signals at δ 1.52 (t, $J = 5.6$ Hz, 3H) and 3.88 (q, $J = 5.8$ Hz, 2H) supported the presence of ethyl group and appearance of strong absorption at 1678 cm$^{-1}$ in IR spectrum confirms the formation of ethyl 2-(quinolin-8-yloxy)acetate (130). Compound 130 readily yielded 2-(quinolin-8-yloxy)acetohydrazide (131) by its reaction with hydrazine monohydrate. The formation of this hydrazide was confirmed by the presence of signals due to NH-NH$_2$ group at δ 4.09 (br, s, NH$_2$, 2H) and 9.71 (s, N-H, 1H) and absence of signals due to ethyl group. Compound 131 was then converted into corresponding thiosemicarbazides (132-135) which was confirmed by the presence of extra signals in aromatic region due to phenyl ring of isothiocyanate along with signals at δ 8.44-8.62 (CSNH), 9.08-9.21 (CONH) and 8.02-8.08 ppm (Ar-NH-) in the $^1$H NMR spectrum. It is interesting to note that thiocarbonyl compounds are present in their thionethiol tautomeric forms in solution as indicated by their IR and $^1$H NMR spectra [56]. These tautomeric forms are also present in dimethyl sulfoxide as suggested by NMR spectral data. It is already reported that the acid (HCl, H$_2$SO$_4$) mediated cyclization of thiosemicarbazides leads to the formation of 1,3,4-thiadiazoles [57]. Cyclization of compounds 132-135 to 136-139 was confirmed from the IR and NMR spectral data. Absorption bands in the region 1601-1605 cm$^{-1}$ (C=N of triazole ring stretching) and the presence of signal as singlet corresponding to thiol
proton (-SH) in the range of δ 14.10-14.18 ppm in $^1$H NMR spectrum confirmed the formation of 3-mercapto-1,2,4-triazole.

Formation of alkylated derivatives 140-149 from the cyclized compound 136-139 were confirmed from the disappearance of –SH signal. Instead, new signals that originated from methylene (4.88-5.02, -S-CH$_2$) and carbonyl group (190-193, C=O) were observed in the $^1$H and 13C NMR spectra of compounds 140-149. The -SH proton is acidic enough and some substitution reaction could be achieved on this group in the presence of a base [16, 34]. Finally the structures were confirmed from mass spectrum.

All the newly synthesized compounds 140-149 were screened for their biological activities (anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities). These novel triazole derivatives have shown varied therapeutic efficacy in vitro and in vivo.

### 2.4.4.1.2. Biological Activity

#### 2.4.4.1.2.1. Anti inflammatory activity

Compounds 142, 143 and 149 from S-substituted phenacyl 1,2,4-triazole exhibited significant anti-inflammatory activity with 68.40, 63.34, 68.40 % and 72.33, 71.87, 75.00 % inhibition at the end of 3 h and 5 h respectively compared to standard drug Indomethacin (55.29 and 69.50 %). The active compounds have been further investigated for their analgesic activity and ulcerogenic studies. Among the above synthesized library of compounds, compound 149 (68.40 and 75.00 % inhibition at 3 h and 5 h respectively) showed highest inhibition in rat paw oedema. The results are given in table 4.2 and figure 4.1.
Table 4.2. Anti-inflammatory activity of quinoline based 1,2,4-triazole derivatives.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Change in paw oedema volume (mL)</th>
<th>Anti-inflammatory activity % Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3h</td>
<td>5h</td>
</tr>
<tr>
<td>140</td>
<td>0.533±0.03***</td>
<td>0.433±0.07***</td>
</tr>
<tr>
<td>141</td>
<td>0.316±0.04**</td>
<td>0.283±0.06**</td>
</tr>
<tr>
<td>142</td>
<td>0.200±0.16***</td>
<td>0.166±0.04***</td>
</tr>
<tr>
<td>143</td>
<td>0.232±0.18***</td>
<td>0.178±0.08***</td>
</tr>
<tr>
<td>144</td>
<td>0.416±0.07***</td>
<td>0.400±0.07***</td>
</tr>
<tr>
<td>145</td>
<td>0.283±0.04**</td>
<td>0.200±0.03**</td>
</tr>
<tr>
<td>146</td>
<td>0.616±0.08***</td>
<td>0.466±0.12***</td>
</tr>
<tr>
<td>147</td>
<td>0.633±0.08***</td>
<td>0.483±0.08***</td>
</tr>
<tr>
<td>148</td>
<td>0.533±0.10***</td>
<td>0.466±0.09***</td>
</tr>
<tr>
<td>149</td>
<td>0.200±0.05***</td>
<td>0.150±0.03***</td>
</tr>
<tr>
<td>Control</td>
<td>0.633±0.05</td>
<td>0.600±0.03</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.283±0.03**</td>
<td>0.183±0.30***</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s ‘t’ test (n=6), *p<0.05, **p<0.01 & ***p<0.001 significantly different from standard; ns, not significant.

Figure 4.1. Anti-inflammatory activity of quinoline based 1,2,4-triazole derivatives.
2.4.4.1.2.2. *Analgesic activity*

The results of this series of compounds, viz. S-substituted phenacyl 1,2,4-triazoles (140-149) conclude that none of the compounds is superior than the standard drug Indomethacin but all the compounds showed moderate to comparable activity. Among the tested compounds, compound 143 and 149 showed comparable analgesic activity (4.58, 5.27 and 4.88, 5.41) with the standard drug Indomethacin (5.79, 6.42) at 30 min. and 60 min respectively. The results are summarized in *table 4.3* and *figure 4.2*. All the data were analyzed by one way ANOVA test followed by Dunnett’s test.

**Table 4.3.** Analgesic activity of quinoline based 1,2,4-triazole derivatives.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mean value of Tail Flick Latency (sec)±SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min.</td>
</tr>
<tr>
<td>142</td>
<td>3.50±0.247</td>
</tr>
<tr>
<td>143</td>
<td>3.46±0.247</td>
</tr>
<tr>
<td>149</td>
<td>3.60±0.450</td>
</tr>
<tr>
<td>Control</td>
<td>2.89±0.170</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3.38±0.321</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s ‘t’ test (n=6), *p<0.05, **p<0.01, ***p<0.001 significantly different from standard; ns, not significant.

**Figure 4.2.** Analgesic activity of quinoline based 1,2,4-triazole derivatives
2.4.4.1.2.3. Ulcerogenic study

Screening of stomach wall of Indomethacin treated group at low power (10x) photomicrograph showed ulceration and necrosis of the mucosa, the necrosed area is marked by arrows. Stomach wall of indomethacin treated high power (40x) photomicrograph of the same section showed necrotic epithelial cells surrounded by fibrinoid matrix of the mucosa, which indicated significant surface epithelial damage, submucosal damage, deep mucosal damage and slightly muscular layer damage. Whereas the test compound 142 treated animal showed significant surface epithelial damage, slightly submucosal damage and no damage of deep mucosal and muscular layer. Compound 149 treated animal exhibited no damage of any layer. All the results are presented in table 4.4 and Figure 4.3.

Table 4.4. Histopathological studies of quinoline based 1,2,4-triazole derivatives

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface Epithelial Damage</th>
<th>Submucosal Damage</th>
<th>Deep Mucosal Damage</th>
<th>Muscular Layer Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
<td>+++</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>149</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

- No damage; +, ++, +++: indicates increasing degree of damage

Continued...
Figure 4.3 Haematoxylin and Eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.
2.4.4.1.2.4. Antimicrobial activity

The antifungal results showed that some of the compounds exhibited good antifungal activity against *A. niger* and *A. flavus*. Compounds 140 and 141 showed good growth of inhibition against *A. niger*. Compounds 143, 144 and 149 were active against *A. flavus*. It was observed that the maximum antimicrobial activity was observed by the tested compounds having R₁ = 3-Cl, 4-Cl, methoxy group in phenyl ring of the 1,2,4-triazole system. Replacement of chloro group in the phenyl ring diminishes the antifungal activity.

The antibacterial results showed that some of the compounds displayed good antibacterial activity against *K. pneumoniae* and *P. aeruginosa*. Compounds 142 and 144 showed good growth of inhibition against both the bacterial strains. It can be concluded that compound 143 was superior to positive controls against *P. aeruginosa*. It was observed that the maximum antibacterial activity was displayed by the tested compounds having 4-Cl, 4-Br groups present in phenyl ring of the 1,2,4-triazole system. Replacement of chloro group in the phenyl ring decreases the antibacterial activity.

**Table 4.5.** Antimicrobial activity of quinoline based 1,2,4-triazole derivatives.

<table>
<thead>
<tr>
<th>Compds</th>
<th>Antifungal Activity (zone of inhibition in mm)</th>
<th>Antibacterial Activity (zone of inhibition in mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>A. flavus</em> 200 (100) µg/disc</td>
<td><em>A. niger</em> 200 (100) µg/disc</td>
</tr>
<tr>
<td>140</td>
<td>10 (8)</td>
<td>14 (12)</td>
</tr>
<tr>
<td>141</td>
<td>12 (8)</td>
<td>18 (11)</td>
</tr>
<tr>
<td>142</td>
<td>10 (8)</td>
<td>8 (6)</td>
</tr>
<tr>
<td>143</td>
<td>14 (10)</td>
<td>12 (10)</td>
</tr>
<tr>
<td>144</td>
<td>14 (12)</td>
<td>11 (8)</td>
</tr>
<tr>
<td>145</td>
<td>(-)</td>
<td>6</td>
</tr>
<tr>
<td>146</td>
<td>(-)</td>
<td>(-)</td>
</tr>
<tr>
<td>147</td>
<td>12 (10)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>148</td>
<td>10 (9)</td>
<td>9</td>
</tr>
<tr>
<td>149</td>
<td>14 (12)</td>
<td>10 (8)</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Fluconazole</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>NT</td>
<td>12</td>
</tr>
</tbody>
</table>

- : no zone of inhibition; nt: not tested
2.4.4.2. Experimental

2.4.4.2.1. Chemistry

All chemicals (reagent grade) used were commercially available. Melting points were measured on a VEEGO-VMP-DS melting point apparatus and are uncorrected. $^1$H NMR was recorded on a Bruker DPX 500, 400, 300 instruments in CDCl$_3$/DMSO-d$_6$ using TMS as internal standard for protons. $^1$H NMR chemical shifts and coupling constants $J$ are given in ppm and Hz respectively. Mass spectra were recorded on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV and Maldi-MS. Mass-spectrometric (MS) data is reported in $m/z$. Elemental analysis was carried out using Elemental Vario EL III elemental analyser. Elemental analysis data is reported in % standard.

General procedure for synthesis of Ethyl 2-(quinolin-8-yloxy)acetate (130) Yield: 75%; Light yellow crystals, m.p. 51-52 °C, $R_f = 0.42$ (n-hexane : ethylacetate: 6:4).

![Chemical structure of Ethyl 2-(quinolin-8-yloxy)acetate](image)

**IR (KBr) cm$^{-1}$**

: 3052, 2921, 1678, 1109.

**$^1$H NMR (300 MHz, CDCl$_3$)**

: $\delta$ 1.52 (t, 3H, $J = 5.6$ Hz), 3.92 (q, 2H, $J = 5.8$ Hz), 5.29 (s, 2H, O-CH$_2$), 7.02 (d, 2H, $J = 8.6$ Hz), 7.24(s, 1H, $J = 7.8$ Hz), 7.36-7.54 (m, 3 H, Ar-H).

**FAB-MS ($m/z$)**

: 231 (M$^+$), 232 (M$^+$+1).

**Elemental Analysis**

: Calculated for molecular formula C$_{13}$H$_{13}$NO$_3$.

Calculated: C, 67.52; H, 5.67; N, 6.06.

Found: C, 67.53; H, 5.68; N, 6.07%.

2-(quinolin-8-yloxy)acetohydrazide (131) Yield: 84%; Light yellow crystals, m.p. 150-152 °C, $R_f = 0.25$(CHCl$_3$: MeOH; 9:1).

![Chemical structure of 2-(quinolin-8-yloxy)acetohydrazide](image)

**IR (KBr) cm$^{-1}$**

: 3352, 3054, 2921, 1684, 1109.
\(^1\)H NMR (300 MHz, CDCl\(_3\)) : δ 4.09 (s, 2H, NH\(_2\)), 4.87 (s, 2H, O-CH\(_2\)), 9.71 (s, 1H, N- H), 8.94 (d, J = 3.9 Hz, 1H), 8.20 (d, J = 8.4 Hz, 1H), 7.17-7.60 (m, 4H, Ar-H).

FAB-MS (m/z) : 217 (M\(^+\)), 218 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula C\(_{12}\)H\(_{12}\)N\(_2\)O\(_2\).
Calculated : C, 66.65; H, 5.59; N, 12.96
Found : C, 66.71; H, 5.56; N, 12.92%.

**General procedure for synthesis of thiosemicarbazide (132-135)**

To a solution of hydrazide 131 in absolute alcohol (50 mL) was added different arylisothiocyanates and the reaction mixture refluxed for 5-8 h. After completion of reaction monitored by TLC, the reaction mixture was cooled to RT and concentrated under reduced pressure, the concentrated solution (25mL) was poured on crushed ice, the precipitate so obtained was filtered off and washed with cold water, dried and crystallized in ethanol to yield the pure thiosemicarbazides (132-135).

**General procedure for the synthesis of 3-mercapto-1,2,4-triazoles (136-139)**

To a solution of thiosemicarbazide (132-135) in absolute alcohol (50 mL) was added triethyl amine (1mL) and the reaction mixture refluxed for 5-8 h. After completion of reaction monitored by TLC, the reaction mixture was cooled to RT and concentrated under reduced pressure, the concentrated solution (25 mL) was poured on crushed ice, the precipitate so obtained was filtered off, washed with cold water, dried and crystallized in ethanol to yield the pure 3-mercapto-1,2,4-triazoles (136-139).

\(4-(3\text{-chlorophenyl})-5\text{-[\{quinolin-8\text{-yloxy}methyl\}]4H-1,2,4\text{-triazole-3-thiol}} \quad (136)\)

**Yield:** 55 %; White crystals, m.p. 218-220 °C, \(R_f = 0.37\) (CHCl\(_3\): MeOH; 9:1).

\[
\text{IR (KBr) cm}^{-1} : 3042, 2926, 1604, 1452, 1374, 1107
\]
$^1$H NMR (300 MHz, DMSO-d$_6$) : $\delta$ 5.12 (s, 2H, O-CH$_2$), 7.21-7.60 (m, 8H, Ar-H), 8.35 (d, $J = 8.4$Hz, 1H, Ar-H), 8.87 (d, $J = 3.6$Hz, 1H, Ar-H), 14.18 (s, 1H, -SH).

Maldi-MS (m/z) : 368 (M$^+$), 369 (M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{18}$H$_{13}$ClN$_4$OS.

Calculated : C, 58.61; H, 3.55; N, 15.19.

Found : C, 58.24; H, 3.46; N, 15.68%

4-(4-chlorophenyl)-5-[[quinolin-8-yloxy]methyl]-4H-1,2,4-triazole-3-thiol (137)


IR (KBr) cm$^{-1}$ : 3038, 2926, 1603, 1452, 1374, 1109.

$^1$H NMR (300 MHz, DMSO-d$_6$) : $\delta$ 5.13 (s, 2H, O-CH$_2$), 6.98-7.54 (m, 7H, Ar-H), 8.33-8.36 (d, $J = 9.3$Hz, 2H, Ar-H), 8.87 (d, $J = 5.7$Hz, 1H, Ar-H), 14.14 (s, 1H, -SH).

Maldi-MS (m/z) : 368 (M$^+$), 369 (M$^+$+1).

Elemental Analysis : Calculated for molecular formula C$_{18}$H$_{13}$ClN$_4$OS.

Calculated : C, 58.61; H, 3.55; N, 15.19.

Found : C, 58.28; H, 3.49; N 15.45%

4-(4-methoxyphenyl)-5-[[quinolin-8-yloxy]methyl]-4H-1,2,4-triazole-3-thiol (138)

Yield: 72 %; White crystals, m.p. 237-238 ºC, $R_f$ = 0.34 (CHCl$_3$: MeOH; 9:1).

IR (KBr) cm$^{-1}$ : 3042, 2922, 1601, 1450, 1371, 1108.
\( ^1H \text{NMR (300 MHz, DMSO-d_6)} \) : \( \delta \) 3.73 (s, 3H, O\text{-CH}_3), 5.12 (s, 2H, O\text{-CH}_2), 6.89 (d, \( J = 8.7 \text{ Hz}, 2\text{H} \)), 7.24 (d, \( J = 7.2\text{Hz}, 2\text{H}, \text{Ar-H} \)), 7.41-7.58 (m, 4H, Ar-H), 8.35 (d, \( J = 8.4 \text{ Hz}, 1\text{H}, \text{Ar-H} \)), 8.86 (d, \( J = 2.8\text{Hz}, 1\text{H}, \text{Ar-H} \)), 14.10 (s, 1H, -SH).

Maldi-MS (m/z) : 364 (M\(^+\)), 365 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula C\(_{19}\)H\(_{16}\)N\(_4\)O\(_2\)S.

Calculated : C, 62.62; H, 4.43; N, 15.37.

Found : C, 62.34; H, 4.36; N, 15.70% 

4-phenyl-5-[(quinolin-8-yloxy)methyl]-4H-1,2,4-triazole-3-thiol (141) Yield: 55%; White crystals, m.p. 224-226 °C, \( R_f \) = 0.34 (CHCl\(_3\); MeOH; 9:1).

IR (KBr) cm\(^{-1}\) : 3056, 2927, 1605, 1454, 1376, 1107.

\( ^1H \text{NMR (300 MHz, DMSO-d_6)} \) : \( \delta \) 5.13 (s, 2H, O\text{-CH}_2), 6.92-7.56 (m, 8H, Ar-H), 8.35 (d, \( J = 6.8 \text{ Hz}, 1\text{H} \)), 8.87-8.88 (d, \( J = 3.4 \text{ Hz}, 1\text{H}, \text{Ar-H} \)), 14.13 (s, 1H, -SH).

Maldi-MS (m/z) : 334 (M\(^+\)), 335 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula C\(_{18}\)H\(_{14}\)N\(_4\)OS.

Calculated : C, 64.65; H, 4.22; N, 16.75.

Found : C, 64.51; H, 4.19; N, 16.63%

**General procedure for synthesis of Quinoline based S-substituted 1,2,4-triazole (140-149)**

To a solution of 3-mercaptop-1,2,4-triazole (136-139, 0.5 mmol) in dry acetone (50 mL) was added different substituted phenacyl bromide (0.5 mmol), potassium carbonate (1 mmol) and the reaction mixture refluxed for 4-8 h. After completion of reaction monitored by TLC, the reaction mixture was filtered in hot condition, concentrated under reduced pressure, the concentrated solution (25 mL) was poured
on crushed ice, the precipitate so obtained was filtered, washed with cold water, dried and crystallized in methanol to yield the pure compound (140-149).

2-[4-(3-chlorophenyl)-5-{(quinolin-8-yloxy)methyl}-4H-1,2,4-triazol-3-ythio]-1-(4-chlorophenyl)ethanone (140) Yield: 54 %; White crystals, m.p. 170 ºC, Rf = 0.25 (CHCl₃: MeOH; 9:1).

IR (KBr) cm⁻¹: 1677, 1587, 1557, 1486, 1455, 1375, 1302, 1112.

¹H NMR (500 MHz, DMSO-d₆): δ 4.96 (s, 2H, S-CH₂), 5.21 (s, 2H, O-CH₂), 7.01-7.02 (d, 2H, J = 7.4 Hz), 7.17-7.19 (d, 2H, J = 6.7 Hz), 7.40-7.57 (m, 8H, Ar-H), 8.31-8.32 (s, 1H, J = 3.6 Hz), 8.86-8.87 (d, 1H, J = 3.4 Hz).

¹³C-NMR (125 MHz, CDCl₃): δ 41.42, 61.00, 111.98, 121.47, 121.92, 126.42, 127.22, 128.36, 129.05, 129.47, 130.47, 133.05, 134.41, 135.86, 139.77, 147.99, 149.41, 152.72, 168.65, 190.00.

Maldi-MS (m/z): 520 (M+), 521 (M⁺+1).

Elemental Analysis: Calculated for molecular formula C₂₆H₁₈Cl₂N₄O₂S.

Calculated: C 59.89, H 3.48, N 10.75.

Found: C 59.29, H 3.27, N 10.42 %

2-[4-(3-chlorophenyl)-5-{(quinolin-8-yloxy)methyl}-4H-1,2,4-triazol-3-ythio]-1-(2,4-dihydroxyphenyl)ethanone (141) Yield: 62 %; White Crystals, m.p. 228-230 ºC, Rf = 0.32 (CHCl₃: MeOH; 9:1).
IR (KBr) cm\(^{-1}\): 3455, 3042, 2922, 1677, 1605, 1456, 1374, 1105.

\(^1\)H NMR (500 MHz, DMSO-d\(_6\)):
\[\delta 4.91 (s, 2H, S-CH\(_2\)), 5.18 (s, 2H, O-CH\(_2\)), 6.86 (d, J = 8.6 Hz, 2H), 7.01-7.02 (d, J = 7.5 Hz, 1H), 7.29 (d, J = 6.6 Hz, 1H), 7.41-7.49 (m, 4H), 7.52 (t, J = 4.5 Hz, 2H), 7.58 (d, J = 2.0 Hz, 1H), 8.19-8.21 (d, J = 8.2 Hz, 1H), 9.03-9.04 (d, J = 2.6 Hz, 1H), 10.52 (s, 1H, OH), 10.61 (s, 1H, OH).

\(^13\)C-NMR (125 MHz, DMSO-d\(_6\)):
\[\delta 58.90, 63.90, 105.00, 121.35, 121.76, 122.10, 125.50, 126.50, 126.71, 126.76, 128.50, 128.80, 130.10, 130.30, 130.38, 134.79, 136.31, 146.36, 148.97, 149.36, 152.56, 191.32.\]

Maldi-MS (m/z): 518 (M\(^+\)), 520 (M\(^+\)+2).

Elemental Analysis:
Calculated for molecular formula C\(_{26}\)H\(_{19}\)ClN\(_4\)O\(_4\)S.
Calculated: C 60.17, H 3.69, N 10.80.
Found: C 60.67, H 3.44, N 11.02%.

2-[4-(3-chlorophenyl)-5-[(quinolin-8-yloxy)methyl]-4H-1,2,4-triazol-3-ylthio]-1-(4-hydroxyphenyl)ethanone (142) Yield: 72%; White Crystals, m.p. 218-220 °C, R\(_f\) = 0.20 (CHCl\(_3\): MeOH; 9:1).

IR (KBr) cm\(^{-1}\): 3450, 3046, 2926, 1674, 1601, 1450, 1371, 1108.

\(^1\)H NMR (200 MHz, DMSO-d\(_6\)):
\[\delta 4.89 (s, 2H, S-CH\(_2\)), 5.31 (s, 2H, O-CH\(_2\)), 6.88 (d, J = 9.0 Hz, 2H), 7.26 (d, J = 6.5 Hz, 1H), 7.82 (s, 1H), 7.42-7.62 (m, 7H), 7.90 (d, J = 8.0 Hz, 2H), 8.88 (d, J = 2.6 Hz, 1H), 10.53 (s, 1H, OH).

\(^13\)C-NMR (125 MHz, DMSO-d\(_6\)):
\[\delta 42.34, 60.00, 111.00, 115.00, 121.00, 122.00, 126.00, 127.00, 129.00, 130.00, 131.00, 133.00,\]
135.00, 139.00, 149.00, 151.00, 152.00, 162.00, 191.00.

**Maldi-MS (m/z)**

: 502 (M⁺), 503 (M⁺+1).

**Elemental Analysis**

: Calculated for molecular formula

\[ \text{C}_{26}\text{H}_{19}\text{ClN}_{4}\text{O}_{3}\text{S}. \]

Calculated


Found

: C 61.69, H 3.76, N 11.02%.

2-[4-(3-chlorophenyl)-5-[(quinolin-8-yloxy)methyl]-1H,2,4-triazol-3-ylthio]-1-(4-bromophenyl)ethanone (143) **Yield**: 63 %; White Crystals, **m.p.** 188-190 ºC; **Rf** = 0.22 (CHCl₃: MeOH; 9:1).

IR (KBr) \text{cm}^{-1}

: 3046, 2926, 1674, 1601, 1450, 1371, 1108.

\[^1\text{H NMR (200 MHz, DMSO-d}_6\text{)}\]

: \( \delta \) 4.95 (s, 2H, S-CH\(_2\)), 5.31 (s, 2H, O-CH\(_2\)), 7.23 (d, \( J = 7.5\) Hz, 2H, Ar-H), 7.42-7.61 (m, 4H, Ar-H), 7.76 (s, 1H, Ar-H), 7.78 (d, \( J = 8.6\) Hz, 2H), 7.96 (d, \( J = 8.5\) Hz, 2H, Ar-H), 8.32 (d, \( J = 4.7\) Hz, 2H, Ar-H), 8.87 (s, 1H, Ar-H).

\[^{13}\text{C-NMR (125 MHz, DMSO-d}_6\text{)}\]

: \( \delta \) 40.70, 61.28, 111.00, 121.00, 122.00, 126.62, 126.94, 127.84, 130.55, 130.87, 131.50, 132.36, 136.30, 149.86, 150.00, 151.00, 162.00, 190.00.

**Maldi-MS (m/z)**

: 565 (M⁺), 566 (M⁺+1).

**Elemental Analysis**

: Calculated for molecular formula

\[ \text{C}_{26}\text{H}_{18}\text{ClBrN}_{4}\text{O}_{2}\text{S}. \]

Calculated

: C 55.19, H 3.21, N 9.9.

Found

: C 54.68, H 3.16, N 9.62%.
2-[4-(4-chlorophenyl)-5-((quinolin-8-ylxy)methyl)-4H-1,2,4-triazol-3-ythio]-1-(4-hydroxyphenyl)ethanone (144) Yield: 72 %; White Crystals, m.p. 180-182 °C, \( R_f = 0.24 \) (CHCl3: MeOH; 9:1).

\[
\text{IR (KBr) cm}^{-1} : 3456, 3045, 2922, 1679, 1605, 1495, 1452, 1370, 1106.
\]

\[ ^1\text{H NMR (200 MHz, DMSO-d}_6) : \delta 4.88 (s, 2H, S-CH\_2), 5.38 (s, 2H, O-CH\_2), 7.20 (d, J = 7.2 Hz, 2H), 7.43-7.55 (m, 3H), 7.63 (d, J = 8.8Hz, 2H), 7.75 (d, J = 9.0 Hz, 2H), 8.29-8.31 (d, 2H, J = 4.4 Hz), 8.81 (d, 1H, J = 3.95Hz). \]

\[ ^1\text{C-NMR (125 MHz, DMSO-d}_6) : \delta 48.5, 79.00, 111.00, 122, 126.42, 129.06, 129.48, 130.32, 131.80, 139.76, 149.65, 150.95, 153.30, 192.34. \]

\[ \text{Maldi-MS (m/z) : 502 (M^+)}, 503 (M^+1). \]

Elemental Analysis : Calculated for molecular formula C\_26H\_19ClN\_4O\_3S.


Found : C 61.69, H 3.76, N 11.02%.

2-[4-(4-chlorophenyl)-5-((quinolin-8-ylxy)methyl)-4H-1,2,4-triazol-3-ythio]-1-(4-bromophenyl)ethanone (145) Yield: 68 %; White Crystals, m.p. 168-170 °C; \( R_f = 0.28 \) (CHCl3: MeOH; 9:1).

\[
\text{IR (KBr) cm}^{-1} : 3045, 2926, 1681, 1601, 1495, 1455, 1370, 1104.
\]

\[ ^1\text{H NMR (200 MHz, DMSO-d}_6) : \delta 4.91 (s, 2H, S-CH\_2), 5.28 (s, 2H, O-CH\_2), 7.20 (d, 2H, J = 7.2 Hz), 7.43-7.55(m, 3H), 7.63 (d,
2H, \( J = 8.8 \) Hz), 7.75 (d, 2H, \( J = 8.4 \) Hz), 7.92 (d, 2H, \( J = 9.0 \) Hz), 8.30 (d, 2H, \( J = 4.4 \) Hz), 8.83 (d, 1H, \( J = 3.9 \) Hz).

\( ^{13}C-NMR \ (125 \ MHz, \text{DMSO-}d_6) : \delta 44.5, 79.00, 112.00, 121.0, 128.00, 126.42, 128.48, 129.00, 131.32, 132.80, 137.72, 148.65, 151.95, 152.32, 191.34.

MALDI MS (m/z) : 565 (M\(^+\)), 566 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula

\( C_{26}H_{18}ClBrN_4O_2S. \)


Found : C 61.69, H 3.76, N 11.06%.

2-[4-(4-methoxyphenyl)-5-{(quinolin-8-yloxy)methyl}-4H-1,2,4-triazol-3-ylthio]-1-phenylethanone (146) Yield: 49 \%; White Crystals, m.p. 172-174 °C, \( R_f = 0.31 \) (CHCl\(_3\): MeOH; 9:1).

IR (KBr) cm\(^{-1}\) : 3042, 2927, 1678, 1515, 1445, 1408, 1379, 1262, 1111.

\( ^1H \) NMR (200 MHz, DMSO-\( d_6 \)) : \( \delta 3.72 \) (s, 3H, O-CH\(_3\)), 4.86 (s, 2H, S-CH\(_2\)), 4.98 (s, 2H, O-CH\(_2\)), 6.81 (d, \( J = 8.8 \) Hz, 2H), 7.24 (d, \( J = 7.3 \) Hz, 1H), 7.36 (d, \( J = 7.3 \) Hz, 2H), 7.46-7.59 (m, 7H), 8.33 (d, \( J = 6.2 \) Hz, 2H), 8.87 (d, \( J = 4.1 \) Hz, 1H).

\( ^{13}C-NMR \ (125 \ MHz, \text{DMSO-}d_6) : \delta 40.50, 56.00, 79.00, 114.14, 121.12, 126.42, 128.00, 129.00, 128.48, 131.32, 132.80, 137.72, 148.65, 151.95, 152.32, 191.38.

MALDI MS (m/z) : 482 (M\(^+\)), 483 (M\(^+\)+1).

Elemental Analysis : Calculated for molecular formula

\( C_{27}H_{22}ClN_4O_3S. \)

Calculated : C 67.2, H 4.6, N 11.61.
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Found: C 67.69, H 4.46, N 11.52%

2-(4-phenyl-5-[(quinolin-8-yloxy)methyl]-4H-1,2,4-triazol-3-ylthio)-1-
phenylethanone (147) Yield: 62%; White Crystals, m.p. 160-162 °C, Rf = 0.25 (n-

IR (KBr) cm⁻¹: 3450, 3046, 2926, 1674, 1601, 1450, 1371, 1108.

¹H NMR (200 MHz, DMSO-d₆): δ 5.02 (s, 2H, S-CH₂), 5.32 (s, 2H, O-CH₂), 7.26-7.28 (d, J = 7.36 Hz, 2H), 7.47-7.70 (m, 9H), 7.69 (d, J = 8.3 Hz, 2H), 8.00 (d, J = 8.30 Hz, 2H), 8.37 (d, J = 7.80 Hz, 1H), 8.93 (1H, J = 2.65 Hz).

¹³C-NMR (125 MHz, DMSO-d₆): δ 42.50, 78.00, 121.00, 126.42, 128.00, 128.48, 129.00, 130.32, 132.80, 134.72, 149.65, 151.95, 152.82, 191.34.

MALDI MS (m/z): 452 (M⁺), 453 (M⁺+1).

Elemental Analysis: Calculated for molecular formula C₂₆H₂₀N₄O₂S
Calculated: C 69.01, H 4.45, N 12.38.
Found: C 68.69, H 4.52, N 12.18%

2-[4-(4-methoxyphenyl)-5-[(quinolin-8-yloxy)methyl]-4H-1,2,4-triazol-3-ylthio]-1-
(4-hydroxyphenyl)ethanone (148) Yield: 68%; White Crystals, m.p. 230-232 °C, Rf
= 0.18 (CHCl₃: MeOH; 9:1).

IR (KBr) cm⁻¹: 3450, 3046, 2926, 1674, 1601, 1450, 1371, 1108.

¹H NMR (200 MHz, DMSO-d₆): δ 3.74 (s, 3H, O-CH₃), 4.88 (s, 2H, S-CH₂), 5.25 (s, 2H, O-CH₂), 6.88 (d, J = 8.60 Hz, 2H), 6.96 (d, J = 8.80 Hz, 2H), 7.24 (d, J = 6.80 Hz, 1H), 7.43 (d, J = 7.30 Hz, 2H).
7.42-7.89 (m, 5H), 7.92 (d, \( J = 8.65 \) Hz, 2H), 8.34 (d, \( J = 8.1 \) Hz, 1H), 8.88 (d, \( J = 3.00 \) Hz, 1H).

\[ ^{13}\text{C-NMR (125 MHz, DMSO-d}_6 \): \delta 44.46, 56.54, 78.00, 111.00, 114.00, 121.00, 125.42, 129.06, 129.48, 130.32, 131.80, 139.76, 149.65, 150.95, 155.30, 159.00, 162.00, 191.00.}

**MALDI MS (m/z):** 498 (M\(^+\)), 499 (M\(^+\)+1).

**Elemental Analysis:** Calculated for molecular formula \( \text{C}_{27}\text{H}_{22}\text{N}_4\text{O}_4\text{S} \).

**Calculated:** C 65.05, H 4.45, N 11.24.

**Found:** C 65.63, H 4.66, N 11.12%.

2-[4-(4-methoxyphenyl)-5-[(quinolin-8-yloxy)methyl]-1H,1,2,4-triazol-3-ythio]-1-(4-bromophenyl)ethane (149) **Yield:** 74%; White Crystals, m.p. 158-160 °C, \( R_f = 0.25 \) (CHCl\(_3\); MeOH; 9:1).

\[ \text{IR (KBr) cm}^{-1} : 3056, 2926, 1674, 1601, 1450, 1371, 1108.} \]

\[ ^{1}\text{H NMR (200 MHz, DMSO-d}_6 \): \delta 3.79 (s, 3H, O-CH\(_3\)), 4.96 (s, 2H, S-CH\(_2\)), 5.27 (s, 2H, O-CH\(_2\)), 6.85 (d, \( J = 8.6 \) Hz, 2H), 7.23 (d, \( J = 6.6\)Hz, 1H), 7.41-7.65 (m, 6H), 7.79 (d, \( J = 8.4 \) Hz, 2H), 7.97 (d, \( J = 8.5 \) Hz, 2H), 8.30 (d, \( J = 1.3 \) Hz, 1H).}

\[ ^{13}\text{C-NMR (125 MHz, DMSO-d}_6 \): \delta 42.50, 55.43, 78.00, 114.00, 120.00, 126.42, 128.00, 128.48, 131.32, 133.00, 137.72, 148.65, 150.00, 152.32, 193.34.}

**MALDI MS (m/z):** 561 (M\(^+\)), 562 (M\(^+\)+1).

**Elemental Analysis:** Calculated for molecular formula \( \text{C}_{27}\text{H}_{21}\text{ClBrN}_4\text{O}_3\text{S} \).

**Calculated:** C 57.76, H 3.77, N 9.98.

**Found:** C 57.24, H 3.68, N 10.04%.
2.4.4.2.2. Biological activity

2.4.4.2.2.1. Anti-inflammatory activity
All the synthesized compounds were evaluated for their anti-inflammatory activity against carrageenan-induced rat paw oedema in Albino Wistar rats weighing 150-200 g. The standard drug Indomethacin was used at dose of 10 mg/kg body weight and test drugs were used equimolar to the standard drug. The method used was the same as discussed in section-1 of this chapter. The results of anti-inflammatory activity are presented in table 4.2 and figure 4.1.

2.4.4.2.2.2. Analgesic activity
The analgesic activity was determined using tail flick method in Albino Wistar rats weighing 150-200 g. Indomethacin (10 mg/kg body weight) was used as standard drug and test drugs were used equimolar to the standard drugs. The same method was used as given in section-1 of this chapter. The results are presented in table 4.3 and figure 4.2.

2.4.4.2.2.3. Histopathological studies
For the histopathological study, same methods were used as given in section-1 of this chapter. The drugs were used at doses three times to the doses used for anti-inflammatory activity. The results are presented in table 4.4 and figure 4.3.

2.4.4.2.2.4. Antimicrobial activity
All the synthesized compounds were evaluated for their antimicrobial activity against various bacterial and fungal strains. Same methods were used as given in section-1 of this chapter. The results are presented in table 4.5.
2.4.4.3. Conclusion
In conclusion, we have synthesized a small library of ten compounds 140-149 successfully using the standard methodology. The newly synthesized compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and antimicrobial studies. Compound 149 has exhibited potent anti-inflammatory, comparable analgesic activity with no ulcer. Other members 142 and 143 from this series also exhibited significant anti-inflammatory activity with reduced ulcer. Compound 149 may be considered as promising candidate for development of new anti-inflammatory agent. From antimicrobial screening it was concluded that most of the compounds from this series showed moderate antimicrobial activity.
2.5. References


$^1$H NMR spectrum of compound 68
$^{13}$C NMR spectrum of compound 68
Mass spectrum of compound 68

Ionization Mode: ESI+
MS Calibration Name: YOKUDELNA_ES+_2000
Reduction History: Correct Base[5.0%], Average(MS[1] 0.100 - 0.117)
Experiment Date/Time: 6/17/2010 2:31:17 PM

Operator Name: admin
IR spectrum of compound 115
$^{1}$H NMR spectrum of compound 115
13C NMR spectrum of compound 115
Mass spectrum of compound 115
IR spectrum of compound 124
$^1$H NMR spectrum of compound 124
\( ^{13}C \) NMR spectrum of compound 124
Mass spectrum of compound 124
$^1$H NMR spectrum of compound 149
\[
\text{\textsuperscript{13}C NMR spectrum of compound 149}
\]
Spectrum Report

Mass spectrum of compound 149