1.0. **Review of Literature**

1.1. **Current advancements on 1,2,4-Triazoles**

In this twenty first century research is directed towards the introduction of new and safe therapeutic agents of clinical importance. The nitrogen containing heterocycles are found in abundance in most of the medicinal compounds [87]. The triazoles are said to be the isosters of imidazoles in which the carbon atom of imidazole is isostERICally replaced by nitrogen. The derivatization of triazole ring is based on the phenomenon of bioisosterism in which replacement of oxygen of oxadiazole nucleus occurs with nitrogen atom in the triazole analogue. Out of the two triazoles 1,2,4-triazole has wide variety of activity [88].

Triazole refers to either one pair of isomeric chemical compound having five membered ring of two carbon atoms and three nitrogen atoms. The triazoles exist in isomeric forms according to the position of nitrogen atoms. Two structural isomeric triazoles are known, the 1,2,3-(1,2,5) and the 1,2,4-(1,3,4), the former being known as osotriazole and the latter as triazole. Each exists in two dissimilar tautomeric forms [89]. The different isomers (Figure 1) are characterized by the position of the nascent hydrogen. Thus 1,2,4- triazoles exist in two forms i.e. 1H and 4H.

![Figure 1. Tautomeric forms of triazoles.](image-url)
1.2. **Clinically used drugs containing 1,2,4-Triazole nucleus**

The 1H-1,2,4-triazole compounds are interesting heterocycles since they possess important pharmacological activities such as antifungal and antiviral activities. The antifungal action of these compounds is based on the inhibition of biosynthesis of ergosterol, the major steroid in fungal membranes, by blocking 14-α-demethylation and subsequently the disruption of the fungal membranes [90-92]. The important antifungal drugs present in market (Figure 2) include fluconazole [93], itraconazole [94], ravuconazole [95], voriconazole [96], ICI 153066 [97] and posaconazole [98]. Triazole-based drugs are more selective for fungi in comparison to mammalian cells than the azole-based antifungal compounds [99].

![Fluconazole](image1)

![Ravuconazole](image2)

![Voriconazole](image3)

![ICI 153066](image4)

![Itraconazole](image5)

![Posaconazole](image6)

**Figure 2. Antifungal drugs**

3-amino-1H-1,2,4-triazoles have been used as herbicides and defoliants. They have been described as catalase inhibitors [100] and blockers for certain ethanol-induced behaviour effects [101]. It has been reported that only certain enantiomers (Figure 3) of triazoles containing oxazolidine rings e.g. 4(R), 5(R)) are active against *Candida albicans* infections in mice [102].
Ribavirin (Figure 4) is an antiviral drug containing 1,2,4-triazole nucleus [103-105]. It is a broad spectrum antiviral agent containing the 3-aminocarbonyl triazole moiety. It is active against both RNA and DNA viruses and is used in an aerosol for lower respiratory tract viral disease as well as in the treatment of influenza, Lassa fever and Hantaan virus [106,107]. Its amidine and guanidine derivatives (R = H-HCl, Me, CN) [108] have also been prepared.

Some triazole derivatives are considered as angiotensin II receptor antagonists (Figure 5). The following two compounds are used to increase the blood pressure [109,110].
Compounds having triazole moieties, such as vorozole, letrozole and anastrozole (Figure 6) act as effective aromatase inhibitors and prevent breast cancer [111].

![Vorozole, Letrozole, Anastrozole](image)

**Figure 6. Aromatase inhibitors**

<table>
<thead>
<tr>
<th>Sno.</th>
<th>Compound</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><img src="image" alt="Compound 1" /></td>
<td>Anti-inflammatory</td>
<td>[112]</td>
</tr>
<tr>
<td>2.</td>
<td><img src="image" alt="Compound 2" /></td>
<td>Anti-inflammatory &amp; gastric ulcerogenic effects</td>
<td>[113]</td>
</tr>
<tr>
<td>3.</td>
<td><img src="image" alt="Compound 3" /></td>
<td>Antimycobacterial</td>
<td>[114]</td>
</tr>
<tr>
<td>4.</td>
<td><img src="image" alt="Compound 4" /></td>
<td>Antimicrobial</td>
<td>[115]</td>
</tr>
<tr>
<td>5.</td>
<td><img src="image" alt="Compound 5" /></td>
<td>Anticonvulsant</td>
<td>[116]</td>
</tr>
<tr>
<td>6.</td>
<td><img src="image" alt="Compound 6" /></td>
<td>Analgesic</td>
<td>[117]</td>
</tr>
</tbody>
</table>

**Table 1.** Biological profile of 1,2,4-Triazoles.
7. Antioxidant [118]

8. Anti-depressant [119]

9. Antiasthmatic [120]

10. Antimigraine [121]

1.3. **Methods for the Synthesis of 1,2,4-Triazoles**

Several methods are available for the synthesis of 1,2,4-triazole and its derivatives, out of which some are described as under.

**Scheme 1**

The cyclisation of the thiosemicarbazides in an alkaline medium results in the formation of 1,2,4-triazoles [122,123].

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

**Scheme 2**

A copper-catalyzed reaction under an atmosphere of air provides 1,2,4-triazole derivatives by sequential N-C and N-N bond-forming oxidative coupling reactions [124].
Scheme 3

Thiosemicarbazides on refluxing with triethylamine in ethanol undergo cyclisation to produce 1,2,4-triazoles [125].

Scheme 4

Thiosemicarbazides on refluxing with 1,1-cyclopropanedicarboxylic acid and thionyl chloride in an alkaline medium undergo cyclisation to form 1,1-bis(3-thio-1,2,4-triazol-5-yl)cyclopropane [126].

Scheme 5

Reaction of various coumarins with hydrazines in anhydrous ethanol furnishes corresponding 1,2,4-triazoles [127].

Scheme 6

A highly regioselective one-pot process provides access to highly diverse 1,3,5-trisubstituted 1,2,4-triazoles from the reaction of carboxylic acids, primary amidines and monosubstituted hydrazines [128].
Scheme 7

An effective 1,3-dipolar cycloaddition for the synthesis of 1,3,5-trisubstituted 1,2,4-triazole derivatives by reaction of oximes with hydrazonoyl hydrochlorides using triethylamine as a base gave the desired 1,3,5-trisubstituted 1,2,4-triazoles in good yields. The reaction was applicable to aliphatic, cyclic aliphatic, aromatic and heterocyclic oxime substrates [129].

Scheme 8

2-chloro-6-methoxy-4-phenyl-quinoline on refluxing with substituted acylhydrazides in a nitrogen atmosphere yield 7-methoxy-1-(4-methoxyphenyl)-5-phenyl-1,2,4-triazolo[4,3] quinolone [130].

Scheme 9

Cyclo condensation reactions of phenyl selanyl propionate, using selanyl group as the precursor of terminal double bond results in the formation of 1,2,4-triazole ring [131].
Scheme 10

1,3,4-oxadiazole on reaction with primary amines give 3,5-disubstituted 1,2,4-trazole [132].

1.4. Reactions of 1,2,4-Triazoles

1. Triazoles on condensation with heteroaromatic acids in the presence of POCl₃ produce triazole thiaodiazoles [133].
2. 1,2,4-triazole-3-thiol on reaction with ethyl bromoacetate in presence of sodium ethoxide or triethyl amine, hydrazine hydrate, CS₂ and KOH leads to the formation of $5-\{[\{(4\text{-phenyl}-5\text{-pyridin}-4\text{yl}-4\text{H}-1,2,4\text{-triazol}-3\text{-yl} \text{thio})\text{methyl}]\text{1,3,4-oxadiazole-2-thiol}\}$ which on reaction with 2-(4-morpholino) ethylamine in presence of formaldehyde solution is converted into corresponding manich base derivatives [134].

1.5. Present work

The 1,2,4-triazole derivatives are an important class of heterocyclic compounds known for their broad spectrum of biological activities such as analgesic, anti-inflammatory and antimicrobial etc [135-137]. With the passage of time the resistance towards anti-inflammatory drugs is increasing and accordingly the demand for the identification of novel lead molecules with potent anti-inflammatory activity and less toxicity is also becoming rigorous. So far many non-steroidal anti-inflammatory drugs (NSAIDs) have been developed to heal the inflammation. There are many NSAIDs present in the market but they are simultaneously associated with many adverse side effects like gastric ulcer [138], kidney damage [139] and hepatotoxicity [140]. During the last few decades, a considerable attention has been laid towards the synthesis of 1,2,4-triazole derivatives [141,142].

The important pharmacological properties of benzoxazolinones have been highlighted previously in the section A of this chapter. In view of the biological importance of benzoxazolinones and 1,2,4-triazoles, we have conjugated these two moieties through a methylene linkage and evaluated them for their anti-inflammatory, analgesic and ulcerogenic activities.
As shown in the figure 7, 2-aminophenol derivatives were refluxed with 1,1-carbonyldiimidazole in dry tetrahydrofuran (THF) for 5-6 h. The benoxazolinone derivatives thus formed were reacted with ethylbromoacetate in dry acetone containing K$_2$CO$_3$ leading to the formation of acetic acid ethyl ester derivatives which on further reaction with hydrazine hydrate in absolute ethanol yielded hydrazide derivatives.

![Figure 7. Schematic representation of the synthetic route.](image-url)

The formation of the ester derivatives was confirmed from the TLC and $^1$H NMR spectra which showed the presence of a singlet for two methylene protons in a range of δ 4.87-4.92. The formation of the ester derivatives was further confirmed by the appearance of two signals at δ 1.21 (t, $J = 6.9$ Hz, 3H) and 4.18 (q, $J = 7.2$ Hz, 2H) whereas the formation of the acetic acid hydrazide derivatives from the ester derivatives was confirmed by the disappearance of these two signals and the
appearance of two new signals in the $^1$H NMR spectra at $\delta$ 9.1 (s, 1H, CONH) and $\delta$ 4.40 (s, 2H, NH$_2$). The hydrazide derivatives were converted into corresponding thiosemicarbazides by reaction with different substituted aryl isothiocyanates in dry ethanol. The formation of the thiosemicarbazides was confirmed from the TLC and $^1$H NMR spectra. The presence of the extra signals in the aromatic region due the protons of the phenyl ring ($\delta$ 7.53-7.73) of the isothiocyanate along with the signals at $\delta$ 9.5-9.9 (CSNH) and 10.13-10.58 (CONH) confirmed the formation of the thiosemicarbazides. The thiosemicarbazides were finally cyclised into respective substituted 3-mercapto-1,2,4-triazoles in dry ethanol in the presence of triethylamine. All the synthesized compounds have been characterised on the basis of detailed spectral data and evaluated for their anti-inflammatory and analgesic activities. The compounds showing significant activity were further evaluated for their gastric ulceration effects.

Table 2. Physical data of novel benzoxazolinone based 1,2,4- triazoles.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
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<td>267-268</td>
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<tr>
<td>3a</td>
<td><img src="image3" alt="Structure" /></td>
<td>60.00</td>
<td>256-257</td>
</tr>
<tr>
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<td>63.63</td>
<td>235-236</td>
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<tr>
<td>5b</td>
<td><img src="image5" alt="Structure" /></td>
<td>52.17</td>
<td>285-286</td>
</tr>
<tr>
<td></td>
<td>Structure</td>
<td>Mass (Da)</td>
<td>pKₐ (units)</td>
</tr>
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<td>---</td>
<td>-----------</td>
<td>-----------</td>
<td>-------------</td>
</tr>
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<td><img src="image" alt="Structure 6b" /></td>
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<td>14d</td>
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<td>59.99</td>
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</table>
2.0. **Results and discussion**

2.1. **Analytical**

A focused library of twenty novel benzoxazolinone based 1,2,4-triazoles (1a - 20e) has been synthesized starting from different ortho-aminophenol derivatives. The conjugation of the benzoxazolinone ring with the 1,2,4-triazole nucleus was supported by the presence of a singlet for two methylene protons (-CH$_2$) in a range of δ 4.66-5.08 in the $^1$H-NMR. The formation of 1,2,4-triazole ring was confirmed by the resonance of the triazole ring proton (SH) at a δ 13.95-14.15 as a singlet in the $^1$H-NMR. The attachment of the benzoxazolinone ring with the 1,2,4-triazole nucleus was confirmed by the presence of the methylene carbon (-CH$_2$) in a range of δ 37.31-38.87 in the $^{13}$C-NMR spectra. The final compounds varied from each other on the basis of nature and position of the substituents on the benzoxazolinone ring and the phenyl ring attached to the 1,2,4-triazole ring. The structure of all the compounds was further confirmed by the IR, $^1$H-NMR, $^{13}$C-NMR spectra and ESI-MS or FAB-MS mass spectral analysis.

2.2. **In silico molecular docking studies**

2.2.1. **Molecular docking studies on COX-2**

In order to get the insights of binding mode of ligands with COX-2, these ligands were docked against COX-2 (PBD NO: 3LN1). Before docking these ligands, the docking methodology of Schrodinger was validated by predicting the binding mode of celecoxib itself against the developed grid. Hydrogen bonding between LEU-338 residue & celecoxib (Figure 8) is highlighted in red color. Figure 8 clearly shows that adapted Schrodinger methodology successfully predicted the binding mode of crystallographic celecoxib with root mean square deviation of 1’A. All the ligands were docked against COX-2 (PBD NO: 3LN1) target protein separately. The predicted binding energies were found to be in the range of -4.48 to -40.38 kcal/mol are summarized in Table 3.
Figure 8. Superimposed binding orientation of the crystallographic celecoxib (green) and docked celecoxib (maroon) as predicted by Schrodinger glide software. The amino acid residues are show in stick model (blue).

Table 3. Docking scores of celecoxib and twenty new ligands with 3LN1.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Glide Score</th>
<th>Glide Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celecoxib</td>
<td>-11.29</td>
<td>-61.11</td>
</tr>
<tr>
<td>1a</td>
<td>-7.21</td>
<td>-37.89</td>
</tr>
<tr>
<td>2a</td>
<td>-7.50</td>
<td>-39.61</td>
</tr>
<tr>
<td>3a</td>
<td>-8.22</td>
<td>-34.35</td>
</tr>
<tr>
<td>4a</td>
<td>-8.26</td>
<td>-10.70</td>
</tr>
<tr>
<td>5b</td>
<td>-7.76</td>
<td>-23.37</td>
</tr>
<tr>
<td>6b</td>
<td>-7.94</td>
<td>-13.77</td>
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<td>7b</td>
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<td>-35.38</td>
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<td>12c</td>
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<td>-6.80</td>
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<tr>
<td>13d</td>
<td>-7.64</td>
<td>-25.96</td>
</tr>
<tr>
<td>14d</td>
<td>-7.97</td>
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<tr>
<td>15d</td>
<td>-8.57</td>
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<tr>
<td>16d</td>
<td>-7.21</td>
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<tr>
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<td>-40.38</td>
</tr>
<tr>
<td>20e</td>
<td>-5.28</td>
<td>-6.76</td>
</tr>
</tbody>
</table>
The molecule 18e shows the hydrogen bond interactions (Figure 9) with TYR 341, ARG 106 and SER 516. Its glide score was found to be -7.88 in comparison to the standard drug celecoxib which exhibited a glide score of -11.29.

![Figure 9. In silico molecular docking of ligand 18e against COX-2 (PBD NO: 3LN1).](image)

The molecule 10c shows the hydrogen bond interactions (Figure 10) with TYR 341 and ARG 106. Its glide score was found to be -8.09 in comparison to the standard drug celecoxib which exhibited a glide score of -11.29.

![Figure 10. In silico molecular docking of ligand 10c against COX-2 (PBD NO: 3LN1).](image)
Figure 11. *In silico* molecular docking of ligand **14d** against COX-2 (PDB NO: 3LN1).

The molecule **14d** shows the hydrogen bond interactions (Figure 11) with TYR 341 and ARG 106. Its glide score was found to be -7.97 in comparison to the standard drug celecoxib which exhibited a glide score of -11.29.

Figure 12. *In silico* molecular docking of ligand **5b** against COX-2 (PDB NO: 3LN1).
The molecule 5b shows the hydrogen bond interactions (Figure 12) with TYR 341 and ARG 106. Its glide score was found to be -7.76 in comparison to the standard drug celecoxib which exhibited a glide score of -11.29.

The ligands 8b, 15d, 19e and 1a do not show Hydrogen bonding (Figure 13) and the interactions are purely hydrophobic and shape driven in nature.

**Figure 13.** In silico molecular docking of ligands 8b, 15d, 19e and 1a against COX-2 (PBD NO: 3LN1).
2.2.2. Molecular docking studies on TNF-α

The *in silico* molecular docking study of these ligands has also been done against TNF-α target by downloading the 2AZ5 ligand from protein data bank (PDB). The binding pocket of 2AZ5 is large and without defined cervices therefore the binding site is a mainly hydrophobic consisting of glycine, leucine, and tyrosine residues. In order to bind to this hydrophobic large pocket, the ligands also need to be hydrophobic in nature and of large size. Before docking the new ligands against the generated grid, the reference ligand was separately docked against the generated grid to validate the grid and docking methodology.

![Figure 14](image)

**Figure 14.** Superimposed binding orientation of the reference ligand (green) and docked reference ligand (maroon) as predicted by Schrodinger glide software.

**Figure 14** shows comparison between the original binding mode of reference ligand against docked binding mode as predicted by Schrodinger Glide software. It can be clearly seen that adapted Schrodinger methodology successfully predicted the binding of crystallographic 2AZ5 ligand with root mean square deviation of 0.003˚A. The **figure 15** shows the structure of the reference ligand 2AZ5.
The molecule 13d shows the hydrogen bond interactions (Figure 16) with GLY 121. Its glide score was found to be -5.44 in comparison to the standard 2AZ5 ligand which exhibited a glide score of –7.1.

The molecule 14d shows the hydrogen bond interactions (Figure 17) with GLY 121. Its glide score was found to be -5.61 in comparison to the standard 2AZ5 ligand which exhibited a glide score of –7.1.
Figure 17. *In silico* molecular docking of ligand 14d against TNF-α protein.

The molecule 15d shows the hydrogen bond interactions (Figure 18) with GLY 121. Its glide score was found to be -5.80 in comparison to the standard 2AZ5 ligand which exhibited a glide score of -7.1.

Figure 18. *In silico* molecular docking of ligand 15d against TNF-α protein.

All the ligands were docked against TNF-α target protein separately. The binding energies range from -29.34 to -45.94 kcal/mol. The predicted glide scores and binding energies are summarized in Table 4.
Table 4. Glide scores of twenty new ligands with 2AZ5.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>Glide Score</th>
<th>Glide Energy</th>
</tr>
</thead>
<tbody>
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</tr>
<tr>
<td>1a</td>
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<tr>
<td>20e</td>
<td>-4.39</td>
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</table>
Hydrogen bonding is not observed in the case of ligands 4a, 7b, 9c and 17e indicating that the interactions (Figure 19) are purely hydrophobic and shape driven in nature.

**Figure 19.** Ligands showing hydrophobic interactions with the TNF-α target protein.
3.0. **Biological activity**

A focused library of twenty compounds has been synthesized and all the compounds have been screened for their anti-inflammatory activity. The compounds 2a and 17e-20e showing significant *in-vivo* anti-inflammatory activity were further screened for their *in-vivo* COX-2, TNF-α, NO and IL-1β inhibitory potential. The compounds showing potent anti-inflammatory activity have further been screened for their analgesic activity by tail immersion method and acetic acid induced writhing test. Finally the ulcerogenic study of the active compounds 2a and 17e-20e has been done to check their tolerance towards gastric mucosa.

3.1. **In vivo anti-inflammatory activity**

All the synthesized compounds were evaluated for their *in vivo* anti-inflammatory activity ([Table 5 and Figure20](#)) by carrageenan induced hind paw edema model. Amongst the twenty compounds, five compounds *viz.* 2a and 17e-20e exhibited significant anti-inflammatory activity with potent percentage inhibition i.e. (58.33, 60.41, 68.75, 55.20, and 58.33 %) in the carrageenan-induced hind paw edema model after 3 h and (52.08, 50.00, 55.20, 41.66 and 52.08 %) after 5 h respectively in comparison to the standard drug indomethacin which exhibited 65.62 % and 60.41 % inhibition after 3 h and 5 h respectively. On the basis of the results obtained from the anti-inflammatory activity, the structure activity relationship (SAR) was deduced on the basis of following points:

- Compounds having substitution on the benzoxazolinone ring showed better activity in comparison to the unsubstituted benzoxazolinone ring i.e. 5CH₃ > 6CH₃ > 6NO₂ >H.

- Compounds having para-substituted -OCH₃ on the phenyl ring attached to the triazolyl ring exhibited better activity in comparison to the unsubstituted phenyl ring which in turn exhibited more activity in comparison to the halogen substituted phenyl ring attached to the triazolyl ring i.e. -OCH₃ > -C₆H₅ > X.

- Amongst the halogens substituted on the phenyl ring attached to the triazolyl ring the compounds containing bulkier group were found to be more potent i.e. Br > Cl > F.

All the data were analysed using one-way ANOVA followed by the Dunnett’s test in the carrageenan induced hind paw edema model in the rats.
**Table 5.** *In vivo* anti-inflammatory activity by the carrageenan induced hind paw edema method.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Change in paw volume (ml) Mean ± SEM</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3hr</td>
<td>5hr</td>
</tr>
<tr>
<td>Control</td>
<td>2ml/kg</td>
<td>0.96±0.06</td>
<td>0.96±0.06</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.05mnoles/kg</td>
<td>0.33±0.04***</td>
<td>0.38±0.04***</td>
</tr>
<tr>
<td>1a</td>
<td>-</td>
<td>0.46±0.07**</td>
<td>0.51±0.04**</td>
</tr>
<tr>
<td>2a</td>
<td>-</td>
<td>0.40±0.12**</td>
<td>0.46±0.09***</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>0.51±0.06*</td>
<td>0.55±0.04**</td>
</tr>
<tr>
<td>4a</td>
<td>-</td>
<td>0.45±0.08**</td>
<td>0.50±0.05**</td>
</tr>
<tr>
<td>5b</td>
<td>-</td>
<td>0.50±0.06*</td>
<td>0.55±0.05**</td>
</tr>
<tr>
<td>6b</td>
<td>-</td>
<td>0.46±0.06**</td>
<td>0.51±0.05**</td>
</tr>
<tr>
<td>7b</td>
<td>-</td>
<td>0.61±0.09</td>
<td>0.65±0.05</td>
</tr>
<tr>
<td>8b</td>
<td>-</td>
<td>0.53±0.08</td>
<td>0.60±0.05*</td>
</tr>
<tr>
<td>9c</td>
<td>-</td>
<td>0.53±0.08</td>
<td>0.56±0.08*</td>
</tr>
<tr>
<td>10c</td>
<td>-</td>
<td>0.45±0.05**</td>
<td>0.55±0.06**</td>
</tr>
<tr>
<td>11c</td>
<td>-</td>
<td>0.66±0.09</td>
<td>0.65±0.09</td>
</tr>
<tr>
<td>12c</td>
<td>-</td>
<td>0.55±0.10</td>
<td>0.61±0.09</td>
</tr>
<tr>
<td>13d</td>
<td>-</td>
<td>0.50±0.09*</td>
<td>0.58±0.06*</td>
</tr>
<tr>
<td>14d</td>
<td>-</td>
<td>0.48±0.12*</td>
<td>0.55±0.08**</td>
</tr>
<tr>
<td>15d</td>
<td>-</td>
<td>0.53±0.12</td>
<td>0.61±0.09</td>
</tr>
<tr>
<td>16d</td>
<td>-</td>
<td>0.51±0.07*</td>
<td>0.60±0.10*</td>
</tr>
<tr>
<td>17e</td>
<td>-</td>
<td>0.38±0.07***</td>
<td>0.48±0.07***</td>
</tr>
<tr>
<td>18e</td>
<td>-</td>
<td>0.30±0.05***</td>
<td>0.43±0.06***</td>
</tr>
<tr>
<td>19e</td>
<td>-</td>
<td>0.43±0.05**</td>
<td>0.56±0.03*</td>
</tr>
<tr>
<td>20e</td>
<td>-</td>
<td>0.40±0.05**</td>
<td>0.46±0.04***</td>
</tr>
</tbody>
</table>

Data is analyzed by one way ANOVA followed by Dunnett’s ‘t’ test and expressed as mean ± SEM from six observations; ***p < 0.001, ** p < 0.01 & *p < 0.05
3.2. *In vivo COX-2 assay*

The compounds exhibiting significant *in vivo* anti-inflammatory activity were screened for their *in vivo* COX-2 inhibitory assay (*Figure 21*). The carrageenan induced edema group exhibited a high level of COX-2 i.e. 13.2 ± 0.20 mole/min/ml. The compound 17e reduced the COX-2 level to 7.8 ± 0.18 mole/min/ml in comparison to the standard drug indomethacin which suppressed the increase in COX-2 level to 8.3 ± 0.16 mole/min/ml. The compounds 2a, 18e, 19e and 20e showed a comparable reduction in the level of COX-2 as compared to indomethacin.
Table 6. Selective COX-2 inhibitory activity of the 1,2,4-triazoles.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$ (µM)</th>
<th>Selectivity index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>COX-1 (µM)</td>
<td>COX-2 (µM)</td>
</tr>
<tr>
<td>2a</td>
<td>127</td>
<td>10.2</td>
</tr>
<tr>
<td>17e</td>
<td>110</td>
<td>2.6</td>
</tr>
<tr>
<td>18e</td>
<td>124</td>
<td>7.5</td>
</tr>
<tr>
<td>19e</td>
<td>132</td>
<td>11.2</td>
</tr>
<tr>
<td>20e</td>
<td>106</td>
<td>5.7</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3.80</td>
<td>7.2</td>
</tr>
</tbody>
</table>

Values are the means ± SEM from three independent experiments using COX assay kits (Cayman Chemicals Inc., Ann Arbor, MI, USA).

The compound 17e (COX-1 IC$_{50}$ = 110 µM; COX-2 IC$_{50}$ = 2.6 µM; SI = 42.30) exhibited potent selective COX-2 inhibition as compared to indomethacin (COX-1 IC$_{50}$ = 3.80 µM; COX-2 IC$_{50}$ = 7.20 µM; SI = 0.53). Selective index (Table 6) of the compounds 2a, 18e, 19e and 20e exhibited the selective nature of these compounds towards COX-2 inhibition as compared to indomethacin.

3.3. In vivo TNF-α assay

The compounds showing significant in-vivo anti-inflammatory activity were further screened for their in-vivo TNF-α activity (Figure 22). The standard drug indomethacin supressed the level of TNF-α to 4.6 ± 0.17 pg/ml from 8.2 ± 0.15 pg/ml in the carrageenan injected group. The compound 19e exhibited potent anti TNF-α activity and reduced the TNF-α level to 4.2 ± 0.18 pg/ml. The other compounds exhibited moderate suppression in the level of TNF-α.
3.4. **In vivo Nitric oxide assay**

The anti-inflammatory activity has also been screened by using *in vivo* nitric oxide (NO) assay. Nitric oxide (NO) is an important signalling molecule, produced as part of the inflammatory response from activated cells and macrophages [143,144]. An increase in the NO level has been previously reported in synovial fluids of patients suffering from rheumatoid arthritis [145]. In the present study, increased NO levels have been detected in carrageenan group similar to those previously reported in synovial fluids of patients with rheumatoid arthritis. Analysis of nitrite estimation is summarised in Figure 23. A significant increase in level of nitrite was observed in carrageenan induced edema group (10.30 ± 0.24 μmol/mg) as compared to control group (3.65 ± 0.14 μmol/mg). All synthesized compounds suppressed the increase in the nitrite level significantly as compared to the edema group. Compound 17e significantly suppressed the increase in the NO level to 5.80 ± 0.18 μmol/mg comparison to indomethacin which showed a reduction to 6.3 ± 0.20 μmol/mg.

![Figure 22. In vivo anti-inflammatory activity by TNF-α assay.](image-url)
3.5. **In vivo IL-1β assay**

The compounds exhibiting significant *in vivo* anti-inflammatory activity have been screened for their *in vivo* anti-inflammatory activity by measuring the IL-1β levels in the serum of the animals (*Figure 24*). Administration of the selected active compounds 2a, 18e, 19e and 20e suppressed the increase in the level of IL-1β significantly when compared with the edema group in which the level of IL-1β was found to be $6.3 \pm 0.18$ pg/ml. The compounds 17e and 18e reduced the IL-1β level to $3.2 \pm 0.14$ pg/ml and $2.9 \pm 0.16$ pg/ml respectively in comparison to indomethacin which showed a reduction to $3.4 \pm 0.15$ pg/ml.
3.6. *In vivo* antinociceptive activity

The compounds showing significant anti-inflammatory activity in comparison to the standard drug indomethacin were further tested for their antinociceptive activity by the writhing test and tail immersion method. The results of the writhing test (Table 7 and Figure 25) indicate that compound 2a, 18e and 20e exhibited potent antinociceptive activity with 47.68, 55.93 and 56.70 % inhibition as compared to the standard drug indomethacin which caused 61.77% inhibition. The results of the tail immersion method are shown in the Table 8.

**Table 7.** *In vivo* antinociceptive activity by the writhing test.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Nos. of Writhes in 10 min.</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2 ml/kg</td>
<td>99.40 ± 2.31</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.05 mmoles/kg</td>
<td>38.00 ± 2.16</td>
<td>61.77%</td>
</tr>
<tr>
<td>2a</td>
<td>-</td>
<td>52.00 ± 2.70</td>
<td>47.68%</td>
</tr>
<tr>
<td>17e</td>
<td>-</td>
<td>64.40 ± 2.15</td>
<td>35.21%</td>
</tr>
<tr>
<td>18e</td>
<td>-</td>
<td>43.80 ± 2.13</td>
<td>55.93%</td>
</tr>
<tr>
<td>19e</td>
<td>-</td>
<td>65.00 ± 2.36</td>
<td>34.60%</td>
</tr>
<tr>
<td>20e</td>
<td>-</td>
<td>43.00 ± 2.16</td>
<td>56.70%</td>
</tr>
</tbody>
</table>

![Figure 25. In vivo antinociceptive activity of novel benzoxazolinone based 1,2,4-triazoles.](image-url)
Table 8. *In vivo* antinociceptive activity by the tail immersion method.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dosage</th>
<th>Basal Reaction time(min)</th>
<th>Reaction time(min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>30</td>
</tr>
<tr>
<td>Control</td>
<td>2ml/Kg</td>
<td>2.48 ± 0.22</td>
<td>2.60 ± 0.21</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.05 moles/Kg</td>
<td>2.50 ± 0.24</td>
<td>3.52 ± 0.19*</td>
</tr>
<tr>
<td>2a</td>
<td>-</td>
<td>2.22 ± 0.13</td>
<td>3.18 ± 0.20</td>
</tr>
<tr>
<td>17e</td>
<td>-</td>
<td>2.62 ± 0.08</td>
<td>3.48 ± 0.24*</td>
</tr>
<tr>
<td>18e</td>
<td>-</td>
<td>2.58 ± 0.21</td>
<td>3.34 ± 0.16</td>
</tr>
<tr>
<td>19e</td>
<td>-</td>
<td>2.84 ± 0.15</td>
<td>3.50 ± 0.13*</td>
</tr>
<tr>
<td>20e</td>
<td>-</td>
<td>2.98 ± 0.14</td>
<td>3.44 ± 0.18*</td>
</tr>
</tbody>
</table>

Data is analyzed by one way ANOVA followed by Dunnett’s ‘t’ test and expressed as mean ± SEM from six observations; ** indicates P < 0.01 & * indicates P < 0.05.

3.7. *Ulcerogenic study*

The compounds showing potential anti-inflammatory and antinociceptive activities were further tested for their gastric ulceration activity (*Table 9 and Figure 26*). When compared with indomethacin, compounds 2a, 17e, 18e, 19e and 20e did not induce any gastric ulceration and rupture of the gastric mucosal layer.

Table 9. Histopathology report of ulcerogenic activity.

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface Epith. Damage</th>
<th>Sup. Mucosal Damage</th>
<th>Deep Mucosal Damage</th>
<th>Muscular Layer Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>2a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17e</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>18e</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>19e</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>20e</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*, No damage; **+, indicates high degree of damage
4.0. Experimental

4.1. Chemistry

All commercial chemicals used as starting materials and reagents were purchased from Merck (India), Spectrochem and Sigma Aldrich and were of AR grade. All melting points were uncorrected and measured using Veego VMP-DS apparatus. IR spectra were recorded as KBr pellets on a Perkin Elmer 1650 spectrophotometer (USA). $^1$H NMR spectra were determined on a Bruker (300 MHz) spectrometer and chemical shifts are expressed as ppm with TMS as internal reference. Mass spectra were recorded on 70 eV (EI Ms-QP 1000EX, Shimadzu, Japan). Column
Chromatography was performed on Silica gel (60-120 mesh). Elemental analysis was carried out using Elementar Vario EL III elemental analyzer. Elemental analysis data is reported in % standard.

4.2. **General procedure for synthesis of benzoxazolinone derivatives:**

The different ortho amino phenol derivatives were dissolved in anhydrous tetrahydrofuran and refluxed with 1,1-carbonyldiimidazole (CDI) for 5-6 h at a temperature of 65 °C. The formation of the four benzoxazolinones was confirmed by comparing their Rf value, m.p. and spectral data with those reported in the literature [68].

4.3. **General procedure for synthesis of acetic acid ethyl ester derivatives:**

Ethylbromoacetate (15 mmol) was added to a solution of benzoxazolinone derivatives (10 mmol) dissolved in 30 mL dry acetone containing (20 mmol) K2CO3 and the mixture was refluxed for 10 h. After the completion of the reaction, the reaction mixture was filtered in hot condition and the filtrate was concentrated under reduced pressure [146]. The final crude product thus obtained was crystallised in a mixture of ethyl acetate containing few drops of n-hexane.
4.4. **General procedure for synthesis of acetic acid hydrazide derivatives:**

The ethanolic solution of the different acetic acid ethyl ester derivatives (10 mmol) was refluxed with hydrazine monohydrate (1.5 mmol) for 3 h. After the completion of the reaction, the reaction mixture was concentrated under reduced pressure and left overnight in the refrigerator. The solid thus obtained was filtered and recrystallised from ethanol [125].

![Chemical structure of acetic acid hydrazide derivatives]

4.5. **General procedure for synthesis of thiosemicarbazides:**

Para substituted arylisothiocyanates (1 mmol) were added into the ethanolic solution of hydrazides (1 mmol) and the reaction mixture was refluxed for 3 h. After the completion of the reaction monitored by TLC, the reaction mixture was concentrated and cooled to get the solid product which was crystallised from ethanol to yield the pure thiosemicarbazide [125].

![Chemical structure of thiosemicarbazides]

4.6. **General procedure for synthesis of 1,2,4-triazoles:**

Triethylamine (1mL) was added to a solution of thiosemicarbazide (10 mmol) in absolute ethanol (40 mL) and refluxed for 6-8 h [125]. After the completion of the reaction monitored by TLC, the reaction mixture was cooled, concentrated and poured on crushed ice. The precipitate thus obtained was filtered off and washed with cold water and crystallised in acetone : water (3:1) to give pure 3-mercapto-1,2,4-triazoles (1a- 20e).
1. 3-(5-Mercapto-4-phenyl-4H-[1,2,4]triazol-3-ylmethyl)-6-nitro-3H-benzooxazol-2-one(1a)

Yield : 70 %

Physical appearance : Green powder

m. p. : 273-274 °C

Rf : 0.57 (toluene: ethyl acetate: formic acid 5:4:1)

IR (KBr) cm\(^{-1}\) : 3043, 1803, 1780, 1626, 1348, 1237, 1152

\(^1\)H NMR (DMSO-d\(_6\), 300 MHz) : \(\delta\) 5.08 (s, 2H), 7.32 (d, 1H, \(J=8.7\) Hz), 7.41-7.48 (m, 5H), 8.21 (d, 1H, \(J=9.0\) Hz), 8.28 (s, 1H), 14.08 (s, 1H)

\(^13\)C NMR (DMSO-d\(_6\), 75 MHz) : \(\delta\) 38.38, 106.36, 109.90, 121.40, 128.22, 130.04, 130.23, 133.21, 136.68, 141.64, 143.18, 146.65, 153.21, 169.30

MS (ESI) m/z : 369 (M)\(^+\)

Elemental Analysis : Molecular formula C\(_{16}\)H\(_{11}\)N\(_5\)O\(_4\)S

Calculated : C, 52.03; H, 3.00; N, 18.96%

Found : C, 52.00; H, 2.98; N, 18.93%
2. 3-(5-Mercapto-4-phenyl-4H-[1,2,4]triazol-3-ylmethyl)-5-methyl-3H-benzooxazol-2-one(2a)

\[
\text{Yield : 73.47%}
\]

Physical appearance : White crystals

m. p. : 267-268 °C

R\(_f\) : 0.70 (toluene : ethyl acetate : formic acid 5:4:1)

IR (KBr) cm\(^{-1}\) : 3366, 3351, 3334, 1743, 1021, 1348

\(^1\)H NMR (DMSO-\(d_6\), 300 MHz) : \(\delta\) 2.30 (s, 3H), 4.95 (s, 2H), 6.86-6.93 (m, 2H), 7.17 (d, 1H, \(J=8.1\) Hz), 7.40-7.49 (m, 5H), 14.01 (s, 1H)

\(^13\)C NMR (DMSO-\(d_6\), 75 MHz) : \(\delta\) 21.46, 37.86, 109.73, 110.17, 123.34, 128.21, 129.99, 130.20, 130.69, 133.21, 133.83, 140.19, 147.25, 153.65, 169.13

MS (ESI) m/z : 339 (M+1)\(^+\)

Elemental Analysis : Molecular formula C\(_{17}\)H\(_{14}\)N\(_4\)O\(_2\)S

Calculated : C, 60.34; H, 4.17; N, 16.56%

Found : C, 60.30; H, 4.15; N, 16.55%

3. 3-(5-Mercapto-4-phenyl-4H-[1,2,4]triazol-3-ylmethyl)-3H-benzooxazol-2-one(3a)

\[
\text{Yield : 60%}
\]

Physical appearance : White crystals

m. p. : 256-257 °C
R_f: 0.81 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) cm\(^{-1}\): 3366, 3351, 3334, 1743, 1021, 1348
\(^1\)H NMR (DMSO-d\(_6\), 300 MHz): δ 5.00 (s, 2H), 7.06-7.10 (m, 2H), 7.13-7.7.21 (m, 2H), 7.28-7.35 (m, 3H), 7.39-7.41 (m, 2H), 14.01 (s, 1H)
\(^{13}\)C NMR (DMSO-d\(_6\), 75 MHz): δ 37.90, 109.92, 110.20, 117.12, 123.17, 124.38, 129.49, 130.59, 130.63, 130.71, 133.83, 142.19, 147.21, 153.33, 161.16
MS (ESI) m/z: 324 (M+1)
Elemental Analysis: Molecular formula C\(_{16}\)H\(_{12}\)N\(_4\)O\(_2\)S
Calculated: C, 52.95; H, 3.73; N, 17.27%
Found: C, 52.94; H, 3.75; N, 17.30%

4. 3-(5-Mercapto-4-phenyl-4H-[1,2,4]triazol-3-ylmethyl)-6-methyl-3H-benzooxazol-2-one(4a)

![Chemical structure of 4a](image)

Yield: 63.63%
Physical appearance: White crystals
m. p.: 235-236 °C
R_f: 0.65 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) cm\(^{-1}\): 3418, 3384, 3334, 1742, 1627, 1387, 1228, 1096
\(^1\)H NMR (DMSO-d\(_6\), 300 MHz): δ 2.32 (s, 3H), 4.96 (s, 2H), 6.79-6.90 (m, 2H), 7.15 (d, 1H, J=8.0 Hz), 7.40-7.74 (m, 5H), 14.01 (s, 1H)
\(^{13}\)C NMR (DMSO-d\(_6\), 75 MHz): δ 21.46, 37.86, 109.73, 110.17, 123.34, 128.21, 129.99, 130.20, 130.69, 133.21, 133.83, 140.19, 147.25, 153.65, 169.13
MS (ESI) m/z: 339 (M+1)
Elemental Analysis: Molecular formula C\(_{17}\)H\(_{14}\)N\(_4\)O\(_2\)S
Calculated : C, 60.34; H, 4.17; N, 16.56%
Found : C, 60.35; H, 4.14; N, 16.58%

5. 3-[4-(4-Fluoro-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-6-methyl-3H-benzooxazol-2-one (5b)

Yield : 52.17 %
Physical appearance : White crystals
m. p. : 285-286 °C
Rf : 0.63 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) cm⁻¹ : 3044, 1792, 1622, 1325, 1263, 1172
¹H NMR (DMSO-d₆, 300 MHz) : δ 2.32 (s, 3H), 4.97 (s, 2H), 6.92-7.01 (m, 2H), 7.16 (s, 1H), 7.33-7.43 (m, 4H), 14.04 (s, 1H)
¹³C NMR (DMSO-d₆, 75 MHz) : δ 21.33, 37.88, 109.53, 110.67, 116.80, 124.65, 128.25, 129.51, 130.55, 130.68, 132.88, 142.26, 147.25, 153.45, 161.17, 169.27
MS (ESI) m/z : 357 (M+1)+
Elemental Analysis : Molecular formula C₁₇H₁₃FN₄O₂S
Calculated : C, 57.29; H, 3.68; N, 15.72%
Found : C, 57.31; H, 3.66; N, 15.70%
6. 3-[4-(4-Fluoro-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-5-methyl-3H-benzooxazol-2-one (6b)

Yield : 83.07 
Physical appearance : White crystals
m. p. : 274-275 °C
Rf : 0.60 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) (cm\(^{-1}\)) : 3443, 1742, 1627, 1318, 1228, 1096
\(^1\)H NMR (DMSO-\(d_6\), 300 MHz) : \(\delta\) 2.29 (s, 3H), 4.95 (s, 2H), 6.92 (d, 2H, \(J=6.9\) Hz), 7.12 (d, 2H, \(J=6.6\) Hz), 7.33- 7.43 (m, 3H), 14.01 (s, 1H)
\(^13\)C NMR (DMSO-\(d_6\), 75 MHz) : \(\delta\) 21.43, 37.79, 109.78, 110.18, 123.35, 129.53, 130.65, 130.75, 133.87, 133.80, 140.19, 147.29, 153.62, 161.20, 169.31
MS (ESI) m/z : 357 (M+1)
Elemental Analysis : Molecular formula C\(_{17}\)H\(_{13}\)F\(_4\)N\(_4\)O\(_2\)S
Calculated : C, 57.29; H, 3.68; N, 15.72%
Found : C, 57.30; H, 3.70; N, 15.71%

7. 3-[4-(4-Fluoro-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-3H-benzooxazol-2-one (7b)
Yield: 65.21 %

Physical appearance: White crystals

m. p.: 277-278 °C

R_f: 0.56 (toluene : ethyl acetate : formic acid 5:4:1)

IR (KBr) cm\(^{-1}\): 3032, 1734, 1569, 1229, 1156

\(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta\) 5.00 (s, 2H), 7.06-7.21 (m, 3H), 7.29-7.43 (m, 5H), 14.01 (s, 1H)

\(^13\)C NMR (DMSO-d\(_6\), 75 MHz): \(\delta\) 37.89, 109.93, 110.19, 123.16, 124.38, 129.49, 130.59, 130.63, 130.71, 142.17, 147.21, 153.33, 161.16, 169.30

MS (ESI) m/z: 343 (M+1)^+

Elemental Analysis: Molecular formula C\(_{16}\)H\(_{11}\)FN\(_4\)O\(_2\)S

Calculated: C, 56.13; H, 3.24; N, 16.37%

Found: C, 56.15; H, 3.23; N, 16.35%

8. 3-[4-(4-Fluoro-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-6-nitro-3H-benzooxazol-2-one(8b)

Yield: 57.81 %

Physical appearance: Green crystals

m. p.: 280-281 °C

R_f: 0.66 (toluene : ethyl acetate : formic acid 5:4:1)

IR (KBr) cm\(^{-1}\): 3347, 3092, 1625, 1448, 1347, 1246, 1152

\(^1\)H NMR (DMSO-d\(_6\), 300 MHz): \(\delta\) 5.07 (s, 2H), 7.01 (d, 2H, \(J\)=8.5 Hz), 7.30-7.31 (m, 3H), 8.20 (dd, 1H, \(J\)=9.1, 2.0 Hz), 8.29 (d, 1H, \(J\)=2.1 Hz), 14.01 (s, 1H)
$^{13}\text{C NMR (DMSO-d}_6, 75 \text{ MHz)}: \delta 38.38, 106.36, 109.90, 121.40, 128.22, 130.04, 130.23, 133.21, 136.68, 141.64, 143.18, 146.65, 153.21, 169.30$

MS (ESI) m/z : 388 (M+1)$^+$

Elemental Analysis : Molecular formula $C_{16}H_{10}FN_5O_4S$

Calculated : C, 49.61; H, 2.60; N, 18.08%

Found : C, 49.63; H, 2.58; N, 18.06%

9. 3-[(4-Chloro-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-6-methyl-3H-benzooxazol-2-one (9c)

Yield : 67.50 %

Physical appearance : White crystals

m. p. : 242-243 °C

$R_f$ : 0.69 (toluene : ethyl acetate : formic acid 5:4:1)

IR (KBr) cm$^{-1}$ : 3328, 1626, 1495, 1324, 1249, 1094

$^1\text{H NMR (DMSO-d}_6, 300 \text{ MHz)}: \delta 2.31$ (s, 3H), 4.98 (s, 2H), 6.95-7.00 (m, 2H), 7.15 (s, 1H), 7.38 (d, 2H, $J$=8.7 Hz), 7.55 (d, 2H, $J$=8.4 Hz), 14.05 (s, 1H)

$^{13}\text{C NMR (DMSO-d}_6, 75 \text{ MHz): \delta 20.84, 37.31, 109.09, 110.16, 124.13, 127.74, 129.56, 129.63, 131.59, 132.39, 134.44, 141.75, 146.66, 152.95, 168.62}$

MS (ESI) m/z : 373(M+1)$^+$

Elemental Analysis : Molecular formula $C_{17}H_{13}CIN_4O_2S$

Calculated : C, 54.77; H, 3.51; N, 15.03%

Found : C, 54.75; H, 3.53; N, 15.05%
10. 3-\{4-(4-Chloro-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-5-methyl-3H-benzooxazol-2-one\}(10c)

\[
\text{Yield} : 42.24\% \\
\text{Physical appearance} : \text{White crystals} \\
\text{m. p.} : 237-238^\circ\text{C} \\
\text{R}_f : 0.60 (toluene : ethyl acetate : formic acid 5:4:1) \\
\text{IR (KBr) cm}^{-1} : 3443, 1742, 1627, 1318, 1228, 1096 \\
\text{\textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 300 MHz)} : \delta 2.30 (s, 3H), 4.97 (s, 2H), 6.84 (s, 1H), 6.92 (d, 1H, \textit{J}=8.4 \text{ Hz}), 7.17 (d, 1H, \textit{J}=8.1 \text{ Hz}), 7.42 (d, 2H, \textit{J}=8.7 \text{ Hz}), 7.56 (d, 2H, \textit{J}=8.7 \text{ Hz}), 14.04 (s, 1H) \\
\text{\textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 75 MHz)} : \delta 21.45, 37.79, 109.75, 110.17, 110.48, 123.18, 123.56, 130.04, 130.33, 133.79, 134.99, 140.21, 147.16, 153.64, 169.25 \\
\text{MS (ESI) m/z} : 373 (M+1)^+ \\
\text{Elemental Analysis} : \text{Molecular formula C}_{17}\text{H}_{13}\text{ClN}_4\text{O}_2\text{S} \\
\text{Calculated} : \text{C}, 54.77; \text{H}, 3.51; \text{N}, 15.03\% \\
\text{Found} : \text{C}, 54.78; \text{H}, 3.52; \text{N}, 15.04\%
11. 3-[4-(4-Chloro-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-3H-benzooxazol-2-one(11c)

Yield : 65.00 %
Physical appearance : White crystals
m. p. : 282-283 °C
Rf : 0.58 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) cm\(^{-1}\) : 3327, 1738, 1567, 1477, 1323, 1104
\(^1\)H NMR (DMSO-d\(_6\), 300 MHz) : δ 5.01 (s, 2H), 7.06-7.18 (m, 3H), 7.30 (d, 1H, \(J=8.1\) Hz), 7.40 (d, 2H, \(J=8.4\) Hz), 7.55 (d, 2H, \(J=8.4\) Hz), 14.00 (s, 1H)
\(^13\)C NMR (DMSO-d\(_6\), 75 MHz) : δ 37.87, 109.96, 110.20, 123.18, 124.38, 130.06, 130.19, 130.62, 132.11, 134.95, 142.16, 147.12, 153.35, 169.13
MS (ESI) m/z : 359 (M+1)\(^+\), 357(M-1)\(^+\)
Elemental Analysis : Molecular formula C\(_{16}\)H\(_{11}\)ClN\(_4\)O\(_2\)S
Calculated : C, 53.56; H, 3.09; N, 15.61%
Found : C, 53.58; H, 3.11; N, 15.60%
12. 3-[4-(4-Chloro-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-6-nitro-3H-benzooxazol-2-one(12c)

Yield : 60.93 %
Physical appearance : Green crystals
m. p. : 256-257°C
R_f : 0.69 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) cm⁻¹ : 3426, 3347, 1625, 1516, 1347, 1246
¹H NMR (DMSO-d₆, 300 MHz) : δ 5.08 (s, 2H), 7.31 (d, 1H, J=8.7 Hz), 7.42-7.49 (m, 4H), 8.21 (d, 1H, J=9.0 Hz), 8.29 (s, 1H), 14.09 (s, 1H)
¹³C NMR (DMSO-d₆, 75 MHz) : δ 38.35, 102.94, 106.35, 109.96, 121.31, 125.67, 129.49, 136.69, 141.65, 143.12, 146.65, 153.22, 160.38, 169.53
MS (ESI) m/z : 404 (M+1)^+
Elemental Analysis : Molecular formula C₁₆H₁₀ClN₅O₄S
Calculated : C, 47.59; H, 2.50; N, 17.34%
Found : C, 47.57; H, 2.52; N, 17.35%
13. 3-{4-(4-Bromo-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-6-methyl-3H-benzooxazol-2-one(13d)

Yield : 61.03 %
Physical appearance : White crystals
m. p. : 240-241 ºC
Rf : 0.60 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) cm\(^{-1}\) : 3426, 3347, 1635, 1503, 1322, 1238
\(^1\)H NMR (DMSO-d\(_6\), 300 MHz) : \(\delta\) 2.32 (s, 3H), 4.99 (s, 2H), 6.92-7.00 (m, 2H), 7.15 (s, 1H), 7.32 (d, 2H, \(J=8.4\) Hz), 7.68 (d, 2H, \(J=8.7\) Hz), 14.05 (s, 1H)
\(^13\)C NMR (DMSO-d\(_6\), 75 MHz) : \(\delta\) 21.36, 37.86, 109.57, 110.66, 123.57, 124.63, 128.24, 130.37, 132.51, 132.89, 133.01, 142.23, 147.14, 153.46, 169.03
MS (ESI) m/z : 417 (M+2)\(^+\), 418 (M+3)\(^+\)
Elemental Analysis : Molecular formula C\(_{17}\)H\(_{13}\)BrN\(_4\)O\(_2\)S
Calculated : C, 48.93; H, 3.14; N, 13.43%
Found : C, 48.95; H, 3.15; N, 13.45%
14. 3-[4-(4-Bromo-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-5-methyl-3H-benzo[1,4]oxazol-2-one(14d)

Yield: 46.25 %
Physical appearance: White crystals
m. p.: 249-250 °C
Rf: 0.66 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) cm⁻¹: 3228, 1762, 1622, 1567, 1219, 1139
¹H NMR (DMSO-d₆, 300 MHz): δ 2.30 (s, 3H), 4.97 (s, 2H), 6.84 (s, 1H), 6.92 (d, 1H, J=8.1 Hz), 7.18 (d, 1H, J=7.8 Hz), 7.36 (d, 2H, J=8.4 Hz), 7.69 (d, 2H, J=8.4 Hz), 14.06 (s, 1H)
¹³C NMR (DMSO-d₆, 75 MHz): δ 21.49, 37.77, 109.76, 110.19, 123.35, 123.61, 130.59, 132.58, 133.01, 133.78, 140.18, 147.15, 153.64, 169.10
MS (ESI) m/z: 417 (M+1)⁺, 419 (M+3)⁺
Elemental Analysis: Molecular formula C₁₇H₁₃BrN₄O₂S
Calculated: C, 48.93; H, 3.14; N, 13.43%
Found: C, 48.91; H, 3.15; N, 13.42%
15. 3-{4-(4-Bromo-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-3H-benzooxazol-2-one(15d)

Yield : 59.70 %

Physical appearance : White crystals

m. p. : 257-258 °C

R<sub>f</sub> : 0.58 (toluene : ethyl acetate : formic acid 5:4:1)

IR (KBr) cm<sup>-1</sup> : 3433, 3322, 1655, 1521, 1310, 1215

<sup>1</sup>H NMR (DMSO-<sup>d</sup>6, 300 MHz) : δ 4.66 (s, 2H), 7.16-7.19 (m, 3H), 7.38 (d, 1H, J=7.8 Hz), 7.89 (d, 2H, J=8.7 Hz), 8.24 (d, 2H, J=8.1 Hz), 14.01 (s, 1H)

<sup>13</sup>C NMR (DMSO-<sup>d</sup>6, 75 MHz) : δ 37.87, 109.47, 110.68, 122.57, 124.53, 128.24, 130.37, 132.51, 132.89, 132.91, 142.23, 147.24, 153.44, 169.15

MS (ESI) m/z : 403 (M+1)<sup>+</sup>, 405 (M+3)<sup>+</sup>

Elemental Analysis : Molecular formula C<sub>16</sub>H<sub>11</sub>BrN<sub>4</sub>O<sub>2</sub>S

Calculated : C, 47.66; H, 2.75; N, 13.89%

Found : C, 47.64; H, 2.76; N, 13.91%
16. 3-[4-(4-Bromo-phenyl)-5-mercapto-4H-[1,2,4]triazol-3-ylmethyl]-6-nitro-3H-benzooxazol-2-one(16d)

\[
\begin{align*}
\text{Br} & \quad \text{N} \\
\text{SH} & \quad \text{N} \\
\text{O}_2\text{N} & \quad \text{N} \\
\text{O} & \quad \text{O}
\end{align*}
\]

**Yield**: 59.09 %

**Physical appearance**: Green crystals

**m. p.**: 246-247 °C

**Rf**: 0.60 (toluene : ethyl acetate : formic acid 5:4:1)

**IR (KBr) cm\(^{-1}\)**: 3343, 1732, 1637, 1322, 1234, 1156

**\(^1\)H NMR (DMSO-\text{d}_6, 300 MHz)**: \(\delta 5.07\) (s, 2H), 7.33-7.48 (m, 4H), 8.21-8.29 (m, 3H), 14.09 (s, 1H)

**\(^{13}\)C NMR (DMSO-\text{d}_6, 75 MHz)**: \(\delta 38.87, 110.37, 111.56, 122.57, 123.51, 127.24, 131.24, 132.51, 132.89, 132.91, 142.23, 147.24, 153.30, 168.04\)

**MS (ESI) m/z**: 448 (M+1)^+, 450 (M+3)^+

**Elemental Analysis**: Molecular formula \(\text{C}_{16}\text{H}_{10}\text{BrN}_{5}\text{O}_{4}\text{S}\)

**Calculated**: C, 42.87; H, 2.25; N, 15.62%

**Found**: C, 42.85; H, 2.26; N, 15.64%
17. 3-{5-Mercapto-4-(4-methoxy-phenyl)-4H-[1,2,4]triazol-3-ylmethyl]-6-methyl-3H-benzoxazol-2-one(17e)

Yield: 63.15 %
Physical appearance: White crystals
m. p.: 219-220 °C
Rf: 0.59 (toluene : ethyl acetate : formic acid 5:4:1)
IR (KBr) cm⁻¹: 3124, 3003, 1607, 1475, 1322, 1254, 1180
¹H NMR (DMSO-d₆, 300 MHz): δ 2.31 (s, 3H), 4.94 (s, 2H), 3.78 (s, 3H), 6.94-7.01 (m, 4H), 7.15 (s, 1H), 7.24 (d, 2H, J=8.7 Hz), 13.95 (s, 1H)
¹³C NMR (DMSO-d₆, 75 MHz): δ 21.32, 37.92, 55.89, 109.55, 110.61, 115.13, 124.59, 125.67, 128.37, 129.34, 132.76, 142.27, 147.47, 153.50, 160.31, 169.34
MS (ESI) m/z: 369 (M⁺)
Elemental Analysis: Molecular formula C₁₈H₁₆N₄O₃S
Calculated: C, 58.68; H, 4.38; N, 15.21%
Found: C, 58.66; H, 4.40; N, 15.23%
18. 3-[5-Mercapto-4-(4-methoxy-phenyl)-4H-[1,2,4]triazol-3-ylmethyl]-5-methyl-3H-benzooxazol-2-one (18e)

![Chemical Structure]

**Yield**: 44.12%

**Physical appearance**: White crystals

**m. p.**: 267-268 °C

**Rf**: 0.63 (toluene : ethyl acetate : formic acid 5:4:1)

**IR (KBr) cm⁻¹**: 3236, 3120, 1622, 1455, 1302, 1264, 1150

**¹H NMR (DMSO-d₆, 300 MHz)**: δ 2.29 (s, 3H), 3.78 (s, 3H), 4.92 (s, 2H), 6.83 (s, 1H), 6.91 (d, 1H, J=7.8 Hz), 7.01 (d, 2H, J=8.1 Hz), 7.18 (d, 1H, J=8.1 Hz), 7.23 (d, 2H, J=8.1 Hz), 13.95 (s, 1H)

**¹³C NMR (DMSO-d₆, 75 MHz)**: δ 21.33, 37.86, 55.79, 109.43, 110.58, 115.13, 124.62, 125.67, 128.47, 129.45, 132.69, 142.27, 147.47, 153.43, 160.27, 169.21

**MS (ESI) m/z**: 369 (M+1)⁺

**Elemental Analysis**: Molecular formula C₁₈H₁₆N₄O₃S

**Calculated**: C, 58.68; H, 4.38; N, 15.21%

**Found**: C, 58.69; H, 4.41; N, 15.20%
19. 3-{5-Mercapto-4-(4-methoxy-phenyl)-4H-[1,2,4]triazol-3-ylmethyl]-3H-benzooxazol-2-one(19e)

\[
\begin{align*}
\text{Yield} & : 57.85 \% \\
\text{Physical appearance} & : \text{White crystals} \\
\text{m. p.} & : 232-233 ^\circ \text{C} \\
R_f & : 0.61 (\text{toluene : ethyl acetate : formic acid 5:4:1}) \\
\text{IR (KBr) cm}^{-1} & : 3312, 1654, 1545, 1245, 1132 \\
^1\text{H NMR (DMSO-d}_6, 300 \text{ MHz}) & : \delta 3.75 (s, 3H), 5.01 (s, 2H), 7.02 (1H, d, J=6.6 Hz), 7.14-7.20 (m, 2H), 7.33-7.41 (m, 3H), 7.52 (t, 2H, J=6.9 Hz), 14.15 (s, 1H) \\
^{13}\text{C NMR (DMSO-d}_6, 75 \text{ MHz}) & : \delta 37.89, 55.79, 109.56, 110.80, 115.14, 124.56, 125.65, 128.27, 129.24, 132.75, 142.37, 147.47, 153.50, 161.32, 169.42 \\
\text{MS (ESI) m/z} & : 355(M+1)^+ \\
\text{Elemental Analysis} & : \text{Molecular formula C}_{17}H_{14}N_{4}O_{3}S \\
\text{Calculated} & : \text{C}, 57.62; \text{H}, 3.98; \text{N}, 15.81\% \\
\text{Found} & : \text{C}, 57.60; \text{H}, 3.97; \text{N}, 15.79\%
\end{align*}
\]
20. 3-\(\text{Mercapto-4-(4-methoxy-phenyl)-4H-[1,2,4]triazol-3-ylmethyl]-6-nitro-3H-benzooxazol-2-one}\((20e)\)

\[
\begin{align*}
\text{Yield} & : 59.99 \% \\
\text{Physical appearance} & : \text{White crystals} \\
\text{m. p.} & : 284-285 ^\circ \text{C} \\
\text{R}_f & : 0.55 \text{ (toluene : ethyl acetate : formic acid 5:4:1)} \\
\text{IR (KBr) cm}^{-1} & : 3347, 1625, 1516, 1448, 1347, 1246 \\
\text{\(1^H\) NMR (DMSO-\text{d}_6, 300 MHz)} & : \delta 3.76 \text{ (s, 3H)}, 5.07 \text{ (s, 2H)}, 7.00 \text{ (d, 2H, J=8.7 Hz)}, 7.28-7.33 \text{ (m, 3H)}, 8.20 \text{ (dd, 1H, J=9.0, 2.1 Hz)}, 8.28 \text{ (d, 1H, J=1.8 Hz)}, 14.01 \text{ (s, 1H)} \\
\text{\(13^C\) NMR (DMSO-\text{d}_6, 75 MHz)} & : \delta 38.35, 55.58, 102.94, 106.35, 109.96, 115.20, 121.31, 125.67, 129.49, 136.69, 141.12, 143.12, 146.65, 153.22, 160.38, 169.53 \\
\text{MS (ESI) m/z} & : 400(M+1)^{+}, 398(M-1)^{+} \\
\text{Elemental Analysis} & : \text{Molecular formula } \text{C}_{17}\text{H}_{13}\text{N}_5\text{O}_5\text{S} \\
\text{Calculated} & : \text{C}, 51.12; \text{H}, 3.28; \text{N}, 17.54\% \\
\text{Found} & : \text{C}, 51.13; \text{H}, 3.30; \text{N}, 15.52\%. 
\end{align*}
\]

5.0. **Methodology used for the *in silico* molecular docking studies**

5.1. **In silico molecular docking against COX-2**

The methodology used for the *in silico* molecular docking against COX-2 protein has already been described in the section A of this chapter.

5.2. **In silico molecular docking against TNF-\(\alpha\)**

The methodology used for the *in silico* molecular docking against TNF-\(\alpha\) protein is same as the one used in the section A of this chapter.
6.0. Biological activity

6.1. Anti-inflammatory assay

The in vivo anti-inflammatory activity has been done using carrageenan-induced hind paw edema method. This method has been described in detail in the section A of this chapter.

6.2. Assay for in vivo TNF-α, IL-1β and COX-2

Levels of the proinflammatory cytokines (IL-1β and TNF-α) [147] and COX-2 [148] in the serum have been determined by using commercially available ELISA kits (eBioscience and Cayman, USA). Assays have been performed in duplicate in accordance to the manufacturer’s guidelines. Cytokine concentrations were expressed as picograms of antigen per millilitre of protein.

6.3. Assay for in vivo NO

Animals were sacrificed and their hind paw tissues were washed with PBS (pH 7.4) and placed on ice as described earlier [149]. 50 µL of the sample was added to 100 µL of Griess reagent and reaction mixture was incubated for about 5-10 minutes at room temperature and protected from light. The optical density was measured at 540 nm in microplate reader according to the reagent manufacturer’s protocol. Calculations were done after generating a standard curve for sodium nitrite in the same buffer as used for preparation of homogenate.

6.4. Antinociceptive activity

6.4.1. Writhing test

This writhing test has been described in detail in the section A of this chapter.

6.4.2. Tail immersion method

The tail immersion method has been described in detail in the section A of this chapter.

6.5. Ulcerogenic activity

The method used for performing the ulcerogenic activity has been described in section A of this chapter.
7.0. Conclusion

We have synthesized a focussed library of twenty compounds comprising benzoxazolinone and 1,2,4-triazole moieties conjugated through a methylene linkage and evaluated them for their anti-inflammatory and antinociceptive activities. The compounds 2a and 17e-20e exhibited potent anti-inflammatory and antinociceptive activities. The compound 17e (COX-1 IC$_{50}$ = 110 µM; COX-2 IC$_{50}$ = 2.60 µM; SI = 42.30) exhibited potent selective COX-2 inhibition as compared to indomethacin (COX-1 IC$_{50}$ = 3.80 µM; COX-2 IC$_{50}$ = 7.20 µM; SI = 0.53). The SI values of the other molecules shows that these molecules can be considered as potent anti-inflammatory agents as predicted by their COX-1/COX-2 selective index. The results of in-vivo TNF-α activity indicated that the compound 19e exhibited a more potent TNF-α inhibition than the standard drug indomethacin. The compound 17e was found to exhibit potent reduction in the level of NO as compared to indomethacin. The compounds 2a and 17e-20e exhibited potent activity without inducing any gastric ulceration as compared to indomethacin.