Chapter III

Synthesis of 1,3,4-oxadiazole/oxadiazoline derivatives and their Biological Activities
3.0. Introduction

Oxadiazoles or furadiazoles [1] are five membered heterocyclic compounds with one oxygen and two nitrogen atoms, having molecular formula C₂H₂N₂O. Four isomers of oxadiazole are known viz. 1,2,3-, 1,2,4-, 1,2,5- and 1,3,4-oxadiazoles. Among these isomeric forms, 1,3,4-oxadiazoles are found to be the most biologically potent.

![Four isomers of oxadiazole](image)

### 1,2,3-Oxadiazole (1)
### 1,2,4-Oxadiazole (2)
### 1,2,5-Oxadiazole (3)
### 1,3,4-Oxadiazole (4)

3.1. Recent Advancements

1,3,4-Oxadiazole/oxadiazoline ring is associated with many types of biological properties such as anti-inflammatory [2-4], hypoglycemic [5], antifungal and antibacterial [6-10]. Some of the oxadiazoline derivatives such as 2,5-diaryl-2,3-dihydro-1,3,4-oxadiazoline analogs of combretastatin-A4 showed potent anticancer activity [11]. Oxadiazolin-5-ones and oxadiazoline-5-thiones also possess various analgesic, antipyretic and antichloristic properties. Some of the 1,3,4-oxadiazole derivatives that show promising anti-inflammatory and antimicrobial activities are shown in table 1.

Table 1 Biologically active 1,3,4-oxadiazole derivatives.

<table>
<thead>
<tr>
<th>S.No.</th>
<th>IUPAC Name</th>
<th>Structure</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2-[5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-yl]-1-(4-phenylphenyl) ethanone</td>
<td><img src="image" alt="Structure 5" /></td>
<td>Anti-inflammatory [12]</td>
</tr>
<tr>
<td>2</td>
<td>5-[2-(dimethylamino)-6-methylpyrimidin-4-ylthio)methyl]-1,3,4-oxadiazole-2(3H)-thione</td>
<td><img src="image" alt="Structure 6" /></td>
<td>Anti-inflammatory [13]</td>
</tr>
<tr>
<td></td>
<td>Chemical Structure</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-------------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td><img src="image3.png" alt="Image" /></td>
<td>5-[(naphthalen-6-yloxy)methyl]-N-phenyl-1,3,4-oxadiazol-2-amine</td>
<td>Anti-inflammatory [14]</td>
</tr>
<tr>
<td>4</td>
<td><img src="image4.png" alt="Image" /></td>
<td>5-(6-methyl-2-Arylpyrimidin-4-yloxy)-3-(morpholinomethyl)-1,3,4-oxadiazole-2(3H)-thione</td>
<td>Anti-inflammatory [15]</td>
</tr>
<tr>
<td>5</td>
<td><img src="image5.png" alt="Image" /></td>
<td>(E)-methyl-2-[2-[(substitutedphenyl)-1,3,4-oxadiazol-2-ythio]phenyl]-2-methoxyiminoacetate</td>
<td>Antifungal [16]</td>
</tr>
<tr>
<td>6</td>
<td><img src="image6.png" alt="Image" /></td>
<td>2-(3,4,5-trimethoxyphenyl)-5-(methylsulfonyl)-1,3,4-oxadiazole</td>
<td>Antifungal [17]</td>
</tr>
<tr>
<td>7</td>
<td><img src="image7.png" alt="Image" /></td>
<td>2-substituted-5-(2-benzylxoyphenyl)-1,3,4-oxadiazole</td>
<td>Anticonvulsant [18]</td>
</tr>
<tr>
<td>8</td>
<td><img src="image8.png" alt="Image" /></td>
<td>3-{2-furyl[4-{4-(2-furyl)[5-(2-naphthylxoymethyl)-2-thioxo-2,3-dihydro-1,3,4-oxadiazol-3 yl]methylamino]phenylsulfonyl }anilino]methyl]-5-(2-naphthylxoymethyl)-2,3-dihydro-1,3,4-oxadiazole-2-thione</td>
<td>Antitubercular [19]</td>
</tr>
<tr>
<td>9</td>
<td><img src="image9.png" alt="Image" /></td>
<td>3-(5-(pyridin-4-yl)-1,3,4-oxadiazol-2-yl)-1H-indole</td>
<td>Anticancer [20]</td>
</tr>
<tr>
<td>10</td>
<td><img src="image10.png" alt="Image" /></td>
<td>2-substituted-1,3,4-oxadiazoles</td>
<td>Anti-inflammatory [21]</td>
</tr>
</tbody>
</table>
In view of biological importance of the 1,3,4-oxadiazole, several methods have been developed for the synthesis of 1,3,4-oxadiazoles. Some common methods for the synthesis of 1,3,4-oxadiazole are listed below.

3.2. Common methods for the preparation of 1,3,4-oxadiazoles.

1,3,4-oxadiazoles have been most commonly synthesized from corresponding hydrazides by using either oxidative ring closure or condensation or by reacting with ortho esters, imido esters, phosgene or carboxylic acids etc. as given below.

3.2.1. Ring closure by means of oxidation reactions.

3.2.1.1. 2,5-disubstituted 1,3,4-oxadiazole (16) has been produced from ceric ammonium nitrate (CAN) catalyzed oxidative cyclization of acylhydrazone (15) [22].

\[
\text{CAN} \quad \begin{array}{c}
\text{R}^1 = \text{Alkyl/Aryl; } \text{R}^2 = \text{Alkyl/Aryl}
\end{array}
\]

3.2.1.2. Synthesis of 2,5-disubstituted 1,3,4-oxadiazoles (18) from hydrazones and semicarbazones (17) by using halogen catalyzed oxidations [23].

3.2.1.3. Hydrazone (19) on cyclization in presence of Chloramin-T gives 1,3,4-oxadiazole (20) [24].
3.2.2. Ring closure by means of condensation reactions.

3.2.2.1. Condensation of carboxylic acids (22) with acylhydrazides (21) gives 2,5-disubstituted 1,3,4-oxadiazole (23) [25].

\[
\begin{align*}
\text{COO} & \quad \text{NH} \\
\text{R}_1 & \quad \text{NH}_2 \\
\text{21} & \quad \text{OH} \\
\text{22} & \quad \text{CDI} \\
\text{Ph}_3\text{P}, \text{CBr}_4 & \quad \text{23}
\end{align*}
\]

Scheme-4

\( \text{R}_1 = \text{Alkyl/Aryl}; \text{R}_2 = \text{Alkyl/Aryl} \)

3.2.2.2. Mono and diacid hydrazides, acylsemicarbazides, and the related compounds produce oxadiazole by ring closure. The well-known conversion of \(N, N'\)-diacid hydrazide (24) to 2, 5-diaryl (alkyl)-1,3,4-oxadiazole (25) has been described [26].

\[
\begin{align*}
\text{RCONHNHCO} & \quad \text{H}_2\text{O} \\
\text{24} & \quad \text{25}
\end{align*}
\]

\( \text{R} = \text{Alkyl/Aryl} \)

3.2.2.3. Amino thiosemicarbazide (26) and thiosemicarbazides (28) give 2-Amino 5-aryl 1,3,4-oxadiazole (27) [27] and \(N\)-substituted 1,3,4-oxadiazoles (29) [21] respectively.
3.2.2.4. 1-Acylhydrazine-2-carboxylic acid (30) esters on heating yield 1,3,4-oxadiazolin 5-one (31) with the elimination of alcohol [28, 29].

\[
\text{RCONHNR'}(\text{CO}_2\text{C}_2\text{H}_5) \xrightarrow{-\text{C}_2\text{H}_5\text{OH}} \text{N}^-\text{N}^=\text{O} \\
\text{30} \quad \text{Scheme-8} \quad \text{31}
\]

R = Alkyl/Aryl; R' = Alkyl/Aryl

3.2.2.5. Acid hydrazide (32) and dimethylcarbamyichloride cyclized in presence of pyridine give 1,3,4-oxadiazole-2-one (33) [30].

\[
\text{N}^-\text{N}^=\text{NH} \\
\text{32} \quad \text{Scheme-9} \quad \text{33}
\]

3.2.3. Synthesis of 1,3,4-oxadiazoles from acid hydrazides by introduction of one carbon fragments.

3.2.3.1. Synthesis from acid hydrazides and ortho esters.

The reaction of acid hydrazide (34) with orthoformic esters proceeds via elimination of alcohol to give 2-substituted 1,3,4-oxadiazoles (36). The reaction is mostly carried out with the reactants at the boiling point of the orthoformic ester or in an inert solvent at a higher temperature. Thus the acid hydrazides can be cyclized only when the substituent R is sufficiently electrophilic. An aromatic group, however, is not necessary [31].

\[
\text{RCONHNH}_2 \xrightarrow{\text{CH(OR')}3} \text{(RCONHN=CHOR)} \xrightarrow{-\text{R'}OH} \text{N}^-\text{N}^=\text{O} \\
\text{34} \quad \text{35} \quad \text{36}
\]

R = Alkyl/Aryl

Scheme-10
3.2.3.2. *Synthesis from acid hydrazides and imido esters or imidochlorides.*

A universal method for the preparation of 2,5-dialkyl (aryl)-1,3,4-oxadiazole (38) is by the reaction of acid hydrazide (37) with imido esters or their hydrochlorides [32].

\[
\text{RCONHNH}_2 \xrightarrow{\text{R'CO(OR')=NHCl}} \text{RCONHNH}_2 \xrightarrow{\text{R'=Alkyl/Aryl}} \text{R'N-N} \xrightarrow{\text{O}} \text{R} \xrightarrow{\text{R'=Alkyl/Aryl}} \text{O} \\
\]

\[\text{R= Alkyl/Aryl; R'=Alkyl/Aryl}\]

Scheme-11

3.2.3.3. *Synthesis from hydrazones with acetic anhydride*

Hydrazone (39) undergoes cyclization with acetic anhydride to produce 1,3,4-oxadiazolin-5-ones (40) [11].

\[\text{H}_2\text{C}^\text{O, reflux} \xrightarrow{\text{Ac}_2\text{O}} \text{H}_2\text{C}_\text{O} \xrightarrow{\text{40}} \text{H}_2\text{C}_\text{O} \]

\[\text{R = Cl, Br, H, Alkyl etc}\]

Scheme-12

3.2.3.4. *Synthesis from acid hydrazides with carboxylic acid in presence of POCl}_3*

2,5-disubstituted 1,3,4-oxadiazoles have been conveniently synthesized from acid hydrazide (41) which undergoes cyclization after reaction with different carboxylic acids in presence of POCl}_3 [33].

\[\text{POCl}_3,\text{RCOOH} \xrightarrow{\text{Reflex}} \text{POCl}_3,\text{RCOOH} \xrightarrow{\text{Reflex}} \text{POCl}_3,\text{RCOOH} \]

\[\text{R= Alkyl/Aryl}\]

Scheme-13

148
3.2.3.5. Synthesis from acid hydrazide with carbon disulphides produce 1,3,4-oxadiazole-2-thione.

The 5-mercapto-1,3,4-oxadiazole (44) have been conveniently synthesized from acid hydrazide (42) which undergoing cyclization after reaction with carbondisulphide in basic medium [34].

\[
\begin{align*}
\text{Scheme-14}
\end{align*}
\]

3.3. Reactivity of the 1,3,4-oxadiazole.

Oxadiazole systems are also play as key intermediates for the synthesis of various bioactive molecules. Several reactions have been explored and these heterocyclic systems are also treated as valuable synthons for synthesizing various substituted 1,2,4-triazoles.

3.3.1. Substitution reactions of 1,3,4-oxadiazoles.

3.3.2. Direct ring substitutions.

Direct alkynylation of 1,3,4-oxadiazole (45) with alkynyl bromides (46) under copper catalysis at room temperature has been achieved [35].

\[
\begin{align*}
\text{Scheme-15}
\end{align*}
\]

The direct introduction of functional groups into the oxadiazole nucleus is possible only in a few cases. Halogenation also has not so far been described although for halogenation the deactivating effect of the oxadiazole nucleus is not so important [36, 37]. Introduction of other functional groups into the oxadiazole nucleus by nucleophilic substitution of substituted 1,3,4-oxadiazoles is also difficult. A few known examples proceed with low yield. Thus 2-phenyl-5-amino-1,3,4-oxadiazole
(49) is obtained by ammonolysis of 2-phenyl-5-methanesulfonyl-1,3,4-oxadiazole (48).

\[
\begin{align*}
\text{H}_3\text{CO}_2\text{S} & \quad \text{N} \quad \text{C}_\text{6H}_5 \\
\text{N} \quad \text{O} & \quad \text{C}_\text{6H}_5 \\
\text{NH}_3 & \quad \text{N} \quad \text{C}_\text{6H}_5 \\
\end{align*}
\]

Scheme-16

3.3.3. Reactions proceeding with ring cleavage.

In contrast to high thermal stability the 1,3,4-oxadiazole ring proves extremely labile to chemical agents. Ring cleavage reactions of 1,3,4-oxadiazoles can be achieved by the action of reducing agents and by nucleophilic reagents.

3.3.3.1. Ring Cleavage of 2-Amino-1,3,4-oxadiazole.

The action of nucleophilic reagents on 2-amino-1,3,4-oxadiazoles (50) leads to acyclic compounds which often cyclize immediately to triazoles. Thus 2-amino-1,3,4-oxadiazoles with caustic alkali yield 1,2,4-triazolinones (53) [38, 39].

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

Scheme-17

3.4. Present Work

The importance of 1,3,4-oxadiazoles in biological system has stimulated our interest in designing and constructing new heterocyclic derivatives using chemical/molecular modification approach. Quinoline moiety holds prominent place as it is a part of many drugs and many naturally occurring alkaloids. 8-Hydroxyquinoline is a well known ligand of divalent ions and was rapidly recognized as a major pharmacophore by D’Angelo’s group [40]. It was the first group to include 8-hydroxyquinoline-7-carboxylic acid (55) into the structure of new HIV-1 IN inhibitors with IC\text{50} in the
range of 0.3-4 μM. A number of biological activities including anti-inflammatory, antiallergic [41], antimalarial [42], antibacterial [43], antiproliferative [44] and anticancer [45, 46] have been associated with quinoline-containing compounds such as (quinine, chloroquine, and mefloquine, fluoroquinolones and streptonigirin).

More recently, Shionogi patented 8-hydroxyquinoline-7-carboxylic acid methyl esters, substituted on position 3 by a substituted benzyl group (56). This compound increases the anti HIV potential with IC\textsubscript{50} value 200 nM against HIV-1 IN [47]. The most recent anti HIV drug (57, 58) (patented by Merck) and quinoline based oxadiazole (59) are under clinical trials [48].

Moreover, Non steroidal anti-inflammatory drugs (NSAIDs) present in the markets contains biphenyl and phenyl nucleus such as flurbiprofen and ibuprofen (60, 61).

In view of the biological importance of 1,3,4-oxadiazole, quinoline and biphenyl moieties as anti-inflammatory and antimicrobial agents, we aim to synthesize some novel oxadiazole based conjugates encompassing quinoline-oxadiazole (68-73)
and biphenyl-oxadiazoline (83-91) moieties. A focused library of novel bis-heterocyclic conjugates has been synthesized and evaluated for their biological activities such as anti-inflammatory, analgesic, ulcerogenic and antimicrobial activities. The synthetic scheme for the synthesis of the above compounds is given below.

SCHEME-I

R -O -H
62-63

\[
\begin{align*}
&\text{dry acetone,} \\
&\text{K}_2\text{CO}_3, \\
&\text{Ethyl chloroacetate/reflux} \\
\end{align*}
\]

R -O
64-65

POCl₃

\[
\begin{align*}
&\text{stirring, 60 °C} \\
&\text{Aromatic aldehyde/reflux} \\
\end{align*}
\]

R -O
66-67

\[
\begin{align*}
&\text{Absolute ethanol,} \\
&\text{Aromatic aldehyde/reflux} \\
\end{align*}
\]

8-Hydroxy quinoline and 4-hydroxy biphenyl were taken as starting materials for the synthesis of these two different series. Alkylation of these two hydroxy compounds with ethyl chloroacetate in presence of potassium carbonate in dry acetone yielded the corresponding alkoxyesters 64, 65. Reaction of compound 64 and 65 with hydrazine hydrate in absolute alcohol yielded hydrazide 66 and 67. Hydrazide of quinoline (66) was then converted into 1,3,4-oxadiazole (68-73) by its reaction with different aromatic acids in presence of POCl₃. Reaction of hydrazide of biphenyl 89 with different aromatic aldehydes in absolute alcohol in presence of few drop of acetic acid yielded hydrazones (74-82) which on cyclization with acetic anhydride yielded 1,3,4-
oxadiazoline (83-91). All the synthesized compounds have been completely characterized on the basis of their detailed spectral data and this entire series of new compounds were evaluated for their biological activities. The physical data of all the novel synthesized compounds are given in table 2.

Table 2. Physical data of 1,3,4-oxadiazole and 1,3,4-oxadiazoline derivatives

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>Structure</th>
<th>Yield (%)</th>
<th>m.p. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td><img src="image1" alt="Structure" /></td>
<td>66</td>
<td>116-118</td>
</tr>
<tr>
<td>69</td>
<td><img src="image2" alt="Structure" /></td>
<td>78</td>
<td>108-110</td>
</tr>
<tr>
<td>70</td>
<td><img src="image3" alt="Structure" /></td>
<td>55</td>
<td>145-147</td>
</tr>
<tr>
<td>71</td>
<td><img src="image4" alt="Structure" /></td>
<td>65</td>
<td>184-186</td>
</tr>
<tr>
<td>72</td>
<td><img src="image5" alt="Structure" /></td>
<td>64</td>
<td>190-192</td>
</tr>
<tr>
<td>73</td>
<td><img src="image6" alt="Structure" /></td>
<td>72</td>
<td>178-180</td>
</tr>
<tr>
<td>83</td>
<td><img src="image7" alt="Structure" /></td>
<td>64</td>
<td>119-121</td>
</tr>
<tr>
<td>84</td>
<td><img src="image8" alt="Structure" /></td>
<td>68</td>
<td>128-130</td>
</tr>
<tr>
<td>85</td>
<td><img src="image9" alt="Structure" /></td>
<td>66</td>
<td>122-124</td>
</tr>
<tr>
<td>86</td>
<td><img src="image10" alt="Structure" /></td>
<td>64</td>
<td>168-170</td>
</tr>
</tbody>
</table>
3.5. Results and discussion

3.5.1. Analytical

Compounds were identified on the basis of their \(^1\)H NMR, \(^{13}\)C NMR, IR and mass spectra. The \(^1\)H NMR of compound 64, showed an additional \(-\text{O}-\text{CH}_2\text{CH}_3\) signals derived from ester structure which was observed at \(\delta 3.88\) and 1.5. The signal due to \(\text{OH}\) group of 8-hydroxyquinoline did not appear. Formation of compound 66 from 64 was confirmed from the presence of proton signals at \(\delta 9.7\) and 4.09 corresponding to \(-\text{CONH}\)- and \(-\text{NH}_2\) groups respectively and also by the disappearance of signal of ethoxy group. The target compounds 68-73 were confirmed from the IR and \(^1\)H NMR spectra which did not exhibited signals due to \(-\text{NH}, -\text{NH}_2\) and \(-\text{C}=\text{O}\) group. Finally the compounds 68-73 were confirmed from their mass spectra.

The hydrazide 67 when reacted with different aromatic aldehydes yielded hydrazones (74-82). In the FT-IR spectrum, disappearance of the NH and NH\(_2\) peaks in the region 3350-3152 cm\(^{-1}\) and absorption in the region 1476-1606 cm\(^{-1}\) due to C=N band indicated the formation of hydrazones. Finally the formation of hydrazones (74-82)
was confirmed from their mass spectra. This hydrazone undergoes cyclization when refluxed with acetic anhydride to give 1,3,4-oxadiazoline derivatives (83-87). These absorption bands in the region 1603-1626 cm⁻¹ indicated the cyclization of the hyrazones into 1,3,4-oxadiazoline. This absorption bands were absent in the IR spectra of hydrazones (74-82). In the \(^1\)H NMR spectrum, the presence of signals at δ 2.03-2.36 ppm supported the presence of acetylated methyl group. The \(^13\)C NMR spectrum revealed signals at δ 66-67 and δ 168-169 which showed the presence 1,3,4-oxadiazoline ring and carbonyl group respectively. Finally the structure was confirmed from mass spectrum.

3.5.2. Biological activities

All the newly synthesized compounds 68-73 and 83-91 were screened for their biological activities viz. anti-inflammatory, analgesic, ulcerogenic and antimicrobial.

3.5.2.1. Anti-inflammatory activity

The compounds 68, 71, 72 and 73 from oxadiazole of quinoline and 84, 88 and 91 from oxadiazoline of biphenyl exhibited significant anti-inflammatory activity compared to standard drug Indomethacin and Ibuprofen respectively. Among all tested compounds, compound 68, 71 and 88 has shown highest inhibition in rat paw oedema (65.87, 65.80, 73.05% and 72.33, 75.00, 72.55 % at 3h and 5h respectively) when compared to the standard drug Indomethacin (55.29 and 69.50 at 3h and 5h respectively).

From the biological data, the Structure Activity Relationship (SAR) can be drawn as follows:

- Compounds having electron withdrawing substitution on the aromatic ring attached to the 1,3,4-oxadiazole/oxadiazoline ring showed potential activity.
- Compounds having bulkier substitutions like methoxy, -chloro groups on the aromatic ring exhibited potential activity.

The results of anti-inflammatory activity are summarized in table 3 and figure 1. All data were analyzed by one-way ANOVA test followed by Dunnett’s test in carrageenan induced rat paw oedema model in rats.
Table 3. Anti-inflammatory activity of 1,3,4-oxadiazole/oxadiazoline derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Change in paw edema volume in ml, after drug treatment (±SEM)</th>
<th>Anti-inflammatory Activity % Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>68</td>
<td>0.216 ± 0.04*** 0.166 ± 0.04***</td>
<td>65.87 72.33</td>
</tr>
<tr>
<td>69</td>
<td>0.366 ± 0.04** 0.316 ± 0.04**</td>
<td>42.18 47.13</td>
</tr>
<tr>
<td>70</td>
<td>0.400 ± 0.05** 0.316 ± 0.03**</td>
<td>36.80 47.30</td>
</tr>
<tr>
<td>71</td>
<td>0.216 ± 0.04*** 0.150 ± 0.04**</td>
<td>65.80 75.00</td>
</tr>
<tr>
<td>72</td>
<td>0.232 ± 0.18*** 0.178 ± 0.08***</td>
<td>63.34 71.87</td>
</tr>
<tr>
<td>73</td>
<td>0.200 ± 0.16*** 0.166 ± 0.04***</td>
<td>68.40 72.33</td>
</tr>
<tr>
<td>83</td>
<td>0.46 ± 0.057** 0.43 ± 0.021**</td>
<td>25.32 26.24</td>
</tr>
<tr>
<td>84</td>
<td>0.25 ± 0.050** 0.18 ± 0.028**</td>
<td>59.40 69.12</td>
</tr>
<tr>
<td>85</td>
<td>0.33 ± 0.05* 0.30 ± 0.033*</td>
<td>46.42 48.54</td>
</tr>
<tr>
<td>86</td>
<td>0.36 ± 0.049* 0.33 ± 0.021*</td>
<td>41.55 42.88</td>
</tr>
<tr>
<td>87</td>
<td>0.28 ± 0.022** 0.15 ± 0.042***</td>
<td>54.54 74.27</td>
</tr>
<tr>
<td>88</td>
<td>0.16 ± 0.042*** 0.16 ± 0.021***</td>
<td>73.05 72.55</td>
</tr>
<tr>
<td>89</td>
<td>0.36 ± 0.042** 0.40 ± 0.036**</td>
<td>41.55 31.38</td>
</tr>
<tr>
<td>90</td>
<td>0.45 ± 0.021** 0.43 ± 0.042**</td>
<td>26.94 26.24</td>
</tr>
<tr>
<td>91</td>
<td>0.28 ± 0.022** 0.15 ± 0.042***</td>
<td>54.54 74.27</td>
</tr>
</tbody>
</table>

Control for 68-73 0.633 ± 0.05 0.600 ± 0.03
Control for 83-91 0.615 ± 0.047 0.582 ± 0.030
Indomethacin 0.283 ± 0.03** 0.183 ± 0.30*** 55.29 69.50
Ibuprofen 0.13 ± 0.033*** 0.11 ± 0.011*** 78.89 80.10

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

Anti-inflammatory activity

Figure 1. Anti-inflammatory activity of 1,3,4-oxadiazoles/oxadiazoline derivatives

3.5.2.2. Analgesic Activity

The compounds which showed significant anti-inflammatory activity were further screened for analgesic activity and ulcerogenic studies. Among tested compounds, compound 88 and 91 showed comparable analgesic activity (4.76, 4.70 and 4.94, 4.79) with the standard drug Indomethacin (5.79, 6.42) at 30 min. and 60 min. respectively. The results of analgesic activity are shown in table 4 and figure 2.

Table 4. Analgesic activity of 1,3,4-oxadiazole/oxadiazoline derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Mean value of Tail Flick Latency (sec) ± S.E.M</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min.</td>
</tr>
<tr>
<td>68</td>
<td>3.26±0.39</td>
</tr>
<tr>
<td>71</td>
<td>3.13±0.17</td>
</tr>
<tr>
<td>84</td>
<td>3.12±0.16</td>
</tr>
<tr>
<td>88</td>
<td>3.18±0.39</td>
</tr>
<tr>
<td>91</td>
<td>3.28±0.39</td>
</tr>
<tr>
<td>Control</td>
<td>2.89±0.17</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>1.98±0.307</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>3.38±0.32</td>
</tr>
</tbody>
</table>

Data analyzed by one way ANOVA followed by Dunnett’s ‘t’ test (n=6), *p<0.05, **p<0.01 & ***p<0.001 significantly different from standard; ns, not significant.
Figure 2. Analgesic activity of 1,3,4-oxadiazole/oxadiazoline derivatives

3.5.2.3. Ulcerogenic Study

The compounds showing better anti-inflammatory and analgesic activity were further studied for ulceration study. The compound 71 containing oxadiazole-quinoline moieties were found to be the best among all the tested compounds having least ulceration. The results are given in Table 5 and Figure 3.

Table 5. Haematoxylin and eosin immunohistochemical staining of gastric ulcers after ulcer induction in rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Surface Epithelial Damage</th>
<th>Submucosal Damage</th>
<th>Deep Mucosal Damage</th>
<th>Muscular Layer Damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>71</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>78</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>81</td>
<td>++</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>88</td>
<td>++</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ibuprofen</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
</tbody>
</table>

+, ++, +++: increase in damage, -: no damage

Continued..
Antimicrobial activity

The antifungal results showed that some of the compounds exhibited good antifungal activity against *A. niger* and *A. flavus*. Compounds 68, 69, 71, 87, 88 and 89 showed inhibitory effect against all the tested fungal strains. Compounds 68 and 69 showed significant zone of inhibition against *A. flavus*, *C. albicans*, *C. krusei*. It can be concluded that none of the synthesized compounds were superior to positive controls against tested microbial strains, but the tested compound 88 from 1,3,4-oxadiazole series has equal inhibitory effect against *A. niger*. The antibacterial results showed that some of the compounds displayed good antibacterial activity against Gram negative and Gram positive bacteria. Compound 71 was found to be most active against all bacterial strains.
Table 4  Antimicrobial activity of 1,3,4-oxadiazole/oxadiazoline derivatives.

<table>
<thead>
<tr>
<th>Comp. No.</th>
<th>Microorganisms and Zone of Inhibition in mm.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Antifungal activity</td>
<td>Antibacterial activity</td>
</tr>
<tr>
<td></td>
<td>Aniger 200 (100) μg/disc</td>
<td>A. flavus 200 (100) μg/disc</td>
</tr>
<tr>
<td>68</td>
<td>12 (8)</td>
<td>20 (12)</td>
</tr>
<tr>
<td>69</td>
<td>10 (6)</td>
<td>18 (14)</td>
</tr>
<tr>
<td>70</td>
<td>16 (10)</td>
<td>10 (8)</td>
</tr>
<tr>
<td>71</td>
<td>14 (12)</td>
<td>12 (9)</td>
</tr>
<tr>
<td>72</td>
<td>16 (10)</td>
<td>10 (8)</td>
</tr>
<tr>
<td>73</td>
<td>12 (8)</td>
<td>12 (10)</td>
</tr>
<tr>
<td>83</td>
<td>10 (8)</td>
<td>(-)</td>
</tr>
<tr>
<td>84</td>
<td>Nt</td>
<td>12 (8)</td>
</tr>
<tr>
<td>85</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>86</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>87</td>
<td>15 (8)</td>
<td>12 (8)</td>
</tr>
<tr>
<td>88</td>
<td>18 (12)</td>
<td>12 (8)</td>
</tr>
<tr>
<td>89</td>
<td>16 (10)</td>
<td>12 (8)</td>
</tr>
<tr>
<td>90</td>
<td>10 (6)</td>
<td>12 (8)</td>
</tr>
<tr>
<td>91</td>
<td>12 (8)</td>
<td>14 (8)</td>
</tr>
<tr>
<td>Flucon.</td>
<td>16 (12)</td>
<td>20 (14)</td>
</tr>
<tr>
<td>Ampi.</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>DMSO</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

NT: not tested; -: no zone of inhibition; Flucon: fluconazole; Ampi: ampicillin.
3.6. EXPERIMENTAL

3.6.1. Chemistry

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

Procedure for synthesis of Ethyl 2-(quinolin-8-yloxy)acetate (65)

To a mixture of 8-Hydroxyquinoline (10 mmol) and ethylchloroacetate (10 mmol) in 50 ml anhydrous acetone was added 15g of K$_2$CO$_3$. The suspension was refluxed for 20 h. After completion of reaction monitored by TLC, the reaction mixture was filtered in hot condition, concentrated under reduced pressure and finally the crude product was crystallized from methanol in cold condition.

Ethyl 2-(quinolin-8-yloxy)acetate (65): Yield: 72%; pale yellow, m.p. 54-56 °C, $R_f$ = 0.25 (n-hexane : ethylacetate; 4:6).

![Chemical structure](image)

IR (KBr) cm$^{-1}$: 3056, 2924, 1681, 1109.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 1.85 (t, $J = 5.8$ Hz, 3H), 3.92 (q, $J = 6.8$ Hz, 2 H), 4.96 (s, O-CH$_2$, 2H), 7.60-8.34 (m, 5H, Ar-H), 8.87 (1H, d, $J = 3.6$ Hz).

FAB-MS ($m/z$): 215 (M$^+$), 216 (M$^+$+1)

Elemental Analysis: Calculated for molecular formula C$_{13}$H$_{13}$NO$_2$

Calculated: C, 72.54; H, 6.09; N, 6.51.

Found: C, 72.59; H, 6.10; N, 6.52%
Procedure for synthesis of 2-(quinolin-8-yloxy)acetohydrazide (67)
The ethanolic solution of (65) (10 mmol) and hydrazine monohydrate (10 mmol) was refluxed for 6 h. After the completion of reaction monitored by TLC, the reaction mixture was cooled, the light yellow flakes so obtained was filtered and recrystallized from alcohol.

2-(quinolin-8-yloxy)acetohydrazide (67) Yield: 84%; Light yellow crystals, m.p.150-152 °C, Rf = 0.25 (CHCl3 : MeOH; 9:1).

\[
\text{IR (KBr) cm}^{-1} : 3352, 3054, 2921, 1684, 1109.
\]

\[
\text{H NMR (300 MHz, CDCl}_3\text{) : } \delta 4.87 (s, 2H, O-CH}_2\text{), 4.09 (s, 2H, NH}_2\text{), 7.17-7.60 (m, 4H, Ar-H), 8.20 (d, } J = 8.4 \text{ Hz, 1H), 8.94 (d, } J = 3.9 \text{ Hz, 1H), 9.71 (s, 1H, N-}
\]

\[
\text{H), FAB-MS (m/z): 216 (M^+), 217 (M^+1).}
\]

Elemental Analysis

- Calculated: C, 60.82; H, 5.10; N, 19.34%
- Found: C, 60.78; H, 5.12; N, 19.36%

General synthesis of 1,3,4-oxadiazole (68-73)
To 10-15 ml POCl3, mixture of 2 mmol of compound 67 and 2 mmol of different aromatic acids were added, the reaction mixture were kept for stirring at 60 °C for 8-14 h. After completion of reaction monitored by TLC, the reaction mixture was concentrated under reduced pressure, poured on crushed ice and neutralized by sodium bicarbonate. The precipitate so obtained was filtered, washed with cold water, dried and purified by crystallization/recolumning (Pentane: ethyl acetate)

8-[(5-(4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methoxy]quinoline (68) Yield: 66 %; White crystals, m.p.116-118 °C, Rf = 0.46 (CHCl3 : MeOH; 9:1).

\[
\text{IR (KBr) cm}^{-1} : 3046, 2924, 1677, 1605, 1108.
\]
Chapter III

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

\(\delta\) 3.79 (s, 3H, O-CH\(_3\)), 5.60 (s, 2H, O-CH\(_2\)), 6.90 (d, \(J = 8.1\) Hz, 2H), 7.36 (d, \(J = 9.6\) Hz, 1H), 7.38-7.42 (m, Ar-H, 3H), 7.92 (d, \(J = 8.4\) Hz, 2H), 8.10 (d, \(J = 8.1\) Hz, 1H), 8.92 (s, Ar-H, 1H).

MALDI-MS (m/z)

333 (M\(^+\)), 334 (M\(^+1\)).

Elemental Analysis

Calculated

\(\text{C, 68.46; H, 4.54; N, 12.61}\%\)

Found

\(\text{C, 68.26; H, 4.28; N, 12.48\%}\)

8-(S-(2-methoxyphenyl)-1, 3, 4-oxadiazol-2-yl)methoxyquinoline (69) Yield: 78% White Crystals; m.p. 108-110 °C, \(R_f = 0.47\) (CHCl\(_3\) : MeOH; 9:1).

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

3048, 2920, 1678, 1604, 1455, 1375, 1109.

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

\(\delta\) 3.82 (s, 3H, O-CH\(_3\)), 4.92 (s, 2H, O-CH\(_2\)), 6.97 (d, \(J = 8.6\) Hz, 1H), 7.25 (d, \(J = 9.00\) Hz, 1H), 7.46-7.56 (m, Ar-H, 5H), 8.20 (d, \(J = 6.9\) Hz, 1H), 8.85 (d, \(J = 2.94\) Hz, 1H), 8.95 (d, \(J = 4.2\) Hz, 1H).

MALDI-MS (m/z)

333 (M\(^+\)), 334 (M\(^+1\)).

Elemental Analysis

Calculated

\(\text{C, 68.46; H, 4.54; N, 12.61}\%\)

Found

\(\text{C, 68.12; H, 4.32; N, 12.51\%}\)

8-(5-(4-bromophenyl)-1, 3, 4-oxadiazol-2-yl)methoxyquinoline (70) Yield: 55%; White crystals, m.p. 145-147 °C, \(R_f = 0.43\) (CHCl\(_3\) : MeOH; 9:1).

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

3046, 2926, 1674, 1601, 1450, 1371, 1108.
\textbf{Chapter III}

\begin{align*}
\text{\textit{H} NMR (500 MHz, CDCl}_3) & : \delta 4.87 (s, 2H, O-CH}_2), 7.16 (d, J = 8.7 \text{ Hz}, 1H), 7.53-7.60 \text{ (m, 4H, Ar-H)}, 8.27 (d, J = 9.3 \text{ Hz}, 2H), 7.78 (d, J = 8.4 \text{ Hz}, 2H), 8.87 (d, J = 5.8 \text{ Hz}, 1H).
\end{align*}

\text{Maldi-MS (m/z)} : 382 (M\textsuperscript{+}), 383 (M\textsuperscript{+}+1).

\text{Elemental Analysis} : \text{Calculated for molecular formula C}_{18}H_{12}BrN_{4}O_{3}S

\text{Calculated} : C, 56.56; H, 3.16; N, 10.99.

\text{Found} : C, 56.14; H, 3.46; N, 10.68%.

\textit{8-\{(5-\{(2,4-dichlorophenoxy)methyl\}_1,3,4-oxadiazol-2-yl)methoxy\}_quinoline} (71)

\text{Yield: 65 \%}; \text{White Crystals, m.p. 184-186 °C, R}_f = 0.42 (CHCl\textsubscript{3}: MeOH; 9:1).

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{image}
\caption{Structure of 8-\{(5-\{(2,4-dichlorophenoxy)methyl\}_1,3,4-oxadiazol-2-yl)methoxy\}_quinoline}
\end{figure}

\text{IR (KBr) cm\textsuperscript{-1}} : 3046, 2926, 1674, 1601, 1450, 1108.

\begin{align*}
\text{\textit{H} NMR (500 MHz, DMSO-d\textsubscript{6})} & : \delta 4.74 (s, 2H, O-CH}_2), 4.87 (s, 2H, O-CH}_2), 6.97 (d, J = 8.7 \text{ Hz}, 1H), 7.16 (s, 1H, Ar-H), 7.26 (d, J = 8.7 \text{ Hz}, 1H), 7.43 (d, J = 4.6 \text{ Hz}, 1H), 7.54-7.57 \text{ (m, 3H, A-H)}, 8.28 (d, J = 7.0 \text{ Hz}, 1H), 8.86 (d, J = 2.9 \text{ Hz}, 1H).
\end{align*}

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

\text{Elemental Analysis} : \text{Calculated for molecular formula C}_{19}H_{13}Cl_{2}N_{3}O_{3}.

\text{Calculated} : C, 56.73; H, 3.26; N, 10.45.

\text{Found} : C, 56.78; H, 3.27; N, 10.46%.

\textit{8-\{(5-\{4-Hydroxyphenyl\}_1,3,4-oxadiazol-2-yl)methoxy\}_quinoline} (72)

\text{Yield: 64 \%}; \text{White Crystals, m.p. 190-192 °C, R}_f = 0.39 (CHCl\textsubscript{3}: MeOH; 9:1).

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{image}
\caption{Structure of 8-\{(5-\{4-Hydroxyphenyl\}_1,3,4-oxadiazol-2-yl)methoxy\}_quinoline}
\end{figure}
IR (KBr) cm$^{-1}$ : 3046, 2926, 1674, 1601, 1450, 1108.

$^1$H NMR (500 MHz, DMSO-d$_6$) : $\delta$ 4.90 (s, 2H, O-CH$_2$), 8.90-6.70 (m, 10H), 10.54 (s, 1H, OH).

Maldi-MS (m/z) : 319 (M$^+$), 320 (M$^+$+1).

Elemental Analysis

Calculated

Found

Calculated for molecular formula C$_{18}$H$_{13}$N$_3$O$_3$.

C, 67.71; H, 4.10; N, 13.16.

C, 67.73; H, 4.11; N, 13.14%.

8-((S)-(3-Chloro-5-nitrophenyl)-1,3,4-oxadiazol-2-yl)methoxy)quinoline (73) Yield: 72%; Light yellow Crystals, m.p. 178-180 °C, R$_f$ = 0.41 (CHCl$_3$ : MeOH; 9:1).

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

$^1$H NMR (500 MHz, DMSO-d$_6$) : $\delta$ 5.68 (s, 2H, O-CH$_2$), 7.49-7.40 (m, 2H), 7.64-7.56 (m, 3H), 8.37 (d, $J = 8.1$ Hz, 1H), 8.73 (m, 2H), 8.89 (d, $J = 3.3$ Hz, 1H).

Maldi-MS (m/z) : 382 (M$^+$), 384 (M$^+$+2).

Elemental Analysis

Calculated

Found

Calculated for molecular formula C$_{18}$H$_{11}$ClN$_4$O$_4$.

C, 56.48; H, 2.90; N, 14.64.

C, 56.50; H, 2.91; N, 14.62%.

General procedure for synthesis of (E/Z)-N'-(substitutedbenzylidene)-2-(4-phenylphenoxy) acetohydrazide (74-82)

The mixture of hydrazide (1 mmol) and aromatic aldehydes (1 mmol) in 50 ml absolute alcohol was refluxed for 4-8 h. After completion of reaction monitored by TLC, it was concentrated, cooled and poured on crushed ice. The precipitate so obtained was filtered, washed with cold water, dried and crystallized from alcohol to yield compound 74-82.
(E/Z)-N'-(4-Chlorobenzylidene)-2-(4-phenylphenoxy)acetohydrazide (74) Yield:
80%; White flakes, m.p. 204-206 °C, Rf = 0.39 (n-hexane : ethyl acetate; 6:4).

IR (KBr) cm⁻¹ : 3350, 3055, 2921, 1674, 1474, 1108.

¹H NMR (300 MHz, CDCl₃) : δ 4.76 (s, 2H, O-CH₂), 6.34 (s, 1H, NH), 7.03 (d, J = 8.5 Hz, 2H), 7.25-7.32 (m, 1H, Ar-H), 7.38-7.41 (m, 2H, Ar-H), 7.52-7.74 (m, 8H, Ar-H), 8.81 (s, 1H, C-H).

FAB-MS (m/z) : 364 (M⁺), 365 (M⁺+1).

Elemental Analysis
Calculated for molecular formula C₂₁H₁₇ClN₂O₂:
C, 69.14; H, 4.70; N, 7.68.

Found : C, 69.12; H, 4.72; N, 7.65%.

(E/Z)-N'-(2-Chlorobenzylidene)-2-(4-phenylphenoxy)acetohydrazide (75) Yield:
75%; White flakes, m.p. 188-190 °C, Rf = 0.36 (n-hexane : ethyl acetate; 6:4).

IR (KBr) cm⁻¹ : 3350, 3059, 2912, 1670, 1473, 1108.

¹H NMR (300 MHz, CDCl₃) : δ 4.73 (s, 2H, O-CH₂), 5.86 (s, 1H, NH), 6.86-7.02 (m, 2H, Ar-H), 7.48-7.54 (m, 10H, Ar-H), 7.78 (1H, m, Ar-H), 8.79 (s, 1H, C-H).

FAB-MS (m/z) : 364 (M⁺), 365 (M⁺+1).

Elemental Analysis : Calculated for molecular formula C₂₁H₁₇ClN₂O₂:
Calculated : C, 69.14; H, 4.70; N, 7.68.

Found : C, 69.16; H, 4.71; N, 7.65%.
Chapter III

(E/Z)-N’-(2-Nitrobenzylidene)-2-(4-phenylphenoxy)acetohydrazide (76) Yield: 76%; White flakes, m.p. 176-178 °C, Rf = 0.30 (n-hexane : ethyl acetate; 6:4).

![Chemical Structure Image]

IR (KBr) cm⁻¹: 3525, 3059, 2912, 1670, 1473, 1108.

¹H NMR (300 MHz, CDCl₃): δ 4.75 (s, 2H, O-CH₂), 5.17 (s, 1H, NH), 7.07 (d, J = 8.7 Hz, 2H), 7.30-7.33 (m, 1H), 7.41 (t, J = 7.2 Hz, 2H), 7.54-7.71 (m, 5H, Ar-H), 8.06 (t, J = 7.6 Hz, 1H), 8.30 (d, J = 7.8 Hz, 1H), 8.79 (s, 1H, C-H).

FAB-MS (m/z): 375 (M⁺), 376 (M⁺+1).

Elemental Analysis:

<table>
<thead>
<tr>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, 67.19; H, 4.56; N, 11.19.</td>
<td>C, 67.17; H, 4.57; N, 11.21%.</td>
</tr>
</tbody>
</table>

(E/Z)-N’-(3-Nitrobenzylidene)-2-(4-phenylphenoxy)acetohydrazide (77) Yield: 74%; White flakes, m.p. 182-184 °C, Rf = 0.31 (n-hexane : ethyl acetate; 6:4).

IR (KBr) cm⁻¹: 3525, 3059, 2912, 1670, 1473, 1108.

¹H NMR (300 MHz, CDCl₃): δ 4.75 (s, 2H, O-CH₂), 5.17 (s, 1H, NH), 7.06 (d, J = 8.6 Hz, 2H), 7.39-7.42 (m, 3H), 7.74-7.56 (m, 5H), 8.30 (d, 1H, J = 7.8 Hz), 8.54 (s, 1H), 8.78 (1H, s, C-H).

FAB-MS (m/z): 375 (M⁺), 376 (M⁺+1).
### Elemental Analysis

<table>
<thead>
<tr>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, 67.19; H, 4.56; N, 11.19.</td>
<td>C, 67.16; H, 4.57; N, 11.21%</td>
</tr>
</tbody>
</table>

(E/Z)-N'-(3, 4, 5-trimethoxybenzylidene)-2-(4-phenylphenoxy)acetohydrazide (78)

Yield: 74%; White flakes, m.p. 186-188 °C, Rf = 0.15 (n-hexane : ethyl acetate; 6:4).

![Structure of (E/Z)-N'-(3, 4, 5-trimethoxybenzylidene)-2-(4-phenylphenoxy)acetohydrazide](image)

**IR (KBr) cm⁻¹**

- 3525, 3059, 2912, 1670, 1473, 1108.

**¹H NMR (300 MHz, CDCl₃)**

- δ 3.78 (s, 9H, O-CH₃), 4.78 (s, 2H, O-CH₂), 6.78-6.87 (m, 5H, Ar-H), 7.31 (d, J = 8.4 Hz, 2H), 7.38-7.52 (m, 4H, Ar-H), 8.78 (s, 1H, C-H), 9.74 (s, 1H, N-H).

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

- 420 (M⁺).

Elemental Analysis

<table>
<thead>
<tr>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, 68.56; H, 5.75; N, 6.66.</td>
<td>C, 68.53; H, 5.78; N, 6.64%</td>
</tr>
</tbody>
</table>

(E/Z)-N'-(4-Hydroxybenzylidene)-2-(4-phenylphenoxy)acetohydrazide (79)

Yield: 68%; White flakes, m.p. 242-244 °C, Rf = 0.15 (n-hexane : ethyl acetate; 6:4).

![Structure of (E/Z)-N'-(4-Hydroxybenzylidene)-2-(4-phenylphenoxy)acetohydrazide](image)

**IR (KBr) cm⁻¹**

- 3525, 3059, 2912, 1670, 1473, 1108.

**¹H NMR (300 MHz, CDCl₃)**

- δ 4.73 (s, 2H, O-CH₂), 6.12 (s, 1H, NH), 6.87-7.06 (m, 5H, Ar-H), 7.43-7.62 (m, 4H, Ar-H), 7.58-7.72 (m, 4H, Ar-H), 8.80 (s, 1H, C-H), 10.05 (s, 1H, O-H).

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

- 346 (M⁺), 347 (M⁺+1).

Elemental Analysis

<table>
<thead>
<tr>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>: Calculated for molecular formula C₂₁H₁₈N₂O₃</td>
<td>: Calculated for molecular formula C₂₁H₁₈N₂O₃</td>
</tr>
</tbody>
</table>
Chapter III

Calculated: C, 72.82; H, 5.24; N, 8.09,
Found: C, 72.80; H, 5.23; N, 8.10%.

(E/Z)-N’-(5-Hydroxy-3,4-di-methoxybenzylidene)-2-(phenylphenoxy)acetohydrazide

(80) Yield: 86%; White Crystals, m.p. 244-246 °C, Rf = 0.13 (n-hexane : ethyl acetate; 6:4).

\[ \text{IR (KBr) cm}^{-1} \]
\[ : 3190, 3028, 2936, 1674, 1588, 1417, 1374, 1240, 1136. \]

\[ ^1\text{H NMR (300 MHz, CDCl}_3 \]
\[ : \delta 3.76 (s, 6H, O-CH}_3 \], 4.68 (s, 2H, O-CH}_2 \], 4.83 (s, 1H, N-H), 6.64 (s, 2H), 7.64-6.88 (m, 10H), 10.12 (s, 1H, OH).

Maldi-MS (m/z)
\[ : 406 (M^+), 407 (M^+\text{+1}). \]

Elemental Analysis
Calculated: C, 67.97; H, 5.46; N, 6.89.
Found: C, 67.99; H, 5.47; N, 6.90%

(E/Z)-N’-(3-Methoxy,4-hydroxybenzylidene)-2-(4-phenylphenoxy)acetohydrazide

(81) Yield: 68%; White flakes, m.p. 210-212 °C, Rf = 0.21 (n-hexane : ethyl acetate; 6:4).

\[ \text{IR (KBr) cm}^{-1} \]
\[ : 3525, 3059, 2912, 1670, 1473, 1108. \]

\[ ^1\text{H NMR (300 MHz, CDCl}_3 \]
\[ : \delta 3.87 (s, 3H, O-CH}_3 \], 4.72 (s, 2H, O-CH}_2 \], 5.80 (s, 1H, NH), 6.86 (d, J = 7.2 Hz, 1H), 6.87-7.06 (m, 2H, Ar-H), 7.32-7.50 (m, 5H, Ar-H), 7.51-7.58 (m, 4H, Ar-H), 8.76 (s, 1H, C-H), 10.12 (s, 1H, O-H).

FAB-MS (m/z)
\[ : 376 (M^+), 377 (M^+\text{+1}). \]
Elemental Analysis

Calculated: C, 70.20; H, 5.36; N, 7.44.

Found: C, 70.22; H, 5.35; N, 7.43%.

\((E/Z)-N'-(2-Hydroxybenzylidene)-2-(4-phenylphenoxy)acetohydrazide\) (82) Yield: 66%; White flakes, m.p. 192-194 °C, \(R_f = 0.16\) (n-hexane : ethyl acetate; 6:4).

\[
\text{IR (KBr) cm}^{-1} : 3450, 3053, 2915, 1676, 1474, 1109.
\]

\[
\text{\(^1H\) NMR (300 MHz, CDCl}_3) : \delta 4.72 (s, 2H, O-CH}_2\), 5.80 (s, 1H, NH), 6.86 (d, \(J = 7.2\) Hz, 1H), 6.87-7.06 (m, 2H, Ar-H), 7.32-7.50 (m, 6H, Ar-H), 7.51-7.58 (m, 4H, Ar-H), 8.76 (s, 1H, C-H), 10.12 (s, 1H, O-H)
\]

\[
\text{FAB-MS (m/z)} : 346 (M^+) , 347 (M^+1).
\]

Elemental Analysis

Calculated: C, 72.82; H, 5.24; N, 8.09.

Found: C, 72.85; H, 5.25; N, 8.10%.

**General procedure for the synthesis of \(1-(5-((4-phenylphenoxy)methyl)-2-(substitutedphenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone\) (83-91).**

To a 10 ml acetic anhydride, hydrazones were added and refluxed for 3-5 h. After the completion of reaction monitored by TLC, the reaction mixture was cooled to room temperature and poured on crushed ice and kept overnight in freeze. The precipitate formed were filtered, washed with cold water, dried and crystallized from ethanol to yield the pure 1,3,4-oxadiazoline (83-91).

\(1-(5-((4-phenylphenoxy)methyl)-2-(4-chlorophenyl)-1,3,4-oxadiazol-3(2H)-yl)ethanone\) (83) Yield: 64%; White crystals, m.p. 116-118 °C, \(R_f = 0.42\) (n-hexane : ethyl acetate; 6:4).
Chapter III

IR (KBr) cm\(^{-1}\) : 3058, 2926, 1697, 1608, 1518, 1378, 1079.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) : \(\delta\) 2.02 (s, 3H, COCH\(_3\)), 5.21 (s, 2H, O-CH\(_2\)), 6.61 (s, 1H, CH, oxadiazole ring), 7.04 (d, \(J = 8.7\) Hz, 2H), 7.31-7.60 (m, 11H, Ar-H).

\(^13\)C NMR (75 MHz, CDCl\(_3\)) : \(\delta\) 20.58, 65.62, 67.08, 115.20, 126.08, 126.28, 126.85, 132.12, 127.34, 127.78, 128.90, 130.18, 139.72, 157.39, 166.58, 168.12.

ES-MS (m/z) : 407 (M\(^+\)+1), 408 (M\(^+\)+2).

Elemental Analysis

<table>
<thead>
<tr>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
</table>
| C, 67.90; H, 4.71; N, 6.89. | C, 67.85; H, 4.72; N, 6.87%.

\(1\)-[5-{(4-phenylphenoxy)methyl}-2-(2-chlorophenyl)-1,3,4-oxadiazol-3(2H)-yl]ethanone (84) Yield: 68\%; White crystals, m.p. 128-130 °C, \(R_f = 0.34\) (n-hexane : ethyl acetate; 6:4).

IR (KBr) cm\(^{-1}\) : 3058, 2926, 1696, 1607, 1518, 1378, 1076.

\(^1\)H NMR (300 MHz, CDCl\(_3\)) : \(\delta\) 2.04 (s, 3H, COCH\(_3\)), 5.21 (s, 2H, O-CH\(_2\)), 6.50 (s, 1H, CH, oxadiazole ring), 7.35-7.64 (m, 13H, Ar-H)

\(^13\)C NMR (75 MHz, CDCl\(_3\)) : \(\delta\) 20.58, 65.62, 67.08, 115.20, 126.08, 126.28, 126.85, 127.34, 127.78, 128.90, 130.18, 139.72, 157.39, 166.58, 168.12.

Maldi-MS (m/z) : 406 (M\(^+\)), 408 (M\(^+\)+2).

Elemental Analysis

<table>
<thead>
<tr>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calculated for molecular formula C(<em>{23})H(</em>{19})ClN(_2)O(_3)</td>
<td>C, 67.90; H, 4.71; N, 6.89.</td>
</tr>
</tbody>
</table>
Chapter III

Found: C, 67.92; H, 4.70; N, 6.91%

I-[5-{(4-phenylphenoxy)methyl}-2-(2-nitrophenyl)-1,3,4-oxadiazol-3(2H)yl]ethanone (85) Yield: 66%; Light yellow crystals, m.p. 122-124 °C, Rf = 0.51 (n-hexane : ethyl acetate; 6:4).

![Chemical structure of I-[5-{(4-phenylphenoxy)methyl}-2-(2-nitrophenyl)-1,3,4-oxadiazol-3(2H)yl]ethanone](image)

**IR (KBr) cm⁻¹**: 3072, 2937, 1676, 1524, 1487, 1378, 1231, 1074.

**¹H NMR (300 MHz, CDCl₃)**: δ 2.33 (s, 3H, COCH₃), 5.21 (s, 2H, O-CH₂), 6.48 (s, 1H, CH, oxadiazole ring), 6.97-7.06 (m, 3H, Ar-H), 7.32-7.76 (m, 9H, Ar-H), 8.08 (m, 1H, Ar-H).

**¹³C NMR (75 MHz, CDCl₃)**: δ 20.61, 65.62, 67.10, 115.20, 120.26, 26.28, 126.85, 126.85, 127.32, 127.76, 127.81, 128.90, 132.14, 133.26, 139.74, 147.82, 157.39, 169.26.

**Maldi-MS (m/z)**: 417 (M⁺), 418 (M⁺+1).

**Elemental Analysis**: Calculated for molecular formula C₂₃H₁₉N₃O₅.

Calculated: C, 66.18; H, 4.59; N, 10.07

Found: C, 66.16; H, 4.57; N, 10.05%

I-[5-{(4-phenylphenoxy)methyl}-2-(3-nitrophenyl)-1,3,4-oxadiazol-3(2H)yl]ethanone (86) Yield: 64%; Light yellow crystals, m.p. 168-170 °C, Rf = 0.48 (n-hexane : ethyl acetate; 6:4).

![Chemical structure of I-[5-{(4-phenylphenoxy)methyl}-2-(3-nitrophenyl)-1,3,4-oxadiazol-3(2H)yl]ethanone](image)

**IR (KBr) cm⁻¹**: 3068, 2934, 1676, 1524, 1491, 1375, 1231, 1074.

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$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.09 (s, 3H, COCH$_3$), 5.24 (s, 2H, O-CH$_2$), 7.02-7.76 (m, 9H, Ar-H), 7.74 (t, $J = 8.1$ Hz, 1H), 8.12-8.29 (m, 3H Ar-H), 8.52 (s, 1H).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 20.61, 65.62, 67.10, 115.22, 122.26, 119.82, 126.28, 126.85, 127.32, 127.76, 127.82, 128.90, 132.18, 133.26, 139.73, 148.54, 157.38, 169.25.

Maldi-MS (m/z): 417 (M$^+$), 418 (M$^+$+1).

Elemental Analysis: Calculated for molecular formula C$_{23}$H$_{19}$N$_3$O$_5$
Calculated: C, 66.18; H, 4.59; N, 10.07.
Found: C 66.20; H 4.60; N 10.08%.

1-[5-{(4-phenylphenoxy)methyl}-2-(3,4,5-trimethoxyphenyl)-1,3,4-oxadiazol-3(2H)-yl] ethanone (87) Yield: 64%; White crystals, m.p. 236-238 $^\circ$C, $R_f = 0.19$ (n-hexane: ethyl acetate; 6:4).

IR (KBr) cm$^{-1}$: 3033, 2912, 1674, 1605, 1491, 1375, 1239, 1124, 1089.

$^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 2.09 (s, 3H, COCH$_3$), 3.67 (s, 9H, O-CH$_3$), 5.19 (s, 2H, O-CH$_2$), 6.85 (d, $J = 7.5$Hz, 2H), 6.97-7.58 (m, 9H, Ar-H).

$^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 20.53, 55.96, 60.15, 66.03, 104.19, 114.94, 115.21, 126.09, 126.28, 126.88, 127.35, 127.75, 128.92, 132.81, 133.29, 139.73, 142.23, 153.19, 157.94, 166.62, 168.08.

Maldi-MS (m/z): 462 (M$^+$), 463 (M$^+$+1).

Elemental Analysis: Calculated for molecular formula C$_{26}$H$_{26}$N$_2$O$_6$
Calculated: C, 67.52; H, 5.67; N, 6.06.
Found: C, 67.50; H, 5.69; N, 6.03%.

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1-[5-{(4-phenylphenoxy)methyl}-2-(4-hydroxyphenyl)-1,3,4-oxadiazol-3(2H)-yl]ethanone (88) Yield: 60%; White crystals, m.p. 166-168 °C, Rf = 0.19 (n-hexane : ethyl acetate; 6:4).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 3240, 3072, 2937, 1676, 1524, 1487, 1378, 1231, 1074. \\
^1\text{H NMR (300 MHz, CDCl}_3 & : \delta 1.91 (3H, s, COCH}_3, 4.68 (2H, s, O-CH}_2, 4.83 \\
& (s, 1H), 7.60-6.85 (13H, m), 10.07 (s, OH, 1H). \\
^13\text{C NMR (75 MHz, CDCl}_3 & : \delta 20.51, 51.86, 64.61, 66.06, 114.97, 115.20, \\
& 126.08, 126.26, 126.85, 127.34, 127.73, 127.81, \\
& 128.90, 133.27, 139.72, 157.39, 166.57, 168.11. \\
\text{Maldi-MS (m/z)} & : 389 (M\(^+\)), 391 (M\(^+\)+2). \\
\text{Elemental Analysis} & : \text{Calculated for molecular formula C}_{23}\text{H}_{20}\text{N}_2\text{O}_4 \\
& : \text{C, 71.12; H, 5.19; N, 7.21} \\
& : \text{Found} \quad : \text{C, 71.10; H, 5.16; N, 7.19\%} \\
\end{align*}
\]

1-[5-{(4-phenylphenoxy)methyl}-2-(3,4-dimethoxy-5-hydroxyphenyl)-1,3,4-oxadiazol-3(2H)-yl]ethanone (89) Yield: 70%; White Crystals, m.p. 238-240 °C, Rf = 0.56 (n-hexane : ethyl acetate; 6:4).

\[
\begin{align*}
\text{IR (KBr) cm}^{-1} & : 3192, 3025, 2936, 1676, 1587, 1417, 1373, 1239, 1134. \\
^1\text{H NMR (300 MHz, CDCl}_3 & : \delta 1.91 (s, 3H, COCH}_3, 3.68 (s, 6H, O-CH}_3, 4.68 (s, \\
& 2H, O-CH}_2, 6.63 (s, 1H), 7.60-6.85 (m, 11H), \\
& 10.07 (s, 1H, OH). \\
\end{align*}
\]

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13C NMR (75 MHz, CDCl3)  
\[ \delta 20.51, 51.86, 64.61, 66.06, 114.97, 115.20, \\
126.08, 126.26, 126.85, 127.34, 127.73, 127.81, \\
128.90, 133.27, 139.72, 157.39, 166.57, 168.11. \]

Maldi-MS (m/z)  
\[ 448 (M^+), 450 (M^+2). \]

Elemental Analysis  
Calculated for molecular formula C25H24N2O6

Calculated: C, 66.95; H, 5.39; N, 6.25

Found: C, 66.99; H, 5.37; N, 6.23%

\[ 1\text{-}[5\text{-}((4\text{-}phenyloxy)methyl)-2\text{-}(3\text{-}methoxy-4\text{-}hydroxyphenyl})\text{-}1,3,4\text{-}oxadiazol-3(2H)\text{-}yl]ethanone \text{ (90) Yield: 66\%; White crystal, m.p. 268-270 °C, Rf = 0.25 (n-hexane : ethyl acetate; 6:4).} \]

IR (KBr) cm⁻¹  
\[ 3292, 3035, 2936, 1676, 1584, 1417, 1373, 1239, 1107. \]

1H NMR (300 MHz, CDCl3)  
\[ \delta 2.33 \text{(s, 3H, COCH₃)}, 3.74 \text{(s, 3H, O-CH₃)}, 5.12 \\
\text{(2H, s, O-CH₂)}, 6.48 \text{(s, 1H), 7.72-6.62 (m, 12H, Ar-H)}, 10.54 \text{(1H, OH).} \]

13C NMR (75 MHz, CDCl3)  
\[ \delta 20.52, 51.89, 56.13, 64.90, 66.52, 114.97, 115.20, \\
126.25, 126.31, 126.92, 127.73, 127.86, 129.31, 132 \\
.35, 132.43, 133.39, 139.73, 143.29, 157.95, 164.44, \\
168.08. \]

Maldi-MS (m/z)  
\[ 418 (M^+), 419 (M^+1). \]

Elemental Analysis  
Calculated for molecular formula C24H22N2O5

Calculated: C, 68.89; H, 5.30; N, 6.69

Found: C, 68.81; H, 5.31; N, 6.70%
I-[5-((4-phenylphenoxy)methyl)-2-(2-hydroxyphenyl)-1,3,4-oxadiazol-3(2H)-yl]ethanone (91) Yield: 60%; White crystals; m.p. 148-150 °C, Rr = 0.23 (n-hexane: ethyl acetate; 6:4).

\[
\text{IR (KBr) cm}^{-1}: 3452, 3052, 1676, 1617, 1236, 1096.
\]

\[^1\text{H NMR (300 MHz, CDCl}_3\text{)}: \delta 2.36 (s, 3H, COCH}_3\text{), 4.84 (2H, s, O-CH}_2\text{), 6.48 (1H, s, C-H, oxadiazole ring), 6.76-7.74 (13H, m, Ar-H), 10.45 (s, 1H, OH).\]

\[^{13}\text{C NMR (75 MHz, CDCl}_3\text{)}: \delta 20.59, 64.91, 66.54, 114.96, 115.22, 129.35, 122.13, 126.27, 126.32, 126.92, 127.73, 127.89, 132.85, 133.37, 139.75, 154.34, 157.95, 164.46, 168.08.\]

Maldi-MS (m/z) : 388 (M^+), 389 (M^+1).

Elemental Analysis : Calculated for molecular formula C_{23}H_{20}N_{2}O_{4}

Calculated : C, 71.12; H, 5.19; N, 7.21

Found : C, 71.15; H, 5.18; N, 7.20%

3.6.2. Biological Activity

3.6.2.1. Anti-inflammatory activity

All the synthesized compounds were evaluated for their anti-inflammatory activity against carrageenan-induced acute paw edema in Albino Wistar rats weighing 150-200 g. Indomethacin and Ibuprofen were used as standard drug (10 mg/kg body weight) for oxadiazole and oxadiazoline derivatives respectively and test drugs were used equimolar to the standard drugs. Same protocol was used as given in section 1, chapter-II [49]. The results are presented in table 3 and figure 1.
3.6.2.2. **Analgesic activity**

The analgesic activity was determined using tail flick method in Albino Wistar rats weighing 150-200 g. The same protocol is used as given in section 1, chapter-II [50]. The results are presented in **table 4** and **figure 2**.

3.6.2.3. **Histopathological studies**

The same protocol is used as given in section 1, chapter-II. The results are given in **table 5** and **figure 3**.

3.6.2.4. **In vitro Antimicrobial activity studies**

The protocol used for antimicrobial studies was same as used in section 1, chapter-II [51]. Ampicillin (200, 100μg) and Fluconazole (200, 100 μg) were used as standard drugs. Dimethyl sulfoxide was used as solvent control. The antimicrobial activity results are summarized in **table 6**.
3.7. Conclusion

In conclusion a small library of fifteen compounds (quinoline based 1,3,4-oxadiazole 68-73 and biphenyl based 1,3,4-oxadiazoline 83-91) has been synthesized. The newly synthesized compounds were evaluated for their anti-inflammatory, analgesic, ulcerogenic and antimicrobial studies. Two compounds 68 and 71 from quinoline based 1,3,4-oxadiazole showed better anti-inflammatory (65.87, 65.80 and 72.33, 75.00%) at 3 h. and 5 h. respectively compared to standard drug indomethacin (55.29 and 69.50%) with comparable analgesic activity. Compound 71 caused no ulceration. Three compounds 84, 88 and 91 from biphenyl based 1,3,4-oxadiazoline also showed moderate anti-inflammatory activity (59.40, 73.05, 54.54 and 69.12, 72.55, 74.27%) at 3 h. and 5 h. respectively compared to standard drug Ibuprofen (78.89 and 80.10%). From antimicrobial screening, it is concluded that compound 71 has shown potential zone of inhibition against all tested microbial strains.
13 CNMR spectrum of compound 71
Mass spectrum of compound 71

Sample Name: M-100017365

Experiment Mass Spectrometry Analyses
IR spectrum of compound 71
HNMR spectrum of compound 88
Mass spectrum of compound 71
IR spectrum of compound 88
3.9. References


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