1.0. Review of Literature

1.1. Pharmacological significance of 2-benzoxazolinone nucleus

2-benzoxazolinone is a cyclic isostere of coumarin whose antimicrobial activity [1,2] has been extensively investigated. African armyworm, *Spodoptera exempta* larvae feed exclusively on graminaceous plants. It was observed that *Zea mays*, a favoured host plant for *Spodoptera exempta* discouraged the feeding of its larva. The active antifeedant principle responsible for this phenomenon was identified as 6-methoxy-2-benzoxazolinone [3]. This compound was previously reported as a host plant resistant factor against European corn borer in *Z. mays* [4]. The identification of 4-acetylbenzoxazolin-2(3H)-one was reported from kernels of a special *Zea mays* hybrid line [5]. This hybrid is tolerant to *Fusarium raminearum* and has insecticidal activity against *Sitophilus zeamais* [6]. A biosynthetic relationship has also been postulated between 4-acetylbenzoxazolin-2(3H)-one, benzoxazolin-2(3H)-one and 6-methoxybenzoxazolin-2(3H)-one in gramineous plants [6-7]. Many investigations on 2-benzoxazolinones showed that compounds with this structure have analgesic, anti-inflammatory, antineoplastic, anticonvulsant and antimicrobial activities [8-15].

It has been reported that chlorinated 2-benzoxazolinone compounds have valuable fungicidal properties and are therefore suitable for the protection of organic material from attack by fungi and from damage due to rot [16]. The literature also reveals that 6-acyl-2-benzoxazolinone derivatives show significant analgesic activity [17]. Zoxazolamine, a benzoxazole analogue, is mainly used as a skeletal muscle relaxant [18]. Moreover, some benzoxazole derivatives have been demonstrated to be potent antimicrobial [19], anti HIV [20], analgesic [21], anti-inflammatory [21] and anticancer agents [22].

It has also been shown that different pharmacodynamic moieties of benzoxazolinones possess potent biological activities such as dopamine receptor agonist [23], cardiotonic [24], antihypertensive [25] and antiulcer activities [26]. A novel series of Mannich bases of 5-nitro-3-substituted piperazinomethyl-2-benzoxazolinones was synthesized and the compounds were examined [27] for their *in vivo* anti-inflammatory and analgesic activities in two different bioassays namely, carrageenan-induced hind paw edema and *p*-benzoquinone-induced abdominal
constriction tests in mice respectively. 4-(5-chloro-2-oxo-3H-benzoazol-3-yl) butanamide derivatives were synthesized and screened for their analgesic and anti-inflammatory activities [28] as well as gastric ulceration potential. Besides these the free-radical scavenging activities of 2-benzoxazolinone derivatives containing thiosemicarbazide, triazole, thiadiazole and hydrazone moieties has also been reported [29].

1.2. General methods for the synthesis of 2-benzoxazolinone nucleus

Scheme 1

Reaction of o-nitro phenol with carbon monoxide catalysed by selenium in presence of DBU and triethylamine generates benzoxazolinone in a one pot synthesis [30].

\[
\text{OH} \quad + \quad 3 \text{CO} \xrightarrow{\text{Se, DBU, Et}_3\text{N}} 360^\circ \text{C} \quad 7\text{h} \quad \text{O} \quad + \quad 2\text{CO}_2
\]

Scheme 2

The methyl ester (1) of 4-methoxysalicylic acid on treatment with hydrazine hydrate produces the hydrazone (2) which is converted into a keto azide (3) on diazotisation with sodium nitrite. The pyrolysis of this keto azide in xylene affords the isocyanate intermediate by Curtius rearrangement which is finally converted into 6-methoxy-2-benzoxazolinone (MBOA) [31].

Scheme 3

The nitration of 3-hydroxyacetophenone (1) followed by reduction with hydrogen resulted into 2-amino-3-hydroxyacetophenone (2) which on treatment with bis(trichloromethyl)carbonate and triethylamine in dry THF gave 4-acetylbenzoxazolin-2(3H)-one [32].
Scheme 4

Benzoxazolinones can be synthesized by either the fusion of substituted $o$-aminophenols with urea [33] or reaction with phosgene [34].

Scheme 5

A solution of an appropriate $o$-aminophenol and 1,1-carbonyldiimidazole in anhydrous THF was refluxed for 4 hours. The workup of the reaction resulted in the final product in high yield [35].

Scheme 6

The condensation of excess 2-hydroxy-4-ethoxyphenylammonium chloride with urea in 1,3-butanediol solvent yielded 6-methoxy-2-benzoxazolinone (MBOA) in significant quantity [36].
1.3. **Recent advancements on 1,2,3-Triazoles**

In medicinal chemistry one of the most important attention has been paid to the synthesis of 1,2,3-triazoles condensed with other heterocycles and investigation of their biological activity. Even in 1935 research was started on the possibility of using 1,2,3-triazolo[4,5-\(d\)] pyrimidines (8-azapurines) as chemotherapeutic agents for the treatment of various diseases and particularly malignant tumors [37]. 1,2,3-triazoles have been shown to possess a number of desirable features in the context of medicinal chemistry. For example triazoles are not only stable to acid and basic hydrolysis but also to reductive and oxidative conditions, which indicates their high aromatic stabilization. 1,2,3-triazole moiety are attractive units because they are stable to metabolic degradation and capable of hydrogen bonding, which can be favourable in the binding of biomolecular targets and can improve their solubility [38]. 1,2,3-triazoles have a high dipole moment (about 5 D) [39] and are able to participate actively in hydrogen bond formation as well as in dipole–dipole and \( \pi \) stacking interactions [40]. The 1,2,3-triazole moiety does not occur in nature, although the synthetic molecules which contain 1,2,3-triazole units show diverse biological activities. Many 1,2,3-triazoles act as potent antimicrobial [41,42], analgesic [43], anti-inflammatory, local anesthetic [44], anticonvulsant [45] and anti-neoplastic [46] agents.

Triazoles are heterocyclic compounds (Figure 1) featuring five member ring of two carbon atoms and three nitrogen atoms as part of the aromatic five-membered ring. Triazole refers either one of a pair of isomeric chemical compounds with molecular formula C\(_2\)H\(_3\)N\(_3\).

![Figure 1. Triazoles](image.png)

The search for new biologically active compounds in the series of condensed 1,2,3-triazoles is still continuing. Thus for example, substances acting against the hepatitis C virus [47] and compounds inhibiting benzodiazepine and adenosine
receptors [48,49] were found. Some biologically active compounds (Figure 2) containing 1,2,3-triazoles are shown below [50].

![Molecules containing 1,2,3-triazoles showing different activities.](image1.png)

**Figure 2.** Molecules containing 1,2,3-triazoles showing different activities.

Potential pharmaceuticals (Figure 3) based on 1,2,3-triazoles include the anticancer compound carboxamidotriazole (CAI) [51], the nucleoside derivative non-nucleoside reverse transcriptase inhibitor tert-butylidimethylsilylspiroaminoxathioledioxide (known as TSAO), β-lactum antibiotic Tazobactum [52] and Cefatrizine.

![Potential pharmaceuticals based on 1,2,3-triazoles.](image2.png)

**Figure 3.** Potential pharmaceuticals based on 1,2,3-triazoles

In the present scenario of a continuous requirement for better drugs in shorter times, it is a challenging task to prepare new molecules that combine high activity and selectivity, drug-likeness and good pharmacokinetic properties.
1.4. **Methods for the Synthesis of 1,2,3-Triazoles**

**Scheme 1**

The most popular reaction that has been adapted to produce the 1,2,3-triazole moiety is the 1,3-dipolar cycloaddition also known as Huisgen cycloaddition, between an azide and a terminal alkyne. This reaction was discovered at the beginning of the 20th century, but the potential of this reaction and its mechanism were only unveiled in the 1960s by Huisgen [53].

\[
\begin{array}{c}
\text{R}_1 \equiv \equiv + \text{N}_3 \text{R}_2 \xrightarrow{\text{Cu (I)}} \text{N}_3 \equiv \equiv \text{N}_3 \text{R}_2 \\
\end{array}
\]

The use of copper as a catalyst rejuvenated the Huisgen reaction. The standard catalytic system uses copper (II) salts e.g. copper sulfate pentahydrate [54] or copper acetate [55] in the presence of a reducing agent, such as sodium ascorbate or metallic copper [54]. This constantly reduces copper (II) to copper (I) maintaining significantly high levels of the catalytic species. A mixture of tert-butanol and water is used as solvent, as under these conditions it is not necessary to use a base to generate the copper acetylide species. It is important to stress that this solvent can also be used for lipophilic compounds.

**Scheme 2**

A palladium catalysed synthesis of 1,2,3-triazoles from alkenyl halides and sodium azides [56] added a new chapter in the Palladium chemistry.

\[
\begin{array}{c}
\text{Ar} \equiv \equiv \text{Br} + \text{NaN}_3 \xrightarrow{\text{Pd}_2\text{dba}_3 / \text{Xanthophos} / \text{Dioxane} 90 \degree \text{C} \; \text{14hr}} \text{HN} \equiv \equiv \text{Ar} \\
\end{array}
\]

**Scheme 3**

The reaction of terminal alkynes with benzyl or alkyl halides and sodium azide in ethanol gives 1,4 disubstituted 1,2,3-triazoles in good yields [57]. It is catalysed by copper immobilized on 3-aminopropyl functionalised silica gel.
Scheme 4

An efficient one pot synthesis of 1,2,3-triazole linked glycoconjugates involving 1,3-dipolar cycloaddition in presence of Cu(I) as a catalyst has been reported [58]. It is an easy method to prepare neoglycoconjugates derived from unprotected saccharides or peracetylated saccharides.

Scheme 5

Pankaja Kadaba [59] synthesized a family of closely related 1,2,3-triazoles as anticonvulsant agents in which the dicarboximide moiety was lacking from the triazole ring, unlike the traditional anticonvulsant agents.

Scheme 6

Terminal alkyne on reaction with iodosobenzene and mixture of sodium azide, cuprous iodide and sodium ascorbate gives 1,2,3-triazole [60].
1.5. **Present work**

In view of the biological importance of benzoxazolinone and 1,2,3-triazoles as anti-inflammatory and antinociceptive agents, we aim to conjugate these two important ligands under one construct through a methylene linkage. Hitherto for the first time, we report the synthesis (Figure 4) of benzoxazolinone and 1,2,3-triazole based *bis*-heterocycles and their anti-inflammatory and analgesic activities.

**Figure 4. Schematic representation of the synthetic route**
Table 1. Pharmacological significance of 1,2,3-triazoles.

<table>
<thead>
<tr>
<th>Sno.</th>
<th>Structure</th>
<th>Activity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><img src="image1.png" alt="Structure 1" /></td>
<td>Antituberculosis</td>
<td>[61]</td>
</tr>
<tr>
<td>2</td>
<td><img src="image2.png" alt="Structure 2" /></td>
<td>Antituberculosis</td>
<td>[62]</td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Structure 3" /></td>
<td>HIV protease inhibitors</td>
<td>[63]</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Structure 4" /></td>
<td>Anticancer</td>
<td>[64]</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Structure 5" /></td>
<td>Antimalarial</td>
<td>[65]</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6.png" alt="Structure 6" /></td>
<td>Antimicrobial</td>
<td>[66]</td>
</tr>
</tbody>
</table>

Thus it can be concluded that 1,2,3-triazoles have emerged as powerful (Table 1) pharmacophores [67].
Table 2. Physical data of novel benzoxazolinone and 1,2,3-triazoles based bis-heterocycles.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td><img src="image" alt="Structure 3a" /></td>
<td>85</td>
<td>180-181</td>
</tr>
<tr>
<td>3b</td>
<td><img src="image" alt="Structure 3b" /></td>
<td>88</td>
<td>194-195</td>
</tr>
<tr>
<td>3c</td>
<td><img src="image" alt="Structure 3c" /></td>
<td>86</td>
<td>203-204</td>
</tr>
<tr>
<td>3d</td>
<td><img src="image" alt="Structure 3d" /></td>
<td>85</td>
<td>175-176</td>
</tr>
<tr>
<td>3e</td>
<td><img src="image" alt="Structure 3e" /></td>
<td>85</td>
<td>173-174</td>
</tr>
<tr>
<td>3f</td>
<td><img src="image" alt="Structure 3f" /></td>
<td>93</td>
<td>181-182</td>
</tr>
<tr>
<td>3g</td>
<td><img src="image" alt="Structure 3g" /></td>
<td>90</td>
<td>166-167</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>Page</td>
<td>Range</td>
</tr>
<tr>
<td>---</td>
<td>-------------------</td>
<td>------</td>
<td>---------</td>
</tr>
<tr>
<td>3h</td>
<td><img src="image" alt="3h Image" /></td>
<td>83</td>
<td>209-210</td>
</tr>
<tr>
<td>3i</td>
<td><img src="image" alt="3i Image" /></td>
<td>90</td>
<td>215-216</td>
</tr>
<tr>
<td>3j</td>
<td><img src="image" alt="3j Image" /></td>
<td>83</td>
<td>141-142</td>
</tr>
<tr>
<td>3k</td>
<td><img src="image" alt="3k Image" /></td>
<td>92</td>
<td>177-178</td>
</tr>
<tr>
<td>3l</td>
<td><img src="image" alt="3l Image" /></td>
<td>76</td>
<td>200-201</td>
</tr>
<tr>
<td>3m</td>
<td><img src="image" alt="3m Image" /></td>
<td>88</td>
<td>229-230</td>
</tr>
<tr>
<td>3n</td>
<td><img src="image" alt="3n Image" /></td>
<td>83</td>
<td>205-206</td>
</tr>
<tr>
<td>3o</td>
<td><img src="image" alt="3o Image" /></td>
<td>83</td>
<td>225-226</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>Page</td>
<td>Reference Range</td>
</tr>
<tr>
<td>---</td>
<td>-------------------</td>
<td>------</td>
<td>-----------------</td>
</tr>
<tr>
<td>3p</td>
<td><img src="image1" alt="Chemical Structure" /></td>
<td>80</td>
<td>163-164</td>
</tr>
<tr>
<td>3q</td>
<td><img src="image2" alt="Chemical Structure" /></td>
<td>82</td>
<td>159-160</td>
</tr>
<tr>
<td>3r</td>
<td><img src="image3" alt="Chemical Structure" /></td>
<td>86</td>
<td>196-197</td>
</tr>
<tr>
<td>3s</td>
<td><img src="image4" alt="Chemical Structure" /></td>
<td>86</td>
<td>207-208</td>
</tr>
<tr>
<td>3t</td>
<td><img src="image5" alt="Chemical Structure" /></td>
<td>88</td>
<td>175-176</td>
</tr>
<tr>
<td>3u</td>
<td><img src="image6" alt="Chemical Structure" /></td>
<td>88</td>
<td>169-170</td>
</tr>
<tr>
<td>3v</td>
<td><img src="image7" alt="Chemical Structure" /></td>
<td>90</td>
<td>179-180</td>
</tr>
</tbody>
</table>
2.0. **Results and discussion**

2.1. **Analytical**

The propargylation of the different benzoazolinone derivatives was confirmed by the presence of a signal in the range of $\delta 5.15 - 5.25$ (s, 2H, CH$_2$) in the $^1$H-NMR spectra. The attachment of the benzoazolinone ring with the triazolly ring was confirmed by the resonance of the methylene carbon (CH$_2$) in a range of $\delta 36.98 - 37.69$ in the $^{13}$C NMR spectra. The formation of triazoles from the propargylated derivatives was confirmed by the resonance of the proton in the triazole ring in the range of $\delta 9.0 - 9.2$ as a singlet. All the products were characterized by IR, $^1$H NMR, $^{13}$C NMR and MALDI-MS/ESI-MS which showed [M + Na]$^+$ or [M + K]$^+$ or [M + 1]$^+$ ions.

3.0. **In silico molecular docking studies**

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

The binding energies were found to be in the range of -29 to -14.68 kcal/mol. The predicted binding energies are summarized in Table 3. Redocking celecoxib with the grid showed similar type of binding modes by forming hydrogen bond with LEU-338 residue with root mean square deviation of 1Å as shown in Figure 5. The figures 6, 7 and 8 summarize the binding modes and interactions of ligands as predicted by the Glide software. According to the docking models, all the molecules were predicted to bind in the binding site of COX-2 protein with comparatively good Glide score. All ligands showed similar type of binding interactions within the binding site of COX-2 as compared with that of celecoxib. ADME properties (absorption, distribution, metabolism and excretion) of the ligands were calculated using QikProp program. Properties like partition coefficient (log P o/w), van der Waals surface area of polar nitrogen and oxygen atoms (PSA) and aqueous solubility (log S) properties were within acceptable ranges as shown above in Table 3. As evident from the Table 3 it is clear that the compounds 3e and 3i exhibited a glide score of -8.68 and -8.56 respectively in comparison to the standard drug celecoxib which showed a glide score of -11.8. It can be observed that the π- π stacking plays a key role in the binding of these molecules with the COX-2 protein.
Table 3. Docking scores of celecoxib and six new ligands.

<table>
<thead>
<tr>
<th>Ligands</th>
<th>GScore</th>
<th>Glide Energy</th>
<th>Mol Wt</th>
<th>Log P o/w</th>
<th>PSA</th>
<th>Log S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Celecoxib</td>
<td>-11.8</td>
<td>-61.11</td>
<td>381.372</td>
<td>3.35</td>
<td>80.46</td>
<td>-5.87</td>
</tr>
<tr>
<td>3e</td>
<td>-8.68</td>
<td>-29.53</td>
<td>340.76</td>
<td>3.11</td>
<td>74.17</td>
<td>-4.22</td>
</tr>
<tr>
<td>3i</td>
<td>-8.56</td>
<td>-24.08</td>
<td>385.21</td>
<td>3.22</td>
<td>75.38</td>
<td>-4.61</td>
</tr>
<tr>
<td>3o</td>
<td>-8.31</td>
<td>-14.68</td>
<td>361.18</td>
<td>3.34</td>
<td>78.75</td>
<td>-4.74</td>
</tr>
<tr>
<td>3q</td>
<td>-7.88</td>
<td>-15.65</td>
<td>344.73</td>
<td>3.23</td>
<td>74.62</td>
<td>-4.38</td>
</tr>
<tr>
<td>3f</td>
<td>-7.86</td>
<td>-24.8</td>
<td>324.31</td>
<td>2.83</td>
<td>75.38</td>
<td>-4.04</td>
</tr>
<tr>
<td>3a</td>
<td>-7.58</td>
<td>-18.89</td>
<td>340.31</td>
<td>3.27</td>
<td>73.61</td>
<td>-4.71</td>
</tr>
</tbody>
</table>

Figure 5. Superimposed binding orientation of the crystallographic celecoxib (green) and docked celecoxib (red) as predicted by Schrodinger glide software. Hydrogen bonding with LEU-338 residue (yellow colour).
Figure 6. Celecoxib redocked with COX-2 protein. Hydrogen bonding occurs with LEU-338 & PHE-504 amino acid residues.
Figure 7. Ligand 3e aligned similarly to celecoxib into the binding pocket of COX-2 target protein having π-π stacking.
Figure 8. Ligand 3i aligned similarly to celecoxib into the binding pocket of COX-2 target protein having π-π stacking.
3.2. **Docking studies on TNF-α**

Docking studies of five compounds were carried out on the selected protein target 2AZ5 (TNF-α dimer). There was no apparent difference in the dock scores of these five molecules, which certainly is not the only criterion for predicting the affinity of a ligand with the target protein (Figure 9). But from the interaction figures of these compounds with the protein, it was observed that the compound 3i is oriented in the binding site in such a fashion that it favours the possibility of two π-π interactions on the two benzene rings with Tyr59 (chain A of the dimer) and Tyr119 (chain B of the dimer) respectively. There is no H-bonding observed in the interaction and the dock score is also in the range of -5 thereby indicating that these are not very strong inhibitors of the protein. The comparative strong inhibitory effect of the compound 3i among others could be ascertained due to the extra stability of the complex because of an extra π-π bond. From the interaction figures it is observed that Tyr59, Tyr119 and Tyr151 play an important role in the binding affinity of these ligands. The compound 3e exhibited (Table 4) a glide score of -5.87 in comparison to the standard drug indomethacin (-5.39) and the cocrystallized ligand which showed a glide score of -7.67. Hence these important parameters should be considered while designing inhibitors of this protein.

**Table 4.** XP Docking and MMGBSA results of molecules for TNF-α.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>XP Glide Score</th>
<th>MMGBSA Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co-crystallized Ligand</td>
<td>-7.67</td>
<td>-65.348</td>
</tr>
<tr>
<td>Rolipram</td>
<td>-5.66</td>
<td>-69.445</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>-5.39</td>
<td>-55.975</td>
</tr>
<tr>
<td>3e</td>
<td>-5.87</td>
<td>-63.048</td>
</tr>
<tr>
<td>3o</td>
<td>-5.38</td>
<td>-65.004</td>
</tr>
<tr>
<td>3f</td>
<td>-5.33</td>
<td>-55.522</td>
</tr>
<tr>
<td>3i</td>
<td>-5.15</td>
<td>-63.353</td>
</tr>
<tr>
<td>3a</td>
<td>-4.76</td>
<td>-53.364</td>
</tr>
</tbody>
</table>
Figure 9. *In silico* molecular docking study against TNF-α.
4. Biological activity

A focused library of twenty two compounds has been synthesized and screened for their anti-inflammatory and analgesic activities. The compounds showing potent anti-inflammatory and analgesic activities have been further evaluated for their gastric risk evaluation. The compounds showing significant in-vivo anti-inflammatory activity have also been screened for their in-vitro COX-2 and TNF-α inhibitory potential.

4.1. In vivo anti-inflammatory activity

All the synthesized compounds have been tested for their in vivo anti-inflammatory activity by carrageenan-induced hind paw edema model. The results obtained indicate that the compound 3f exhibited potent anti-inflammatory activity with 81.39% and 80.62% inhibition after 3 h and 5 h as compared to indomethacin which showed 79.06% and 82.25% inhibition after 3 h and 5 h respectively (Table 5 & Figure 10). The compound 3a exhibited 74.00% inhibition at 3 h post-carrageenan and 76.74% inhibition 5 h post-carrageenan administration as compared to indomethacin. Whereas the compounds 3i and 3e showed a time-dependent increase in the inhibition of inflammation (73.25% and 71.76% inhibition at 3 h post-carrageenan and 74.80 and 72.86% inhibition at 5 h post-carrageenan respectively). The compound 3o showed comparable anti-inflammatory activity with 67.44% and 65.11% inhibition after 3 h and 5 h respectively.

The structure activity relationship of the synthesized compounds is analysed on the basis of the nature of the substituents on the benzoxazolinone ring along with the position and nature of substitutions on aryl group attached to the 1,2,3-triazolyl ring. The compounds having substituted benzoxazolinone ring system exhibited more potent anti-inflammatory activity as compared to those having unsubstituted benzoxazolinone ring. The compounds 3t-3v conferred reduced activity whereas 3s did not show any significant anti-inflammatory activity. It has been observed that compounds 3a, 3f, 3i, 3k, 3o and 3u having weak electron withdrawing halogen atoms on the aromatic ring attached to the triazolyl ring conferred greater activity in comparison to compounds 3b, 3c, 3g, 3h, 3p and 3s having para or meta substituted NO2 group. This is further substantiated by the docking studies where the glide scores of halogen containing compounds are better than that of nitro containing compounds. Compounds 3d, 3t and 3u containing the electron donating groups (OCH3, OC2H5) as
well as compounds 3v, 3m and 3r containing pyridyl substitutions showed significant loss in the anti-inflammatory activity.

**Table 5. In vivo anti-inflammatory activity of 1,2,3-triazoles.**

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose</th>
<th>Change in paw volume(ml)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mean ± SEM</td>
<td>3hr</td>
</tr>
<tr>
<td>Control</td>
<td>2 ml/kg</td>
<td>0.866±0.049</td>
<td>0.866±0.049</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.05 mmoles/kg</td>
<td>0.183±0.031***</td>
<td>0.150±0.022**</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>0.216±0.047**</td>
<td>0.200±0.051**</td>
</tr>
<tr>
<td>3b</td>
<td>-</td>
<td>0.350±0.138</td>
<td>0.366±0.130*</td>
</tr>
<tr>
<td>3c</td>
<td>-</td>
<td>0.383±0.125</td>
<td>0.350±0.133**</td>
</tr>
<tr>
<td>3d</td>
<td>-</td>
<td>0.466±0.114</td>
<td>0.416±0.122*</td>
</tr>
<tr>
<td>3e</td>
<td>-</td>
<td>0.250±0.042**</td>
<td>0.233±0.042**</td>
</tr>
<tr>
<td>3f</td>
<td>-</td>
<td>0.166±0.021***</td>
<td>0.166±0.033***</td>
</tr>
<tr>
<td>3g</td>
<td>-</td>
<td>0.416±0.094</td>
<td>0.383±0.060*</td>
</tr>
<tr>
<td>3h</td>
<td>-</td>
<td>0.416±0.130</td>
<td>0.383±0.140*</td>
</tr>
<tr>
<td>3i</td>
<td>-</td>
<td>0.233±0.042**</td>
<td>0.216±0.030**</td>
</tr>
<tr>
<td>3j</td>
<td>-</td>
<td>0.283±0.060**</td>
<td>0.300±0.051**</td>
</tr>
<tr>
<td>3k</td>
<td>-</td>
<td>0.316±0.047</td>
<td>0.283±0.047**</td>
</tr>
<tr>
<td>3l</td>
<td>-</td>
<td>0.36±0.055</td>
<td>0.35±0.050</td>
</tr>
<tr>
<td>3m</td>
<td>-</td>
<td>0.480±0.070</td>
<td>0.46±0.061</td>
</tr>
<tr>
<td>3n</td>
<td>-</td>
<td>0.300±0.141*</td>
<td>0.283±0.047**</td>
</tr>
<tr>
<td>3o</td>
<td>-</td>
<td>0.283±0.060**</td>
<td>0.300±0.085**</td>
</tr>
<tr>
<td>3p</td>
<td>-</td>
<td>0.333±0.138</td>
<td>0.333±0.042**</td>
</tr>
<tr>
<td>3q</td>
<td>-</td>
<td>0.200±0.052***</td>
<td>0.216±0.030**</td>
</tr>
<tr>
<td>3r</td>
<td>-</td>
<td>0.366±0.133</td>
<td>0.333±0.666**</td>
</tr>
<tr>
<td>3s</td>
<td>-</td>
<td>0.400±0.106</td>
<td>0.433±0.122</td>
</tr>
<tr>
<td>3t</td>
<td>-</td>
<td>0.633±0.117</td>
<td>0.600±0.165</td>
</tr>
<tr>
<td>3u</td>
<td>-</td>
<td>0.466±0.117</td>
<td>0.483±0.060</td>
</tr>
<tr>
<td>3v</td>
<td>-</td>
<td>0.533±0.111</td>
<td>0.483±0.113</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.
Figure 10. *In vivo* anti-inflammatory activity of novel *bis*-heterocycles.

4.2. **In vitro COX assay**

All the above synthesized compounds were evaluated for their anti-inflammatory activity by biochemical selective COX-2 inhibitory assay. It was found some compounds which were showing significant *in-vivo* anti-inflammatory activity also exhibited potent *in-vitro* COX-2 activity. Compounds 3a, 3f, 3i, 3o and 3e showed significant COX-2 inhibition as compared to the standard drug celecoxib (Figure 11 & Table 6). The gastric tolerance of these compounds may be related to their selective percentage COX-2 inhibition (Table 7). The compounds 3a (COX-1 IC$_{50}$ = 389.2 µM; COX-2 IC$_{50}$ = 5.6 µM; SI = 69.5), 3f (COX-1 IC$_{50}$ = 174.72 µM; COX-2 IC$_{50}$ = 2.4 µM; SI = 72.8) and 3i (COX-1 IC$_{50}$ = 188.20 µM; COX-2 IC$_{50}$ = 2.78 µM; SI = 67.7) exhibited potent selective COX-2 inhibition as compared to celecoxib (COX-1 IC$_{50}$ = 25.74 µM; COX-2 IC$_{50}$ = 0.32 µM; SI = 80.43). The COX-1/COX-2 selective index (SI value) of the compounds 3a, 3f and 3i shows the selective nature of these compounds towards COX-2 inhibition as compared to celecoxib.
Figure 11. *In vitro* COX-2 activity of 1,2,3-triazole based benzoxazolinones.

Table 6. *In vitro* COX-2 inhibition of the 1,2,3-triazole based benzoxazolinones.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Concentration</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>3a</td>
<td>10μM</td>
<td>53.66</td>
</tr>
<tr>
<td>3f</td>
<td>10μM</td>
<td>59.48</td>
</tr>
<tr>
<td>3i</td>
<td>10μM</td>
<td>56.56</td>
</tr>
<tr>
<td>3o</td>
<td>10μM</td>
<td>49.54</td>
</tr>
<tr>
<td>3e</td>
<td>10μM</td>
<td>52.36</td>
</tr>
<tr>
<td>3q</td>
<td>10μM</td>
<td>33.56</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>10μM</td>
<td>66.36</td>
</tr>
</tbody>
</table>
**Table 7.** Selectivity index (SI) COX-1/COX-2 of the 1,2,3-triazole based benzoxazolinones.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$ (µM)</th>
<th>Selectivity index (SI) COX-1/COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(COX-1)</td>
<td>(COX-2)</td>
</tr>
<tr>
<td>3a</td>
<td>389.2</td>
<td>5.6</td>
</tr>
<tr>
<td>3f</td>
<td>174.72</td>
<td>2.4</td>
</tr>
<tr>
<td>3i</td>
<td>188.20</td>
<td>2.78</td>
</tr>
<tr>
<td>3e</td>
<td>207.36</td>
<td>3.6</td>
</tr>
<tr>
<td>3o</td>
<td>111.34</td>
<td>2.7</td>
</tr>
<tr>
<td>3q</td>
<td>138.25</td>
<td>3.1</td>
</tr>
<tr>
<td>Celecoxib</td>
<td>25.74</td>
<td>0.32</td>
</tr>
</tbody>
</table>

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

4.3. *In vitro TNF-α assay*

The compounds showing significant *in-vivo* anti-inflammatory activity were further screened for their *in-vitro* TNF-α activity (**Figure 12 & Table 8**). The compound 3i showed significant (p < 0.001), TNF-α inhibitory activity with 50.95% inhibition as compared to the standard drug indomethacin which exhibited 64.01%, (p < 0.001) inhibition.
**Figure 12.** *In vitro* TNF-α inhibitory activity of novel *bis*-heterocycles.

**Table 8.** *In vitro* TNF-α inhibition of the 1,2,3-triazole based benzoxazolinones.

<table>
<thead>
<tr>
<th>Test Compound</th>
<th>Concentration(μg/ml)</th>
<th>Mean ± SEM</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS Control</td>
<td>1</td>
<td>3.14 ± 0.03</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>1</td>
<td>1.13 ± 0.07***</td>
<td>64.01</td>
</tr>
<tr>
<td>3a</td>
<td>1</td>
<td>2.66 ± 0.09</td>
<td>15.28</td>
</tr>
<tr>
<td>3i</td>
<td>1</td>
<td>1.54 ± 0.11***</td>
<td>50.95</td>
</tr>
<tr>
<td>3o</td>
<td>1</td>
<td>2.58 ± 0.21*</td>
<td>17.83</td>
</tr>
<tr>
<td>3e</td>
<td>1</td>
<td>2.50 ± 0.15*</td>
<td>20.38</td>
</tr>
<tr>
<td>3f</td>
<td>1</td>
<td>2.42 ± 0.05**</td>
<td>22.92</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.
4.4. *Antinociceptive assays*

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 (Figure 13 & Table 9) indicate that compound 3a exhibited potent antinociceptive activity with 41.83% inhibition as compared to the standard drug indomethacin which caused 44.69% inhibition. The results of the tail immersion method (Table 10) demonstrate that the compounds 3a and 3f ($p<0.01$) showed significant antinociceptive activity in comparison to the standard drug indomethacin.

![Figure 13](image.png)

**Figure 13.** *In vivo* antinociceptive activity by writhing test.

**Table 9.** *In vivo* antinociceptive activity by 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>81.66 ± 1.89</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.05 mmoles/kg</td>
<td>45.16 ± 1.32</td>
<td>44.69%</td>
</tr>
<tr>
<td>3e</td>
<td>-</td>
<td>55.66 ± 1.28</td>
<td>31.83%</td>
</tr>
<tr>
<td>3i</td>
<td>-</td>
<td>53.16 ± 1.35</td>
<td>34.90%</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>47.50 ± 1.25</td>
<td>41.83%</td>
</tr>
<tr>
<td>3f</td>
<td>-</td>
<td>52.71 ± 1.24</td>
<td>35.45%</td>
</tr>
<tr>
<td>3q</td>
<td>-</td>
<td>54.00 ± 1.41</td>
<td>33.87%</td>
</tr>
</tbody>
</table>
Table 10. *In vivo* antinociceptive activity by 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>2 ml/kg</td>
<td>2.00 ± 0.23</td>
<td>2.22 ± 0.20</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>0.05 mmoles/kg</td>
<td>2.08 ± 0.22</td>
<td>3.12 ± 0.11 *</td>
</tr>
<tr>
<td>3e</td>
<td>-</td>
<td>1.90 ± 0.20</td>
<td>2.18 ± 0.11</td>
</tr>
<tr>
<td>3i</td>
<td>-</td>
<td>2.28 ± 0.14</td>
<td>2.62 ± 0.26</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>2.30 ± 0.21</td>
<td>3.08 ± 0.24 *</td>
</tr>
<tr>
<td>3f</td>
<td>-</td>
<td>2.48 ± 0.20</td>
<td>3.10 ± 0.28 *</td>
</tr>
<tr>
<td>3q</td>
<td>-</td>
<td>2.44 ± 0.15</td>
<td>2.88 ± 0.13</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.

4.5. *Ulcerogenic studies*

The compounds showing potential anti-inflammatory and antinociceptive activity were further tested for their gastric ulceration activity (Figure 14 & Table 11). When compared with indomethacin, compounds 3a, 3e, 3f, 3i and 3o did not induce any gastric ulceration and rupture of the gastric mucosal layer.

Table 11. 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>3e</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3i</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3a</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3f</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3q</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

(-) No damage, (+++) indicates high degree of damage
Figure 14. Haematoxylin and eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.
5.0. **Quantitative Structure Activity Relationship Studies (QSAR)**

To find the important features responsible for COX-II inhibitory activity, a molecule having activity greater than 1.55 against COX-II (9 molecules) was selected for pharmacophore development. The common pharmacophore was developed by matching the five pharmacophoric sites to all nine active molecules. More than 200 hypotheses were possible, however after scoring; the best thirty four pharmacophores with different combination of variants were obtained. The selection of these pharmacophores depended on the survival score of pharmacophore. The final selection of hypothesis amongst the different hypothesis solely depends on the ability of hypothesis to discriminate between active set and inactive set molecules. To identify the pharmacophore model with ability to distinguish between active and inactive molecule, all the survived pharmacophores in survival scoring step were subjected to mapping with inactive ligand. If the inactive ligand scored well, it meant that this hypothesis is not valid because it does not discriminate active and inactive ligands. By taking all these points into consideration a final survival inactive score was calculated by subtracting inactive score from survival score of these pharmacophore. Finally the model with maximum inactive survival score was selected for further flexible alignment and atom based 3D QSAR development. In this study we get a five featured pharmacophore, AAARR, with highest survival inactive score (Table 12).

<table>
<thead>
<tr>
<th>ID</th>
<th>Survival</th>
<th>Survival inactive</th>
<th>Site</th>
<th>Vector</th>
<th>Volume</th>
<th>Matches</th>
<th>Energy</th>
<th>Activity</th>
<th>Inactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAARR</td>
<td>4.378</td>
<td>2.737</td>
<td>0.48</td>
<td>0.939</td>
<td>0.819</td>
<td>9</td>
<td>0.825</td>
<td>1.774</td>
<td>1.641</td>
</tr>
</tbody>
</table>

These arrangements along with their distances in five featured pharmacophore, AAARR, are shown in figure 15a. The AAARR pharmacophore was used for further alignment and 3D QSAR studies. The figure 15b represents the alignment used in 3D QSAR study.
A three-PLS factor model was developed with good statistical result and predictive ability for dataset (Table 13). Due to fewer molecules in the training set it’s not good practice to develop a model with further increasing PLS factor. Table 14 shows the result for the generated model and fitness plot is presented in Figure 16.

**Table 13.** Predicted activity data for training and test set for model generated by PLS factor 3.

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Ligand Name</th>
<th>QSAR Set</th>
<th>Actual Activity</th>
<th>PLS Factor</th>
<th>Predicted Activity</th>
<th>Pharm Set</th>
<th>Fitness</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>training</td>
<td>1.92</td>
<td>3</td>
<td>1.71</td>
<td>active</td>
<td>1.56</td>
</tr>
<tr>
<td>2</td>
<td>A</td>
<td>training</td>
<td>1.819</td>
<td>3</td>
<td>1.78</td>
<td>active</td>
<td>2.26</td>
</tr>
<tr>
<td>3</td>
<td>Q</td>
<td>training</td>
<td>1.774</td>
<td>3</td>
<td>1.7</td>
<td>active</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>I</td>
<td>test</td>
<td>1.774</td>
<td>3</td>
<td>1.75</td>
<td>active</td>
<td>2.26</td>
</tr>
<tr>
<td>5</td>
<td>E</td>
<td>training</td>
<td>1.73</td>
<td>3</td>
<td>1.76</td>
<td>active</td>
<td>1.84</td>
</tr>
<tr>
<td>6</td>
<td>K</td>
<td>training</td>
<td>1.61</td>
<td>3</td>
<td>1.71</td>
<td>active</td>
<td>2.27</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>test</td>
<td>1.61</td>
<td>3</td>
<td>1.68</td>
<td>active</td>
<td>2.27</td>
</tr>
<tr>
<td>8</td>
<td>J</td>
<td>training</td>
<td>1.572</td>
<td>3</td>
<td>1.57</td>
<td>active</td>
<td>2.64</td>
</tr>
<tr>
<td>9</td>
<td>O</td>
<td>training</td>
<td>1.572</td>
<td>3</td>
<td>1.57</td>
<td>active</td>
<td>2.79</td>
</tr>
<tr>
<td>10</td>
<td>R</td>
<td>training</td>
<td>1.5</td>
<td>3</td>
<td>1.52</td>
<td></td>
<td>2.79</td>
</tr>
<tr>
<td>11</td>
<td>P</td>
<td>test</td>
<td>1.499</td>
<td>3</td>
<td>1.58</td>
<td></td>
<td>2.52</td>
</tr>
<tr>
<td>12</td>
<td>C</td>
<td>training</td>
<td>1.464</td>
<td>3</td>
<td>1.42</td>
<td></td>
<td>2.49</td>
</tr>
<tr>
<td>13</td>
<td>L</td>
<td>training</td>
<td>1.464</td>
<td>3</td>
<td>1.55</td>
<td></td>
<td>2.28</td>
</tr>
<tr>
<td>14</td>
<td>B</td>
<td>training</td>
<td>1.431</td>
<td>3</td>
<td>1.43</td>
<td></td>
<td>2.33</td>
</tr>
<tr>
<td>15</td>
<td>G</td>
<td>training</td>
<td>1.402</td>
<td>3</td>
<td>1.38</td>
<td></td>
<td>2.4</td>
</tr>
<tr>
<td>16</td>
<td>H</td>
<td>test</td>
<td>1.396</td>
<td>3</td>
<td>1.56</td>
<td></td>
<td>2.47</td>
</tr>
<tr>
<td>17</td>
<td>4</td>
<td>training</td>
<td>1.328</td>
<td>3</td>
<td>1.37</td>
<td></td>
<td>2.21</td>
</tr>
</tbody>
</table>
Table 14. Statistical parameter of the best 3D QSAR model.

<table>
<thead>
<tr>
<th>ID</th>
<th>Factor</th>
<th>SD</th>
<th>R²</th>
<th>F</th>
<th>P</th>
<th>RMSE</th>
<th>Q²</th>
<th>Pearson-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>AAARR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>0.1971</td>
<td>0.4103</td>
<td>10.4</td>
<td>0.005601</td>
<td>0.1085</td>
<td>0.688</td>
<td>0.8561</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>0.146</td>
<td>0.6981</td>
<td>16.2</td>
<td>0.0002284</td>
<td>0.1365</td>
<td>0.5055</td>
<td>0.7415</td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>0.0946</td>
<td>0.8822</td>
<td>32.5</td>
<td>2.638e-006</td>
<td>0.0913</td>
<td>0.7791</td>
<td>0.9522</td>
</tr>
</tbody>
</table>

Figure 16. Fitness Plot of training and test set molecules.

The model generated by 3D QSAR (Figure 17) with blue and red cubes indicates positive and negative coefficient with activity. In atom type QSAR we can check the contribution of each atom in the sense of afore said six categories. In our selected molecules for QSAR study we have only two types of atoms in our molecule viz hydrophobic and electron withdrawing type of atom as there is no ionization state generated at user defined pH. Visual analysis of QSAR model (Figure 17a) for
hydrophobic atom type contribution shows the blue cubes at aromatic ring (except para position) present on triazollyl ring and 5\textsuperscript{th} and 6\textsuperscript{th} position of benzoxazolinone ring indicate that hydrophobic atom type at this position is responsible for COX-2 inhibition. The hydrophobic substitution at para position on aromatic ring is detrimental to COX-2 inhibition because red colour cube are indication of negative effect of hydrophobic atom type with activity. Figure 17b illustrates that the electron withdrawing character is necessary at A2, A3 and A4 which is present on triazollyl ring and benzoxazolinone ring respectively. The red cubes at the para position of benzene ring are indicative of negative potential of electron withdrawing group at that position.

Figure 17. Atom type 3D QSAR visualization of model: (a) Hydrophobic atom type contribution (b) electron withdrawing atom type contribution.

In overall conclusion of QSAR study it was found that the hydrophobic substitution at 6\textsuperscript{th} position is more contributing parameter compared to 5\textsuperscript{th} position. The increasing hydrophobicity of substitution at para position of benzene ring decreases activity for COX-2 inhibition. It motivates the further designing of molecules with more hydrophobic substitution at 5\textsuperscript{th}, 6\textsuperscript{th} position and on benzene ring except at para position.

6.0. Experimental

6.1. Chemistry

All commercial chemicals used as starting materials and reagents were purchased from Merck (India), Spectrochem and Sigma Aldrich and were of analytical 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 against 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.

6.2. General procedure for synthesis of benzoxazolinone derivatives:

The different ortho amino phenol derivatives were dissolved in anhydrous tetrahydrofuran and reacted with 1,1-carbonyldiimidazole (CDI) for 5-6 hrs at a temperature of 65°C [68].

\[
\text{OH} \quad \text{NH}_2 \quad + \quad \begin{array}{c}
\text{Dry THF} \\
\text{Reflux}
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{N} \\
\text{O}
\end{array} \\
R = \text{H, 6CH}_3, \text{5CH}_3, \text{5Cl}
\]

6.3. General procedure for synthesis of propargylated derivatives:

Benzoxazolinone derivatives were dissolved in dry acetone containing K$_2$CO$_3$ and refluxed in the presence of propargyl bromide for 6 hrs. The progress of the reaction was monitored by TLC. After the completion, the reaction mixture was filtered. The filtrate was concentrated and poured on ice to get different pure propargylated derivatives.

\[
\text{R} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{O} \\
\rightarrow \quad \begin{array}{c}
\text{Br} \\
\text{K}_2\text{CO}_3 \\
\text{Acetone / Reflux}
\end{array} \quad \rightarrow \quad \begin{array}{c}
\text{N} \\
\text{O}
\end{array} \\
R = \text{H, 6CH}_3, \text{5CH}_3, \text{5Cl}
\]
6.4. **General procedure for synthesis of 1, 2, 3-triazole derivatives:**

Propargylated derivatives were dissolved in 20 mL of t-Butanol: water (1:1) solvent at ambient temperature. CuSO\(_4\).5H\(_2\)O was then charged and the reaction mixture was stirred for 5 min. Reaction mixture was light blue in colour. Sodium ascorbate was now added at once to the reaction mixture and stirred for 15 min. Reaction mixture colour was changed to dark yellow. After 15 min, azide was added. The reaction mixture was allowed to stir for further 8 h at ambient temperature. After the completion of the reaction, monitored by TLC, reaction mixture was quenched with water and extracted with ethyl acetate. Combined organic layers were dried over anhydrous sodium sulphate, filtered and concentrated under reduced pressure to obtain the required product.

\[
\text{R N} \hspace{1cm} \text{O} \hspace{1cm} \text{O} \hspace{1cm} \text{N3} \\
\text{R1} \\
\begin{array}{c}
\text{CuSO}_4 \cdot 5\text{H}_2\text{O} \\
\text{Sodium Ascorbate} \\
tert \text{Butanol : H}_2\text{O} \\
1:1
\end{array}
\]

\[
\text{R = H, 6CH}_3, 5\text{CH}_3, 5\text{Cl}
\]

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

\[
\begin{array}{c}
\text{O} \\
\text{N} \\
\text{O} \\
\text{N} \\
\text{N} \\
\text{Cl}
\end{array}
\]

**Yield**: 85%

**Physical appearance**: Yellowish brown crystals

**m. p.**: 180-181 °C

**R\(_f\)**: 0.52 (toluene: ethyl acetate: formic acid; 5:4:1)

**IR (KBr) cm\(^{-1}\)**: 3178, 3045, 1503, 1441, 1238, 1175, 1047, 825

**\(^1\)H NMR (DMSO-\(d_6\), 300 MHz)**: δ 2.32 (s, 3H), 5.17 (s, 2H), 7.01 (d, 1H, \(J=7.5\) Hz), 7.19 (d, 2H, \(J=8.1\) Hz), 7.66 (d, 2H, \(J=8.4\) Hz), 7.92 (d, 2H, \(J=8.7\) Hz), 8.92 (s, 1H)

**\(^{13}\)C NMR (DMSO-\(d_6\), 75 MHz)**: δ 21.35, 37.48, 109.73, 110.66, 112.46, 122.21, 122.58, 124.67, 128.66, 130.32, 132.62, 133.50, 135.72, 142.53, 143.13, 156.15
Chapter II

Section A

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

Yield : 88%
Physical appearance : Yellowish crystals
m. p. : 194-195 °C
Rf : 0.74 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm\(^{-1}\) : 3176, 3068, 1759, 1597, 1441, 1242, 1172, 854
\(^1\)H NMR (DMSO-d\(_6\), 300 MHz) : δ 2.32 (s, 3H), 5.21 (s, 2H), 7.02 (d, 1H, \(J=5.4\) Hz), 7.19-7.21 (m, 2H), 8.20 (d, 2H, \(J=6.8\) Hz), 8.44 (d, 2H, \(J=6.8\) Hz), 9.12 (s, 1H)
\(^13\)C NMR (DMSO-d\(_6\), 75 MHz) : δ 22.345, 36.98, 110.731, 112.64, 113.45, 122.61, 123.68, 125.65, 129.77, 131.52, 133.63, 134.46, 136.75, 143.56, 144.15, 157.25

MS (MALDI) m/z : 390 (M+K)
Elemental Analysis : Molecular formula C\(_{17}\)H\(_{13}\)N\(_5\)O\(_4\)
Calculated : C, 58.12; H, 3.73; N, 19.93%
Found : C, 58.15; H, 3.80; N, 19.95%
3. 6-Methyl-3-[1-(3-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzoazol-2-one (3c)

![Chemical structure of 6-Methyl-3-[1-(3-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzoazol-2-one (3c)]

**Yield**: 86%

**Physical appearance**: Yellow powder

**m. p.**: 203-204 °C

**R_f**: 0.62 (toluene: ethyl acetate: formic acid; 5:4:1)

**IR (KBr) cm⁻¹**: 3234, 3143, 1756, 1533, 1172, 1045, 809, 737

**¹H NMR (DMSO-d₆, 300 MHz)**: δ 2.32 (s, 3H), 5.21 (s, 2H), 7.02 (d, 1H, J=5.9 Hz), 7.18-7.21 (m, 2H), 7.88 (t, 1H, J=6.1 Hz), 8.32 (d, 1H, J=6.2 Hz), 8.39 (d, 1H, J=6.0 Hz), 8.70 (t, 1H, J=1.5 Hz), 9.14 (s, 1H)

**¹³C NMR (DMSO-d₆, 75 MHz)**: δ 22.40, 37.38, 110.11, 111.32, 123.15, 127.13, 128.49, 129.32, 132.12, 134.34, 135.46, 136.24, 141.34, 142.34, 155.31

**MS (ESI) m/z**: 351 (M+1)^+

**Elemental Analysis**

- **Calculated**: C, 58.12; H, 3.73; N, 19.93%
- **Found**: C, 58.10; H, 3.72; N, 19.90%

---

4. 3-[1-(4-Ethoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-methyl-3H-benzoazol-2-one (3d)

![Chemical structure of 3-[1-(4-Ethoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-methyl-3H-benzoazol-2-one (3d)]

**Yield**: 85%

**Physical appearance**: White crystals

**m. p.**: 175-176 °C

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

**IR (KBr) cm⁻¹**: 3478, 3147, 2977, 1756, 1615, 1178, 1048, 804
5H NMR (DMSO- d6, 300 MHz) : δ 1.34 (t, 3H, J=6.3 Hz), 2.32 (s, 3H), 4.08 (q, 2H, J=6.6 Hz), 5.15 (s, 2H), 7.00-7.11 (m, 3H), 7.19 (d, 2H, J=6.6 Hz), 7.75 (d, 2H, J=8.4 Hz), 8.78 (s, 1H)

13C NMR (DMSO-d6, 75 MHz) : δ 15.01, 21.35, 37.52, 64.00, 109.75, 110.64, 115.72, 122.17, 122.39, 124.65, 128.69, 132.58, 142.54, 142.65, 154.11, 159.05

MS (ESI) m/z : 350 (M)+

Elemental Analysis
Calculated : C, 65.13; H, 5.18; N, 15.99%

Found : C, 65.11; H, 5.15; N, 15.97%

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

Yield : 85%

Physical appearance : Brown crystals

m. p. : 173-174 °C

Rf : 0.70 (toluene: ethyl acetate: formic acid; 5:4:1)

IR (KBr) cm-1 : 3178, 3045, 1503, 1441, 1238, 1175, 1047, 825

1H NMR (DMSO-d6, 300 MHz) : δ 2.33 (s, 3H), 5.20 (s, 2H), 7.02 (d, 1H, J=8.1 Hz), 7.20-7.22 (m, 2H), 7.5-7.6 (m, 3H), 7.76 (d, 1H, J=7.8 Hz), 8.68 (s, 1H)

13C NMR (DMSO-d6, 75 MHz) : δ 21.35, 37.36, 109.80, 110.66, 124.64, 126.57, 128.68, 128.68, 128.87, 128.93 131.00, 132.21, 132.60, 134.79, 141.83, 142.52, 154.10

MS (ESI) m/z : 341(M+1)+

Elemental Analysis
Calculated : Molecular formula C17H13ClN4O2

Found : C, 59.92; H, 3.85; N, 16.44%
6. 3-[1-(4-Fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-6-methyl-3H-benzoaxazol-2-one (3f)

Yield: 85%

Physical appearance: Brown crystals

m. p.: 181-182 °C

Rf: 0.72 (toluene: ethyl acetate: formic acid; 5:4:1)

IR (KBr) cm\(^{-1}\): 3685, 3132, 1768, 1445, 1236, 1172, 1046, 835

\(^1\)H NMR (DMSO-\(d_6\), 300 MHz):
- \(\delta\) 2.32 (s, 3H), 5.17 (s, 2H), 7.02 (d, 1H, \(J=7.8\) Hz), 7.19 (d, 2H, \(J=7.5\) Hz), 7.44 (t, 2H, \(J=8.7\) Hz), 7.90-7.94 (m, 2H), 8.88 (s, 1H)

\(^13\)C NMR (DMSO-\(d_6\), 75 MHz):
- \(\delta\) 21.34, 37.49, 109.73, 110.65, 117.03, 117.34, 122.73, 122.85, 122.97, 124.66, 128.66, 132.61, 133.49, 142.54, 142.98, 154.10, 163.76.

MS (ESI) m/z: 324 (M)+

Elemental Analysis:
- Calculated: C, 62.96; H, 4.04; N, 17.28%
- Found: C, 62.94; H, 4.02; N, 17.26%

7. 5-Methyl-3-[1-(4-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzoaxazol-2-one (3g)

Yield: 90%

Physical appearance: Brown crystals

m. p.: 166-167 °C

Rf: 0.71 (toluene: ethyl acetate: formic acid; 5:4:1)

IR (KBr) cm\(^{-1}\): 3628, 3067, 1774, 1519, 1381, 1246, 1082, 855
$^1$H NMR (DMSO-$d_6$, 300 MHz) : $\delta$ 2.33 (s, 3H), 5.21 (s, 2H), 6.95 (d, 1H, $J$=8.4 Hz), 7.17-7.25 (m, 2H), 8.21 (d, 2H, $J$=8.4 Hz), 8.44 (d, 2H, $J$=8.4 Hz), 9.12 (s, 1H)

$^{13}$C NMR (DMSO-$d_6$, 75 MHz) : $\delta$ 21.52, 37.41, 109.81, 110.38, 121.22, 122.92, 123.24, 126.03, 131.00, 133.92, 141.15, 143.70, 147.24, 154.24.

MS (ESI) m/z : 352.1 (M+1)$^+$

Elemental Analysis:

**Calculated**: Molecular formula $C_{17}H_{13}N_5O_4$

**Found**: C, 58.12; H, 3.73; N, 19.93%

**Experimental**: C, 58.09; H, 3.72; N, 19.91%

8. 5-Methyl-3-[1-(3-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one (3h)

![Chemical Structure](image)

**Yield**: 83%

**Physical appearance**: Yellow crystals

**m. p.**: 209-210 °C

**R$_f$**: 0.70 (toluene: ethyl acetate: formic acid; 5:4:1)

**IR (KBr) cm$^{-1}$**: 3668, 3243, 1776, 1535, 1494, 1250, 1194, 805

$^1$H NMR (DMSO-$d_6$, 300 MHz) : $\delta$ 2.33 (s, 3H), 5.21 (s, 2H), 6.95 (d, 1H, $J$=8.1Hz), 7.17 (s, 1H), 7.24 (d, 1H, $J$=8.1 Hz), 7.88 (t, 1H, $J$=8.1 Hz), 8.33 (d, 1H, $J$=8.1 Hz), 8.41 (d, 1H, $J$=1.2 Hz), 8.71 (t, 1H, $J$=2.1 Hz), 9.15 (s, 1H)

$^{13}$C NMR (DMSO-$d_6$, 75 MHz) : $\delta$ 21.51, 37.46, 109.81, 110.37, 115.25, 122.93, 123.23, 123.68, 126.56, 130.99, 132.99, 133.91, 137.50, 140.50, 143.51, 148.96, 154.25

MS (ESI) m/z : 351 (M)$^+$

Elemental Analysis: Molecular formula $C_{17}H_{13}N_5O_4$
Calculated: C, 58.12; H, 3.73; N, 19.93%
Found: C, 58.08; H, 3.69; N, 19.90%

9. 3-[1-(4-Bromo-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzooxazol-2-one (3i)

Yield: 90%
Physical appearance: Yellow crystals
m. p.: 215-216 °C
Rf: 0.58 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm⁻¹: 3135, 3028, 1767, 1625, 1385, 1248, 1183, 835
¹H NMR (DMSO-d₆, 300 MHz): δ 2.33 (s, 3H), 5.17 (s, 2H), 6.94 (d, 1H, J=7.8 Hz), 7.16 (s, 1H), 7.23 (d, 1H, J=8.1 Hz), 7.79 (d, 2H, J=9.0 Hz), 7.86 (d, 2H, J=9.0 Hz), 8.94 (s, 1H)
¹³C NMR (DMSO-d₆, 75 MHz): δ 21.51, 37.43, 109.79, 110.40, 115.08, 122.46, 123.21, 123.68, 131.02, 133.89, 136.16, 140.53, 143.20, 156.45
MS (MALDI) m/z: 424 (M+K)⁺
Elemental Analysis: Molecular formula C₁₇H₁₃BrN₄O₂
Calculated: C, 53.00; H, 3.40; N, 14.54%
Found: C, 52.98; H, 3.38; N, 14.51%. 
10. 3-[1-(2-Chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzoxazol-2-one (3j)

Yield : 83%
Physical appearance : Brown crystals
m. p. : 141-142 °C
Rf : 0.61 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm\(^{-1}\) : 3271, 3035, 1761, 1622, 1435, 1249, 1036, 823
\(^1\)H NMR (DMSO-d\(_6\), 300 MHz) : \(\delta\) 2.33 (s, 3H), 5.20 (s, 2H), 6.94 (d, 1H, \(J=7.5\) Hz), 7.16 (s, 1H), 7.23 (d, 1H, \(J=8.1\) Hz), 7.5-7.7 (m, 3H), 7.77 (d, 1H, \(J=7.5\) Hz), 8.71 (s, 1H)
\(^{13}\)C NMR (DMSO-d\(_6\), 75 MHz) : \(\delta\) 21.50, 37.33, 109.78, 110.54, 123.17, 126.66, 128.89, 128.96, 131.01, 132.23, 133.83, 134.81, 140.46, 141.80, 154.24
MS (MALDI) m/z : 341 (M+1)\(^+\), 363 (M+Na)\(^+\)
Elemental Analysis : Molecular formula C\(_{17}\)H\(_{13}\)ClN\(_4\)O\(_2\)
Calculated : C, 59.92; H, 3.85; N, 16.44%
Found : C, 59.89; H, 3.83; N, 16.41%

11. 3-[1-(4-Fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzoxazol-2-one (3k)

Yield : 92%
Physical appearance : White crystals
m. p. : 177-178 °C
Rf : 0.62 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm\(^{-1}\) : 3154, 3104, 1768, 1623, 1428, 1247, 1104, 843
$^1$H NMR (DMSO-d$_6$, 300 MHz) : $\delta$ 2.33 (s, 3H), 5.17 (s, 2H), 6.95 (d, 1H, $J$=7.8 Hz), 7.17 (s, 1H), 7.23 (d, 1H, $J$=8.1 Hz), 7.42-7.47 (m, 2H), 7.91-7.95 (m, 2H), 8.89 (s, 1H)

$^{13}$C NMR (DMSO-d$_6$, 75 MHz) : $\delta$ 21.51, 37.42, 109.78, 110.40, 117.04, 122.69, 126.66, 122.88, 122.99, 123.20, 131.02, 133.49, 133.90, 140.47, 143.01, 154.25

MS (MALDI) m/z : 347 (M+Na)$^+$

Elemental Analysis : Molecular formula C$_{17}$H$_{13}$FN$_4$O$_2$

Calculated : C, 62.96; H, 4.04; N, 17.28%

Found : C, 62.93; H, 4.02; N, 17.26%

12. 3-[(1-(2-Fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzooxazol-2-one (3I)

Yield : 76%

Physical appearance : Yellow crystals

m. p. : 200-201°C

R$_f$ : 0.48 (toluene: ethyl acetate: formic acid; 5:4:1)

IR (KBr) cm$^{-1}$ : 3138, 3027, 1769, 1496, 1253, 1192, 1092 829

$^1$H NMR (DMSO-d$_6$, 300 MHz) : $\delta$ 2.33 (s, 3H), 5.20 (s, 2H), 6.95 (d, 1H, $J$=7.8 Hz), 7.17 (s, 1H), 7.24 (d, 1H, $J$=8.1 Hz), 7.63-7.67 (m, 3H), 8.31 (d, 1H, $J$=8.4 Hz), 9.00 (s, 1H)

$^{13}$C NMR (DMSO-d$_6$, 75 MHz) : $\delta$ 21.52, 37.33, 109.79, 110.43, 122.87, 123.22, 131.02, 133.91, 140.49, 141.81, 143.25, 150.26, 151.92, 152.29, 154.33, 164.50

MS (ESI) m/z : 324 (M)$^+$

Elemental Analysis : Molecular formula C$_{17}$H$_{13}$FN$_4$O$_2$

Calculated : C, 62.96; H, 4.04; N, 17.28%

Found : C, 62.95; H, 4.02; N, 17.26%
13. 5-Methyl-3-(1-pyridin-3-yl-1H-[1,2,3]triazol-4-ylmethyl)-3H-benzoxazol-2-one (3m)

Yield: 88%

Physical appearance: Brown crystals

m. p.: 229-230°C

\[ \text{R}_f \] : 0.67 (toluene: ethyl acetate: formic acid; 5:4:1)

\[ \text{IR (KBr) cm}^{-1} \]: 3145, 3067, 1772, 1623, 1240, 1132, 1043, 851

\[ \text{\textsuperscript{1}H NMR (DMSO-d\textsubscript{6}, 300 MHz)} \]: \( \delta \) 2.33 (s, 3H), 5.20 (s, 2H), 6.94 (d, 1H, \( J=7.8 \) Hz), 7.20-7.24 (m, 2H), 7.43 (t, 1H, \( J=7.5 \) Hz), 7.53-7.60 (m, 2H), 7.82 (t, 1H, \( J=7.5 \) Hz), 8.74 (s, 1H)

\[ \text{\textsuperscript{13}C NMR (DMSO-d\textsubscript{6}, 75 MHz)} \]: \( \delta \) 21.50, 37.26, 109.77, 110.45, 117.47, 123.18, 124.99, 125.14, 125.89, 126.05, 131.05, 140.46, 142.40, 152.59, 154.25, 155.91

\[ \text{MS (ESI) m/z} \]: 307 (M)\(^+\)

Elemental Analysis:

Calculated: C, 62.53; H, 4.26; N, 22.79%

Found: C, 62.51; H, 4.24; N, 22.76%

14. 3-[1-(4-Chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-5-methyl-3H-benzoxazol-2-one (3n)

Yield: 83%

Physical appearance: Yellow crystals

m. p.: 205-206°C

\[ \text{R}_f \] : 0.68 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm$^{-1}$: 3135, 30216, 1754, 1495, 1246, 1134, 1088, 895

$^1$H NMR (DMSO-d$_6$, 300 MHz): $\delta$ 2.33 (s, 3H), 5.18 (s, 2H), 6.94 (d, 1H, $J$=7.5 Hz), 7.16-7.25 (m, 2H), 7.66 (d, 2H, $J$=7.8 Hz), 7.93 (d, 2H, $J$=8.1 Hz), 8.94 (s, 1H)

$^{13}$C NMR (DMSO-d$_6$, 75 MHz): $\delta$ 21.51, 37.43, 109.78, 110.39, 122.23, 122.53, 123.20, 130.31, 131.02, 133.50, 135.72, 140.48, 143.17, 154.24

MS (ESI) m/z: 341 (M+1)$^+$

Elemental Analysis: Molecular formula C$_{17}$H$_{13}$ClN$_4$O$_2$

Calculated: C, 59.92; H, 3.85; N, 16.44%

Found: C, 59.88; H, 3.82; N, 16.42%

15. 5-Chloro-3-[1-(4-chloro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one (3o)

Yield: 83%

Physical appearance: Yellow crystals

m. p.: 225-226 °C

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

IR (KBr) cm$^{-1}$: 3206, 3123, 1780, 1610, 1248, 1092, 827

$^1$H NMR (DMSO-d$_6$, 300 MHz): $\delta$ 5.22 (s, 2H), 7.2 (dd, 1H, $J$=1.8 and 8.4 Hz), 7.41 (d, 1H, $J$=8.7 Hz), 7.53 (d, 1H, $J$=2.1 Hz), 7.67 (d, 2H, $J$=8.7 Hz), 7.92 (d, 2H, $J$=8.7 Hz), 8.94 (s, 1H)

$^{13}$C NMR (DMSO-d$_6$, 75 MHz): $\delta$ 37.15, 109.87, 111.07, 121.75, 122.11, 128.05, 129.83, 131.98, 133.03, 135.22, 140.73, 142.40, 153.41

MS (ESI) m/z: 360 (M)$^+$

Elemental Analysis: Molecular formula C$_{16}$H$_{10}$Cl$_2$N$_4$O$_2$

Calculated: C, 53.21; H, 2.79; N, 15.51%
Found : C, 53.18; H, 2.76; N, 15.49%

16. 5-Chloro-3-[1-(3-nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzo oxazol-2-one (3p)

Yield : 80%
Physical appearance : Yellow crystals
m. p. : 163-164 °C
Rf : 0.58 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm\(^{-1}\) : 3250, 3182, 1781, 1540, 1484, 1352, 1256, 823
\(^1\)H NMR (DMSO-\(d_6\), 300 MHz) : \(\delta\) 5.25 (s, 2H), 7.21 (dd, 1H, \(J=2.1\) and 8.7 Hz), 7.42 (d, 1H, \(J=8.7\) Hz), 7.53 (d, 1H, \(J=2.1\) Hz), 7.89 (t, 1H, \(J=8.10\) Hz), 8.33-8.41(m, 2H), 8.70 (t, 1H, \(J=2.1\) Hz), 9.15 (s, 1H)
\(^1^3\)C NMR (DMSO-\(d_6\), 75 MHz) : 37.69, 110.36, 111.60, 122.64, 122.96, 123.71, 126.56, 126.59, 128.57, 132.99, 137.49, 140.14, 143.21, 149.68, 153.91
MS (ESI) m/z : 371 (M)\(^+\)
Elemental Analysis : Molecular formula C\(_{16}\)H\(_{10}\)ClN\(_5\)O\(_4\)
Calculated : C, 51.70; H, 2.71; N, 18.84%
Found : C, 51.67; H, 2.69; N, 18.82%
17. 5-Chloro-3-[1-(2-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one (3q)

![Chemical Structure of 5-Chloro-3-[1-(2-fluoro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one (3q)]

Yield: 82%
Physical appearance: White crystals
m. p.: 159-160 °C
Rf: 0.56 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm⁻¹: 3152, 3109, 1780, 1618, 1492, 1112, 1048, 846
¹H NMR (DMSO-d₆, 300 MHz): δ 5.24 (s, 2H), 7.20 (d, 1H, J=7.5 Hz), 7.40 (d, 2H, J=8.7 Hz), 7.56 - 7.82 (m, 4H), 8.75 (s, 1H)
¹³C NMR (DMSO-d₆, 75 MHz): δ 37.47, 109.94, 111.43, 114.68, 121.66, 123.54, 124.61, 132.22, 135.98, 137.12, 141.53, 143.56, 145.25, 155.35
MS (ESI) m/z: 345 (M+1)⁺
Elemental Analysis: Molecular formula C₁₆H₁₀ClFN₄O₂
Calculated: C, 55.75; H, 2.92; N, 16.25%
Found: C, 55.73; H, 2.89; N, 16.23%

18. 5-Chloro-3-(1-pyridin-3-yl-1H-[1,2,3]triazol-4-ylmethyl)-3H-benzooxazol-2-one (3r)

![Chemical Structure of 5-Chloro-3-(1-pyridin-3-yl-1H-[1,2,3]triazol-4-ylmethyl)-3H-benzooxazol-2-one (3r)]

Yield: 86%
Physical appearance: White crystals
m. p.: 196-197 °C
Rf: 0.48 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm⁻¹: 3066, 3012, 1788, 1611, 1488, 1244, 1096, 807
$^1$H NMR (DMSO-d$_6$, 300 MHz): $\delta$ 5.24 (s, 2H), 7.19-7.22 (m, 1H), 7.41 (d, 1H, $J=8.4$ Hz), 7.55 (s, 1H), 7.61 (dd, 1H, $J=8.4$ and 5.1 Hz), 8.31 (d, 1H, $J=8.1$ Hz), 8.69 (s, 1H), 9.01 (s, 1H), 9.12 (s, 1H)

$^{13}$C NMR (DMSO-d$_6$, 75 MHz): $\delta$ 37.46, 109.77, 110.45, 117.47, 123.79, 124.16, 124.69, 126.05, 131.05, 132.65, 140.46, 142.40, 143.62, 153.59

MS (ESI) m/z: 328 (M+1)$^+$

Elemental Analysis: Molecular formula C$_{15}$H$_{10}$ClN$_5$O$_2$

Calculated: C, 54.97; H, 3.08; N, 21.37%

Found: C, 54.95; H, 3.07; N, 21.36%

19. 3-[(3-Nitro-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzooxazol-2-one (3s)

![Chemical structure of 3s]

Yield: 86%

Physical appearance: Yellow crystals

m. p.: 207-208 °C

$R_f$: 0.63 (toluene: ethyl acetate: formic acid; 5:4:1)

IR (KBr) cm$^{-1}$: 3173, 3090, 1781, 1533, 1439, 1248, 1081, 816

$^1$H NMR (DMSO-d$_6$, 300 MHz): $\delta$ 5.24 (s, 2H), 7.12-7.24 (m, 2H), 7.36 (t, 2H, $J=7.8$ Hz), 7.88 (t, 1H, $J=8.4$ Hz), 8.30-8.41 (m, 2H), 8.70 (t, 1H, $J=2.1$ Hz), 9.15 (s, 1H)

$^{13}$C NMR (DMSO-d$_6$, 75 MHz): $\delta$ 37.66, 109.78, 111.27, 116.55, 123.93, 124.33, 125.68, 127.56, 131.99, 132.66, 136.51, 140.50, 143.51, 148.96, 155.25

MS (ESI) m/z: 336 (M)$^+$, 338 (M+2)$^+$

Elemental Analysis: Molecular formula C$_{16}$H$_{11}$N$_5$O$_4$

Calculated: C, 56.98; H, 3.29; N, 20.76%

Found: C, 56.96; H, 3.27; N, 20.74%
20. 3-[1-(4-Ethoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzo oxazol-2-one (3t)

Yield : 88%
Physical appearance : Yellow crystals
m. p. : 175-176 °C
R_f : 0.58 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm\(^{-1}\) : 3172, 3123, 1774, 1521, 1488, 1252, 1050, 802
\(^1\)H NMR (DMSO-\textit{d}_6, 300 MHz) : \(\delta\) 1.34 (t, 3H, \(J=6.9\ \text{Hz}\)), 4.08 (q, 2H, \(J=6.9\ \text{Hz}\)), 5.18 (s, 2H), 7.08-7.21 (m, 4H), 7.34 (t, 2H, \(J=8.7\ \text{Hz}\)), 7.75 (d, 2H, \(J=9.0\ \text{Hz}\)), 8.78 (s, 1H)
\(^13\)C NMR (DMSO-\textit{d}_6, 75 MHz) : \(\delta\) 15.01, 37.54, 64.00, 110.15, 115.73, 122.18, 122.43, 122.92, 124.41, 130.19, 131.08, 142.45, 146.61, 148.96, 154.00, 159.05
MS (ESI) m/z : 336 (M)\(^+\)
Elemental Analysis : Molecular formula C\(_{18}\)H\(_{16}\)N\(_4\)O\(_3\)
Calculated : C, 64.28; H, 4.79; N, 16.66%
Found : C, 64.27; H, 4.77; N, 16.64%

21. 3-[1-(4-Methoxy-phenyl)-1H-[1,2,3]triazol-4-ylmethyl]-3H-benzo oxazol-2-one (3u)

Yield : 88%
Physical appearance : Yellow crystals
m. p. : 169-170 °C
R_f : 0.60 (toluene: ethyl acetate: formic acid; 5:4:1)
IR (KBr) cm\(^{-1}\) : 3215, 3137, 1765, 1486, 11241, 1044, 830
\[^{1}\text{H NMR (DMSO-d}_6, 300 MHz)\]:  \(\delta\) 3.81 (s, 3H), 5.19 (s, 2H), 7.10-7.21 (m, 4H), 7.35 (t, 2H, \(J=7.8\) Hz), 7.77 (d, 2H, \(J=9.0\) Hz), 8.81 (s, 1H)

\[^{13}\text{C NMR (DMSO-d}_6, 75 MHz)\]:  \(\delta\) 37.53, 56.02, 110.17, 115.32, 122.19, 124.08, 130.34, 132.09, 143.17, 160.11

\textbf{MS (ESI) m/z}  : 322 (M\(^+\))

\textbf{Elemental Analysis}  : Calculated for molecular formula \(\text{C}_{17}\text{H}_{14}\text{N}_4\text{O}_3\)
\textbf{Calculated} : C, 63.25; H, 4.38; N, 17.38%
\textbf{Found} : C, 63.23; H, 4.35; N, 17.36%

22. 3-(1-Pyridin-3-yl-1H-[1,2,3]triazol-4-ylmethyl)-3H-benzoazol-2-one (3v)

![Chemical Structure]

\textbf{Yield} : 90%
\textbf{Physical appearance} : Brown crystals
\textbf{m. p.} : 179-180°C
\textbf{R}_f  : 0.55 (toluene: ethyl acetate: formic acid; 5:4:1)
\textbf{IR (KBr) cm}^{-1}  : 3182, 3106, 1757, 1584, 1439, 1151, 1022, 811

\[^{1}\text{H NMR (DMSO-d}_6, 300 MHz)\]:  \(\delta\) 5.23 (s, 2H), 7.12-7.24 (m, 2H), 7.35 (t, 2H,  \(J=6.3\) Hz), 7.62 (d, 1H, \(J=4.5\) Hz), 8.30 (d, 1H, \(J=7.5\) Hz), 8.69 (s, 1H), 8.99 (s, 1H), 9.12 (s, 1H)

\[^{13}\text{C NMR (DMSO-d}_6, 75 MHz)\]:  \(\delta\) 37.48, 110.15, 110.19, 122.94, 124.44, 124.99, 128.44, 131.04, 141.78, 142.44, 143.17, 150.26, 153.99

\textbf{MS (ESI) m/z}  : 294 (M+1)\(^+\)

\textbf{Elemental Analysis} : Molecular formula \(\text{C}_{15}\text{H}_{11}\text{N}_5\text{O}_2\)
\textbf{Calculated} : C, 61.43; H, 3.78; N, 23.88%
\textbf{Found} : C, 61.42; H, 3.76; N, 23.85%
7.0. **Biological activity**

7.1. **Anti-inflammatory assay**

The synthesized compounds were tested for their anti-inflammatory activity using carrageenan induced hind paw edema method. The rat paw edema was induced by subcutaneous injection of 0.1 ml of 1% freshly prepared saline solution of carrageenan into the right hind paw of rats [69]. The standard drug, indomethacin (0.05 mmoles/kg) was given orally as a positive control. The control group was administered orally with 0.9% of 0.1 ml of saline solution only. The test groups were administered orally with equimolar dosage of the synthesized compounds as the standard drug, 1 h before the administration of carrageenan. The paw volumes were measured using plethysmometer [70] at interval of 3 h and 5 h.

7.2. **Assay for in vitro COX 2**

Recombinant human COX-2 has been expressed in insect cell expression system. The enzymes have been purified by using conventional chromatographic techniques. Enzymatic activities of COX-2 was measured according to the method of Copeland [71], with slight modifications using a chromogenic assay based on the oxidation of N,N,N,N-tetra methyl-p-phenylene diamine (TMPD) during the reduction of PGG$_2$ to PGH$_2$ [72]. Briefly, the assay mixture contained Tris HCl buffer (100 mM, Ph 8.0), hematin (15 mM), EDTA (3 mM), enzyme (100 mg COX-2) and the test compound. The mixture was pre-incubated at 25°C for 1 min and then the reaction was initiated by the addition of arachidonic acid and TMPD, in total volume of 1 ml. The enzyme activity was determined by estimating the velocity of TMPD oxidation for the first 25 s of the reaction by following the increase in absorbance at 603 nm. A low rate of nonenzymatic oxidation observed in the absence of COX-2 was subtracted from the experimental value while calculating the percent inhibition.

7.3. **Assay for in vitro TNF-α**

Macrophages cells were grown in in RPMI 1640 containing 10% FBS, 1 M HEPES, 2 mM glutamine, 100 U/mL penicillin and 100 mg/mL streptomycin was obtained by passage through a stainless mesh. Supernatants of cell culture collected from form macrophages cells seeded with varying concentrations of compounds was assayed for
the pro-inflammatory cytokine levels using ELISA kits (e-Biosciences). For this, macrophages were pre-incubated with cytochalasin D (2.5 µM /1 × 10⁶ cells) for 30 min before the start of the experiment and continued till the end. Cytokine levels were measured according to the protocol of the manufacturer. Cells were cultured in 96-well plates at 2 × 10⁶ cells/mL and cytokines were then measured from the supernatants by ELISA. The assay was performed according to the manufacturer’s instruction with multipoint analysis [73]. Briefly, 100µL of diluted capture antibody was added to each well in a 96 well plate and was allowed to adhere overnight for 4°C. Plates were washed and then blocked with 1× PBS supplemented with 10% FBS for 1 h at room temperature. After washing, serial dilutions of the standard and samples were prepared in the plates and were then incubated for 2 h at room temperature. Then, plates were washed and working detector solution (including detector antibody and avidin–horse radish peroxidase reagent) was added into each well. Plates were then sealed and incubated for 1 h at room temperature. After washing, 100 µL of tri-methyl benzidine (TMB) substrate was added into each well. Stock solution (2 N H₂SO₄) was finally added after incubation in the dark for 30 min at room temperature. The absorbance was read at 450 nm. The result was analyzed using softmax program and values determined against the standard provided by the manufacturer.

7.4. Antinociceptive assays

7.4.1. Writhing test

The writhing test in mice was carried out using the method of Koster et al [74]. The writhes were induced by intraperitoneal injection of 0.6% acetic acid (v/v) (80 mg/kg). The standard drug i.e. indomethacin was given orally at a dose 0.05 mmoles/kg of body weight. The test compounds were administered orally at an equimolar dosage to groups of six animals each, 30 min before chemical stimulus. The numbers of muscular contractions were counted over a period of 20 min after acetic acid injection. The data represents the total number of writhes observed during 20 min and is expressed as writhing numbers.
7.4.2. **Tail immersion method**

In the present study analgesia was assessed by employing tail immersion method [75]. Prior to the experiment the animals were screened for the sensitivity test by immersing the tail of the rats gently in hot water maintained at 55°C [76]. The animals flicking their tail from hot water in 5 s were selected for the study in order to avoid any thermal injury to the tail. The selected rats were then divided into six groups of six rats each. The control group was administered orally with 0.9% of 0.1 ml of saline solution only. The standard drug indomethacin and the test compounds were given orally at a dose of 0.05 mmoles/kg of body weight. After administration of the drugs, the reaction time was measured at 30, 60 and 120 min. The basal reaction time was calculated as the reaction time prior to the drug administration.

7.5. **Ulcerogenic activity**

The test compounds having anti-inflammatory and analgesic activities comparable with the indomethacin were further tested for their ulcerogenic risk evaluation. This was done at three times higher dose in comparison to the dose used for anti-inflammatory activity, i.e. 0.15 mmoles/kg body weight of indomethacin and the test compounds were used. Each group had three animals which were later sacrificed. When compared with indomethacin, these compounds did not cause any gastric ulceration and disruption of gastric epithelial cells at the above mentioned oral dose. Hence gastric tolerance towards the test compounds was better than that of indomethacin.

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

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

COX-2 inhibiting ligands were further analyzed by molecular docking studies to gain insights in specific docking pose of these ligands with respect to COX-2 target. As animal studies were carried out with reference to celecoxib drug, co-crystallized structure of COX-2 protein (PDB No: 3LN1) with celecoxib was considered for the docking studies [77]. All new ligands were separately docked against COX-2 target using Schrodinger software. Having retrieved the protein from Protein Data Bank, it was optimized and minimized by Protein Preparation Wizard. Undesirable water
molecules and other defects in the target protein were rectified to obtain a low energy and structurally correct target protein. This was followed by grid generation which involved selecting celecoxib as the reference ligand. The generated grid was initially redocked with celecoxib to verify the docking methodology. Crystallised celecoxib forms hydrogen bonding with LEU-338 & PHE-505 residue in the binding pocket of target protein [78]. Further COX-2 inhibiting ligands were prepared by LipPrep module to obtain low energy 3D structurally correct molecules. These molecules were docked against the validated grid of target protein. Docking was carried out using Standard precision, followed by Extra precision mode and “write XP descriptor” information.

8.2. **In silico molecular docking against TNF-α**

All the computational studies were carried out in the Schrodinger suite 2010 molecular modeling software. The 2D structure of the ligands (3a, 3e, 3f, 3i and 3o) was built in the maestro window [79]. The structures were then converted to their respective 3D structures, with various conformers, tautomers and ionization states using the Ligprep and Confgen modules [80-83]. The 3D crystal structure 2AZ5 [84], solved at a resolution of 2.10˚A, was downloaded from Protein Data Bank for carrying out the docking studies. The crystal structure constitutes four chains A,B,C and D. Chains A and B were homologous to chains C and D and hence, chains A and B were used for docking studies, while chains C and D were discarded for computational purpose. The coordinates of TNF-α in complex with this ligand were obtained from protein data bank. The protein was prepared for docking using the protein preparation wizard. In the pre-process step hydrogen’s were added to the protein with assigning bond order, creating disulfide bond and water residues were removed beyond 5˚A from the heteroatom. Further, the interactions of water residues with protein and heteroatom were checked and only those water residues were kept, which were interacting with protein as well as heteroatom. Then the heteroatom was extracted and protein was refined by assigning H-bonds and minimization at OPLS 2005 force field. A grid was generated at active site, identified on the bases of already co-crystallised ligand to the receptor using receptor grid generation module. Docking studies were then carried out with the co-crystallised ligand, in order to confirm the
protocol. The conformation of the co-crystallised ligand matched best with the docked conformation using extra precision (XP) docking algorithm of Glide module.

9.0. Quantitative Structure Activity Relationship Studies (QSAR)

With continuing interest in the development of Cox-II inhibitors, a 3D QSAR was carried on the same series. To carry QSAR studies the biological activity were transmitted by using following formula [85].

Activity = -logc + logi

Where c is molar concentration = concentration (μg/ml)*0.001/molecular weight.

Logi = log[%inhibition/(100-%inhibition)].

To establish a relation between spatial 3D pharmacophoric feature and Cox II inhibition, we have used PHASE 3.4 module of Schrodinger software.

9.1. Methodology

A pharmacophore model and 3D QSAR study was carried out by using PHASE 3.4 module of Schrodinger molecular modelling software (Phase, version 3.4, Schrödinger, 2012). A phase produces step by step common pharmacophore and 3D QSAR model which is as follows.

a) Prepare ligand

b) Create pharmacophoric site

c) Find common pharmacophore

d) Score hypothesis

e) Build QSAR model.

Phase provides two methodologies to carry 3D QSAR viz, pharmacophore based and atom based. For dataset with different chemical scaffold pharmacophore based 3D QSAR methodology gives best result, but for same scaffold atom based is preferred. We carried our QSAR studies using atom based methodology.
9.2. **Ligand Preparation**

Ligprep 2.5 was used for preparing the entire molecule (LigPrep, version 2.5, Schrödinger, 2012). Phase incorporates two steps for preparing ligand. In first step all structures are cleaned by attaching hydrogen, converting 2D structure to 3D, producing most probable ionization state at a user defined pH, generating all possible stereoisomers. In second step all the thermally accessible conformations for ligand were generated by using Macro model 9.9 conformation search engine (MacroModel, version 9.9, Schrödinger, 2012). The OPLS-2005 force field was used for optimizing the geometry of molecules.

9.3. **Create pharmacophoric site**

A pharmacophoric site was created for all ligands along with their generated conformations. Phase provides facility to create six pharmacophoric sites on a molecule which is; Hydrogen bond acceptor (A), Hydrogen bond donor (D), Hydrophobic group (H), Negatively charged group (N), Positively charged group (P) and Aromatic ring (R).

9.4. **Find common pharmacophore**

The data set was divided into active and inactive set to develop a common pharmacophore. The activity threshold for active set was set for 1.55 and for inactive set was kept for 1.300 (Table 13). Phase develops common pharmacophore by utilizing only active set of molecules. The common pharmacophoric feature was identified from the set of all conformers of active set molecules. Common pharmacophore is developed by utilizing a tree-based partitioning technique that groups together similar pharmacophores according to their intersite distances, i.e., the distances between pairs of sites in the pharmacophore. The pharmacophore which satisfies all user defined criteria will survive. To find common pharmacophore in this study, both the maximum number of sites and minimum number of sites was set to 5.

9.5. **Score hypothesis**

All the developed pharmacophores were subjected to scoring to identify the best candidate hypothesis. Two types of scoring *viz*, survival scoring and inactive scoring
were used to score developed pharmacophore. The scoring algorithm included the contribution from the alignment of site point, vectors, volume overlap, selectivity, number of ligand matches, relative conformational energy and activity [86]. We kept only those pharmacophores whose alignment was below 0.7 °A.

9.6. **Build QSAR model**

A 3D QSAR model was developed for selected hypothesis by utilizing atom based QSAR methodology. The molecules which were not a part of dataset during pharmacophore development and nonaligned molecules were aligned at this stage by using flexible alignment methodology. The data set (Table 13) was divided randomly into training set (70%) and test set (30%). In atom based QSAR methodology the atoms are differentiated into six categories: polar hydrogen were designated as hydrogen bond donor (D), hydrogen, carbon and hydrogen attached to carbon were categorised as hydrophobic (H), atom with positive and negative charges were categorised as positive ion (P) and negative ion (N) respectively, the oxygen, nitrogen and halogens were classified as electron withdrawing (W) and all other type of atom were categorized as miscellaneous (X). The all aligned training set molecule for the purpose of QSAR development were placed in a regular grid of cube. Each cube gets a weightage of zero or one for different types of atoms occupying that cube. If the centre of cube falls within the radius of the atom or site then it would get a weightage of one, otherwise zero. Using this binary data as independent variable, the partial least square (PLS) QSAR model was developed. A QSAR model was developed on 1˚A grid and three PLS factor. The predictive power of developed model was checked against a test set of five molecules.

10. **Conclusion**

We have synthesized a focussed library of novel *bis*-heterocycles encompassing benzoxazolinone-1,2,3-triazole moieties conjugated through a methylene linkage and evaluated them for their anti-inflammatory and antinociceptive activities. The compounds 3e, 3i, 3a, 3f and 3q exhibited potent anti-inflammatory and antinociceptive activities without exhibiting any gastric ulceration. The selective COX-2 inhibitory potential of compound 3f (COX-1/COX-2 SI = 72.8) along with other molecules strongly suggests 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 vitro* TNF-α activity indicated that the compound 3i has comparable TNF-α inhibition similar to the standard drug indomethacin. The *in silico* molecular docking study on the TNF-α protein showed that the compound 3i is oriented in the binding site in such a manner that it favours the possibility of two π-π interactions on the two benzene rings with Tyr59 (chain A of the dimer) and Tyr119 (chain B of the dimer) respectively.