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# Design, Synthesis of some Novel 1,3,4-Oxadiazole derivatives bearing Benzimidazole Nucleus and Biological evaluation of their possible *in-vitro* Anti-inflammatory and Antioxidant activity

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**Abstract:** Some novel 1,3,4-oxdiazole derivatives bearing benzimidazole nucleus were synthesized and their *in-vitro* anti-inflammatory and antioxidant activity by inhibition of protein denaturation screening, 2, 2-Diphenyl-1-Picryl Hydrazide screening methods respectively. The compound **7.XX** and **7.XXIX** were found to be highly active in low concentration and compounds **7.VIII, 7.IX, 7.X, 7.XIX, 7.XXII, 7. XXIV** and **7.XXV** were found to be moderately active at higher concentration as compared to ascorbic acid in 2, 2-diphenyl-1-picryl hydrazide method. *In-vitro* anti-inflammatory by inhibition of protein denaturation method the compounds **7.VIII, 7.XX**, **7.XXII and 7.XXIX** were found to be highly active in low concentration and compounds **7.IX, 7.X, 7.XIX, 7. XXIV, 7.XXV** and **7.XXXIII** were found to be moderately active at higher concentration as compared to diclofenac sodium.
**Key Words:** o-phenylenediamine, 4-cyanobenzaldehyde, imines intermediate, 1,3, 4-oxadiazole.

## 1. Introduction

The structural and therapeutic diversity coupled with commercial capability of small molecules has enthralled organic and medicinal chemists. There has been significant interest in the chemistry of oxadiazole ring systems, which is a core structure in various synthetic pharmaceuticals displaying a broad spectrum of biological activity. In the past few years, research for new non-steroidal anti-inflammatory agents has been reported that many compounds having a 1,3,4-oxadiazole derivatives are known for their anti-inflammatory activity.[1-3]

Oxadiazole derivatives play a significant role in various pharmaceuticalapplications.[4-6] As an important class of heterocyclic compound, 1,3,4-oxadiazoles show broad spectrum of biological activities.[7-12] Among these, a few differently substituted1,3,4-oxadiazoles have exhibited potent antitumor activities particularly.[13-15] Of the various human diseases, cancer has proven to be one of the most intractable diseases to which humans are subjected, and as yet no practical and generally effective drugs or methods of control are available. Therefore, identification of novel potent, selective and less toxic anti-cancer agent's remains one of the most pressing health problems.[16] Targeting tubulin in rapidly dividing tumor cells has been a well validated strategy for cancer therapy.[17, 18] Benzimidazole derivatives are well known for their anti-inflammatory activity and more recently have been discovered to have anticancer effect.[19, 20] Therefore, in the present research it was planned to incorporate the Benzimidazole moiety with 1,3,4-oxadiazole to have better antioxidant and anti-inflammatory activity.

## 2. Materials and methods

### 2.1. Chemistry

Melting points were determined by open capillary method and were uncorrected. The IR spectra (in KBr) were recorded on a Shimadzu IR Affinity-1 spectrophotometer. $^{1}$H NMR and $^{13}$CNMR spectra were recorded on a Perkin–Elmer EM 300 MHz spectrometer using TMS as internal standard. The mass spectra were recorded on a JEOL JMS-D 300 spectrometer operating at 70 eV. Purity of the compounds was checked by TLC silica coated plates obtained from Merck.

General procedure for the preparation of 4-{1-[(4-acetyl-5-(substituted)-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile **6 (I-IV)**

A mixture of imine intermediate **4 (I-IV)** (0.01 mole) and excess of acetic anhydride (10 ml) was refluxed for 3-4 h. The acetic anhydride was distilled off and the residue was poured on to crushed ice. The solid thus obtained was collected by filtration, washed with water and recrystallized from ethanol. The purity of the product was confirmed by a single spot on TLC plate.

General procedure for the preparation of novel 4-{5-substituted-1-[(4,5-disubstituted)-4,5-dihydro-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl}benzonitrile **7 (V-XXXIII).**

To a solution of imine intermediate **5 (V-XXXIII)** (0.01 mole) in ethanol (15 ml) and Chloramines-T (0.01 mole) was added. The reaction mixture was exposed to microwave at 300 W intermittently at 30 sec intervals for specified time. After complete conversion as indicated by TLC, the reaction mixture was cooled and digested with cold water.

## 3. Biological Evaluation

**3.1.** Antioxidant and free radical scavenging activity assays

**3.1.1.** DPPH assay

This experimental procedure was adapted from.[21] In an ethanol solution of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical, test compounds at different concentrations were added. The reaction mixtures were shaken vigorously and then kept in the dark for 30 min. The absorbance of the resulting solutions was measured in 1 cm cuvettes, using a UV/VIS spectrophotometer at 351nm against blank without DPPH. Decreasing of DPPH solution absorbance indicates an increase of DPPH radical scavenging activity. This activity is given as % DPPH radical scavenging that is calculated in the equation:

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}} \text{ X } 100$$

The DPPH solution without sample solution was used as control. All tests were run in triplicate and averaged. Ascorbic acid was used as positive control in (Table 2 and Figure 2).

**3.2.** In vitro anti-inflammatory activity

**3.2.1.** Inhibition of protein denaturation method

Test solution (0.5 mL) consists of 0.45 mL of BSA (5% w/v aqueous solution) and 0.05 mL of different concentration of test solutions (50, 100, 150, 200 µg/mL). Test control solution (0.5 mL) consists of 0.45 mL of BSA (5% w/v aqueous solution) and 0.05 mL of distilled water. Product control solution (0.5 mL) consists of 0.45 mL of distilled water and 0.05 mL of different concentration of test solutions (50, 100, 150, 200 µg/mL). Standard solution (0.5 mL) consists of 0.45 mL of BSA (5%w/v aqueous solution) and 0.05 mL different concentration of Diclofenac Sodium's (50, 100, 150, 200 µg/ mL). All the above solutions were adjusted to pH 6.3 using 1N hydrochloric acid. The samples were incubated at 37$^{0}$C for 20 min and the temperature was increased to keep the

samples at $57^0$C for 3 min. After cooling, 2.5 mL of phosphate buffer saline was added to the above solutions. The absorbance was measured using UV Visible spectrophotometer at 417 nm.[22] The percentage inhibition of protein denaturation was calculated as,

$$\text{Percentage inhibition} = \frac{\text{O. D of Test Solution - O. D of Product Control}}{\text{O. D of Test Control}} \text{ X } 100$$

The results were compared with Diclofenac Sodium in (Table 3 and Figure 3).

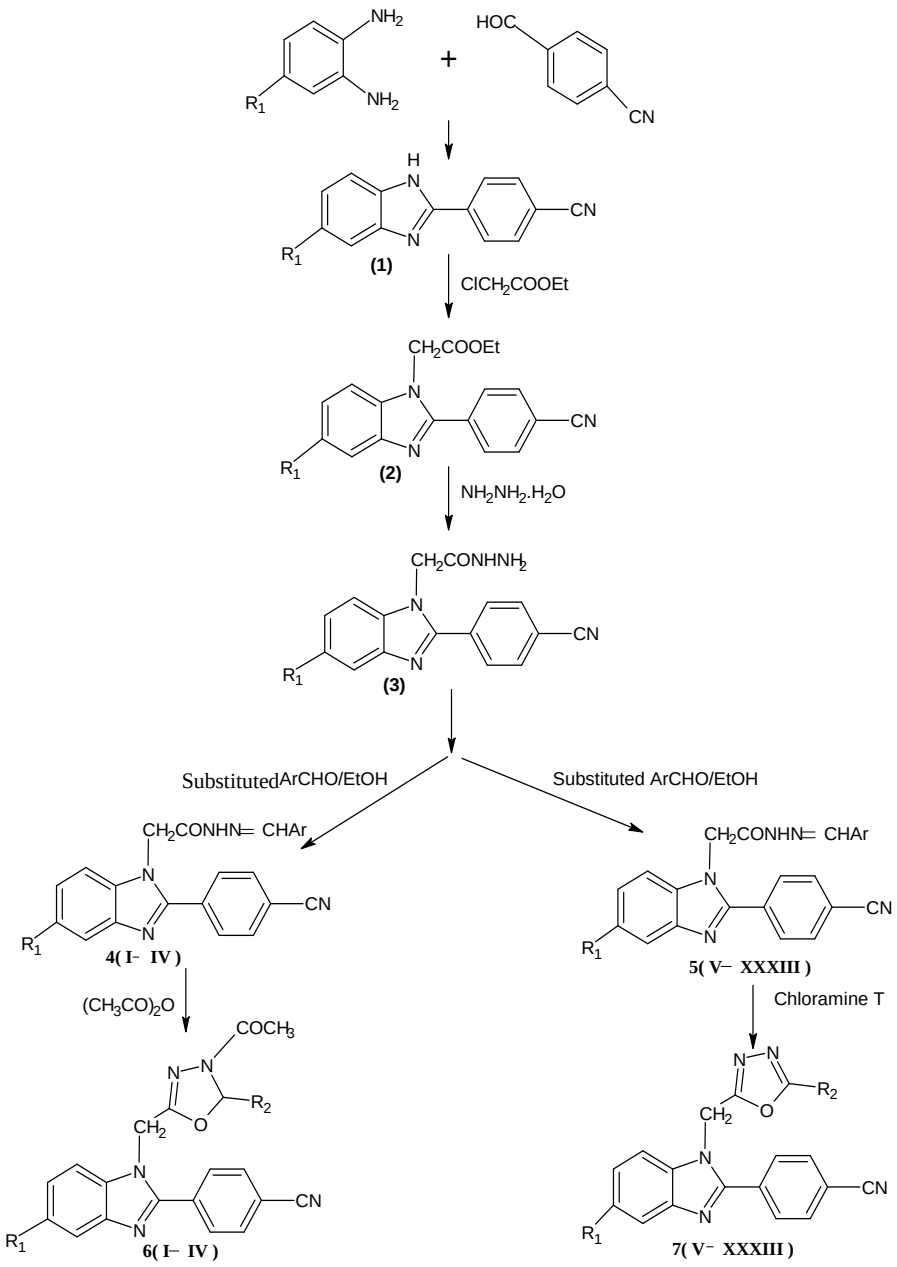
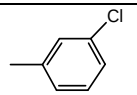
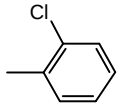
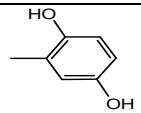
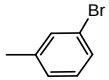
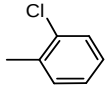
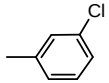
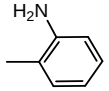
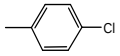
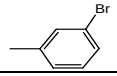
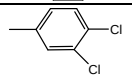
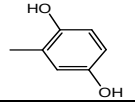
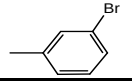
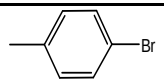
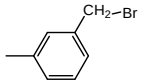
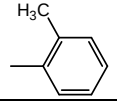
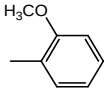
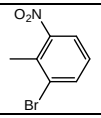
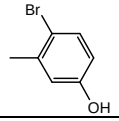
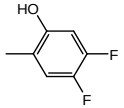
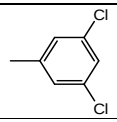
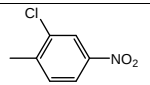
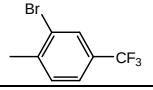
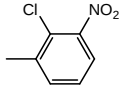
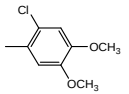
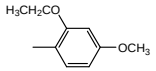



**Figure 1:** Scheme I Synthetic route for the preparation of the compounds.

**Table 1:** Characterization Data Scheme I

| Compound Code | $R_1$ | $R_2$ | $R_3$ | Molecular Formula | Molecular Weight | Yield (% ) | Melting point ($^oC$) | $R_f$ value |
|---|---|---|---|---|---|---|---|---|
| **6. I** | $-NO_2$ |  | $-COCH_3$ | $C_{25}H_{17}ClN_6O_4$ | 500.89328 | 25 | 273-275 | 0.7311 |
| **6. II** | $-NO_2$ |  | $-COCH_3$ | $C_{25}H_{17}ClN_6O_4$ | 500.89328 | 30 | 270-272 | 0.7272 |
| **6. III** | $-NO_2$ |  | $-COCH_3$ | $C_{25}H_{18}N_6O_6$ | 498.44702 | 35 | 213-215 | 0.7647 |
| **6. IV** | $-NO_2$ |  | $-COCH_3$ | $C_{25}H_{17}BrN_6O_4$ | 545.34428 | 32 | 210-212 | 0.6860 |
| **7. V** | $-NO_2$ |  | - | $C_{23}H_{13}ClN_6O_3$ | 456.84072 | 30 | 269-271 | 0.7701 |
| **7. VI** | $-NO_2$ |  | - | $C_{23}H_{13}ClN_6O_3$ | 456.84072 | 26 | 274-276 | 0.7294 |
| **7. VII** | $-NO_2$ |  | - | $C_{23}H_{15}N_7O_3$ | 437.4103 | 20 | 243-245 | 0.7311 |
| **7. VIII** | $-NO_2$ |  | - | $C_{23}H_{13}ClN_6O_3$ | 456.84072 | 28 | 275-277 | 0.7272 |
| **7. IX** | -Cl |  | - | $C_{23}H_{13}BrClN_5O$ | 490.73922 | 31 | 209-211 | 0.7647 |
| **7. X** | $-NO_2$ |  | - | $C_{23}H_{12}Cl_2N_6O_3$ | 491.28578 | 30 | 276-278 | 0.6860 |
| **7. XI** | $-NO_2$ |  | - | $C_{23}H_{14}N_6O_5$ | 454.39446 | 33 | 211-213 | 0.7701 |
| **7. XII** | $-NO_2$ |  | - | $C_{23}H_{13}BrN_6O_3$ | 501.29172 | 28 | 211-213 | 0.7294 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **7. XIII** | $-NO_2$ |  | - | $C_{23}H_{13}BrN_6O_3$ | 501.29172 | 20 | 183-185 | 0.7311 |
| **7. XIV** | $-NO_2$ |  | - | $C_{24}H_{15}BrN_6O_3$ | 515.3183 | 26 | 208-210 | 0.7272 |
| **7. XV** | $-NO_2$ |  | - | $C_{24}H_{16}N_6O_3$ | 436.42224 | 30 | 193-195 | 0.7647 |
| **7. XVI** | $-NO_2$ |  | - | $C_{24}H_{16}N_6O_4$ | 452.42164 | 35 | 223-225 | 0.6860 |
| **7. XVII** | $-NO_2$ |  | - | $C_{23}H_{12}BrN_7O_5$ | 546.28928 | 23 | 256-258 | 0.7701 |
| **7. XVIII** | $-NO_2$ |  | - | $C_{23}H_{13}BrN_6O_4$ | 517.29112 | 31 | 243-245 | 0.7294 |
| **7. XIX** | $-NO_2$ |  | - | $C_{23}H_{12}F_2N_6O_4$ | 474.3759864 | 20 | 254-256 | 0.7311 |
| **7. XX** | $-NO_2$ |  | - | $C_{23}H_{12}Cl_2N_6O_3$ | 491.28578 | 36 | 285-287 | 0.7272 |
| **7. XXI** | $-NO_2$ |  | - | $C_{23}H_{12}ClN_7O_5$ | 501.83828 | 33 | 276-278 | 0.7647 |
| **7. XXII** | $-NO_2$ |  | - | $C_{24}H_{12}BrF_3N_6O_3$ | 569.2896896 | 25 | 252-254 | 0.6860 |
| **7. XXIII** | $-NO_2$ |  | - | $C_{23}H_{12}ClN_7O_5$ | 501.83828 | 31 | 263-265 | 0.7701 |
| **7. XXIV** | $-NO_2$ |  | - | $C_{25}H_{17}ClN_6O_5$ | 516.89268 | 30 | 224-226 | 0.7294 |
| **7. XXV** | $-NO_2$ |  | - | $C_{26}H_{20}N_6O_5$ | 496.4742 | 19 | 214-216 | 0.7311 |

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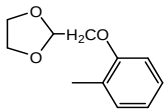
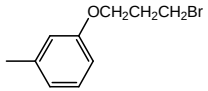
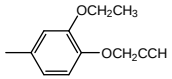
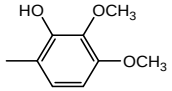
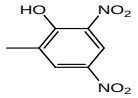
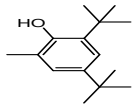
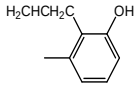
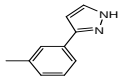
|---|---|---|---|---|---|---|---|---|
| **7. XXVI** | $-NO_2$ |  | - | $C_{27}H_{20}N_6O_6$ | 524.4843 | 20 | 219-221 | 0.7272 |
| **7. XXVII** | $-NO_2$ |  | - | $C_{26}H_{19}BrN_6O_4$ | 559.37086 | 25 | 223-225 | 0.7647 |
| **7. XXVIII** | $-NO_2$ |  | - | $C_{28}H_{20}N_6O_5$ | 520.4956 | 23 | 222-224 | 0.6860 |
| **7. XXIX** | $-NO_2$ |  | - | $C_{25}H_{18}N_6O_6$ | 498.44702 | 19 | 212-214 | 0.7701 |
| **7. XXX** | $-NO_2$ |  | - | $C_{23}H_{12}N_8O_8$ | 528.39018 | 34 | 262-264 | 0.7294 |
| **7. XXXI** | $-NO_2$ |  | - | $C_{31}H_{30}N_6O_4$ | 550.6077 | 19 | 221-223 | 0.7311 |
| **7. XXXII** | $-NO_2$ |  | - | $C_{26}H_{18}N_6O_4$ | 478.45892 | 36 | 226-228 | 0.7272 |
| **7. XXXIII** | $-NO_2$ |  | - | $C_{26}H_{16}N_8O_3$ | 488.45704 | 37 | 222-224 | 0.7647 |

**Table 2:** The $IC_{50}$±SEM (standard error of mean) Values of Ascorbic Acid and Compounds (6.I to 7.XXXIII)

| Compound Code | $IC_{50}$ Value(µg/ml) | $IC_{50}$ Value±SEM (µg/ml) |
|---|---|---|
| 7.VIII | 160 | 160±0.9614 |
| 7.IX | 300 | 300±0.9865 |
| 7.X | 220 | 220±1.0425 |
| 7. XIX | 165 | 165±0.67 |
| 7. XX | 116 | 116±1.025 |
| 7. XXII | 147 | 147±0.96 |
| 7. XXIV | 148 | 148±1.052 |
| 7. XXV | 137 | 137±1.16 |
| 7. XXIX | 103 | 103±0.96 |
| Ascorbic acid | 109 | 109±0.7296 |

Values represent the mean of triplicates.

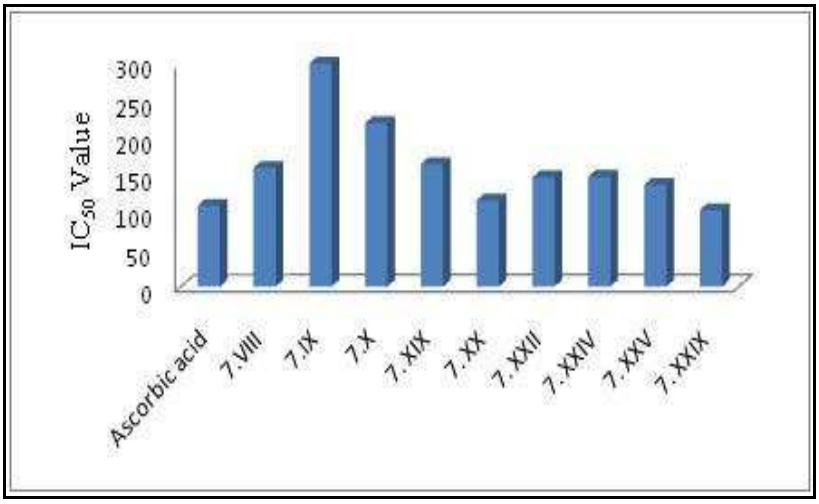



**Figure 2:** The $IC_{50}$ values of compounds (6.I to 7.XXXIII) and ascorbic acid (standard) in 2, 2-Diphenyl-1-Picryl Hydrazide screening of antioxidant activity. The results are (mean±SEM) of three experiments, performed in triplicate.

**Table 3:** The $IC_{50}$±SEM (standard error of mean) Values of Diclofenac Sodium and Compounds (6.I to 7.XXXIII)

| Compound Code | $IC_{50}$ Value(µg/ml) | $IC_{50}$ Value±SEM(µg/ml) |
|---|---|---|
| 7. V | 137.4 | 137.4±0.881917 |
| 7. VI | 119.8 | 119.8±1.527525 |
| 7.VIII | 64.98 | 64.98±1.154701 |
| 7.X | 76.77 | 76.77±0.5773503 |
| 7. XIX | 86.48 | 86.48±0.5773503 |
| 7. XX | 67.59 | 67.59±0.8819171 |
| 7. XXII | 61.02 | 61.02±1.20185 |
| 7. XXIV | 99.56 | 99.56±1.154701 |
| 7. XXV | 99.16 | 99.16±0.8819171 |
| 7. XXVIII | 100 | 100±0.5773503 |
| 7. XXIX | 67.5 | 67.5±0.9865 |
| Diclofenac Sodium | 57.08 | 57.08±0.8819 |

Values represent the mean of triplicates.

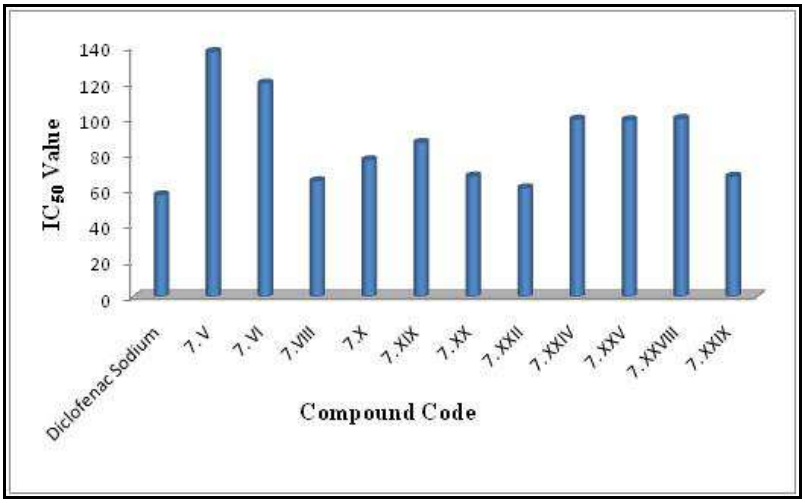



**Figure 3:** The $IC_{50}$ values of compounds (6.I to 7.XXXIII) and diclofenac sodium (standard) in inhibition of protein denaturation screening of *In-Vitro* Anti-Inflammatory activity. The results are (mean±SEM) of three experiments, performed in triplicate.

## 4. Results and discussion

For the synthesis of the target compounds the reaction sequences outlined in Scheme 1, were followed. 4-{5-substituted-1-[(4, 5-disubstituted)-4, 5-dihydro-1, 3, 4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile **7 (V-XXXIII)** had been synthesized employing conventional techniques. Substituted o-phenylene diamine was reacted with appropriately substituted 4-cyanobenzaldehydes in the presence of sodium metabisulfite to furnish substituted 2-(4-Cyanophenyl)-1H-benzimidazoles **(1)**. These substituted 2-(4-Cyano phenyl)-1H-benzimidazoles were further treated with ethyl chloroacetate in KOH/DMSO gave the N-alkylated product, (2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid ethyl esters **(2)**. To endow2-(4-cyanophenyl)-benz imidazol-1-yl)-acetic acid hydrazides **(3)** reaction were occurred between Hydrazine hydrate and the esters **(2)**. A mixture of 2-(4-cyanophenyl)-benzimidazol-1-yl)-acetic acid hydrazides and respective aldehydes were react to generate imines intermediates **4 (I-IV) & 5 (V-XXXIII)**. The imines intermediate and excess of acetic anhydride react to produce 4-{1-[(4-acetyl-5-(substituted)-4, 5-dihydro-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benz imidazol-2-yl} benzo nitrile **6 (I-IV)** in Figure 1. The imines intermediate in ethanol and Chloramines-T react to produce the products **7 (V-XXXIII)** in Figure 1.

**7. V:** 4-{1-[(5-(2-chlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

Yield 30%; m.p.:269-271; IR ($cm^{-1}$): 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1242 (C-O-C), 733 (C-Cl)

$^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 7.361-8.437 (m, 11H, aromatic protons), $^{13}C$:49, 112, 114, 119, 120, 126, 129, 130, 131, 132, 134, 136, 141, 144, 145, 156, 166, 167; MS: m/z 457 ($M^+$)

**7. VI:** 4-{1-[(5-(3-chlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile

Yield 26%; m.p.:274-276; IR ($cm^{-1}$): 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1242 (C-O-C), 733 (C-Cl)

$^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 7.332-8.437 (m, 11H, aromatic protons), $^{13}C$:49, 112, 114, 119, 120, 127, 128, 129, 131, 133, 134, 136, 141, 144, 145, 158, 165, 167; MS: m/z 457 ($M^+$)

**7. VIII:** 4-{1-[(5-(4-chlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 28%; m.p.:275-277; IR ($cm^{-1}$): 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1242 (C-O-C), 733 (C-Cl). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 7.362-8.43 (m, 11H, aromatic protons), $^{13}C$:49, 112, 114, 119, 120, 127, 129, 130, 131, 134, 136, 137, 141, 145, 156, 164, 167; MS: m/z 457 ($M^+$)

**7. IX:** 4-{1-[(5-(3-bromophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-chloro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 31%; m.p.:209-211; IR ($cm^{-1}$): 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1331 (C-N), 1242 (C-O-C), 733 (C-Cl), 575 (C-Br). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 7.137-8.11 (m, 11H, aromatic protons), $^{13}C$: 49, 112, 114, 119, 123, 124, 125, 128, 130, 131, 132, 134, 137, 144, 158, 164, 167; MS: m/z 491, 493 ($M^+$)

**7. X:** 4-{1-[(5-(3, 4-dichlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 30%; m.p.:276-278; IR ($cm^{-1}$): 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1242 (C-O-C), 733 (C-Cl). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 7.36-8.43 (m, 10H, aromatic protons), $^{13}C$: 49, 112, 114, 119, 120, 125, 128, 131, 132, 133, 134, 136, 141, 144, 145, 158, 165, 167; MS: m/z 491 ($M^+$)

**7. XIX:**4-{1-[(5-(4,5-difluoro-2-(1,3,4-oxadiazol-2-yl)phenol)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 20%; m.p.:254-256; IR ($cm^{-1}$): 3655 (O-H), 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1356 (C-F), 1352 (N=O), 1331 (C-N), 1242 (C-O-C). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 6.84-8.43 (m, 9H, aromatic protons), 4.71 (s, 1H, OH), $^{13}C$: 49, 101, 110, 112, 114, 116, 119, 120, 131, 134, 136, 141, 144, 145, 151, 155, 158, 167, 168; MS: m/z 474 ($M^+$)

**7. XX:** 4-{1-[(5-(3, 5-dichlorophenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 36%; m.p.:285-287; IR ($cm^{-1}$): 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1242 (C-O-C), 733 (C-Cl). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 7.36-8.43 (m, 9H, aromatic protons), $^{13}C$: 49, 112, 114, 119, 120, 127, 129, 131, 134, 135, 136, 139, 141, 144, 145, 158, 164, 167; MS: m/z 491 ($M^+$)

**7. XXII:** 4-{1-[(5-(2-bromo-4-(trifluoromethyl) phenyl)-1,3,4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 25%; m.p.:252-254; IR ($cm^{-1}$): 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1356 (C-F), 1352 (N=O), 1331 (C-N), 1242 (C-O-C), 575 (C-Br). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 7.36-8.43 (m, 10H, aromatic protons), $^{13}C$: 49, 112, 114, 119, 120, 124, 126, 127, 128, 131, 134, 135, 136, 141, 145, 158, 164, 167; MS: m/z 569, 571 ($M^+$)

**7. XXIV:** 4-{1-[(5-(2-chloro-4, 5-dimethoxyphenyl)-1, 3, 4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 30%; m.p.:224-226; IR ($cm^{-1}$): 3062 (Ar-C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1242 (C-O-C), 733 (C-Cl). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 3.77 (s, 6H, $-CH_3$), 7.36-8.43 (m, 9H, aromatic protons), $^{13}C$: 49, 56, 112, 113, 114, 117, 118, 119, 120, 127, 131, 134, 136, 144, 145, 151, 158, 165, 167; MS: m/z 517 ($M^+$)

**7.XXV:**4-{1-[(5-(2-ethoxy-4-methoxyphenyl)-1,3,4-oxadiazol-2-yl)methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 19%; m.p.:214-216; IR ($cm^{-1}$): 3062 (Ar-C-H), 2849 (C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1305 (C-C), 1242 (C-O-C). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 4.148-5.126 (s, 4H, $-CH_2$), 1.45-3.82 (s, 6H, $-CH_3$), 6.62-8.43 (m, 10H, aromatic protons), $^{13}C$: 15, 49, 56, 64, 97, 105, 108, 112, 114, 119, 120, 127, 131, 134, 136, 141, 144, 145, 157, 158, 164, 166, 167; MS: m/z 496 ($M^+$)

**7. XXVIII:** 4-{1-[(5-[3-ethoxy-4-(prop-2-yn-1-yloxy) phenyl]-1, 3, 4-oxadiazol-2-yl) methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 23%; m.p.:222-224; IR ($cm^{-1}$): 3062 (Ar-C-H), 2849 (C-H), 2119 (C≡C), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1305 (C-C), 1242 (C-O-C). $^{1}H$ NMR data ($CDCl_3$) δ (ppm) and $^{13}C$ NMR data (ppm): 2.41 (s, 1H, -CH), 4.09-5.126 (s, 6H, $-CH_2$), 1.488 (s, 3H, $-CH_3$), 6.87-8.43 (m, 10H, aromatic protons), $^{13}C$: 15, 49, 57, 65, 76, 79, 110, 111, 112, 114, 119, 120, 123, 131, 134, 136, 141, 144, 145, 149, 157, 158, 163, 167; MS: m/z 520 ($M^+$)

**7.XXIX:**4-{1-[(5-[2,3-dimethoxy-6-(1,3,4-oxadiazol-2-yl)phenol]methyl]-5-nitro-1*H*-benzimidazol-2-yl} benzonitrile. Yield 19%; m.p.:212-214; IR ($cm^{-1}$): 3655 (O-H), 3062 (Ar-C-H), 2849 (C-H), 2210 (C≡N), 1640 (C=N), 1596, 1473, 1439 (C=C), 1352 (N=O), 1331 (C-N), 1242 (C-O-C). $^{1}$H NMR data ($CDCl_3$) δ (ppm) and $^{13}$C NMR data (ppm): 5.126 (s, 2H, $-CH_2$), 3.75-3.85 (s, 6H, $-CH_3$), 6.70-8.47 (m, 9H, aromatic protons), 5.44 (s, 1H, OH), $^{13}$C: 49, 57, 60, 95, 107, 112, 114, 119, 120, 121, 131, 134, 136, 141, 142, 144, 145, 149, 157, 158, 167; MS: m/z 498 ($M^+$)

The compound 7.XX (116±1.025) µg/ml and 7.XXIX (103±0.96) µg/ml were found to be highly active in low concentration and compounds 7.VIII (160±0.9614) µg/ml, 7.IX (300±0.9865) µg/ml, 7.X (220±1.0425) µg/ml, 7.XIX (165±0.67) µg/ml, 7.XXII (147±0.96) µg/ml, 7. XXIV (148±1.052) µg/ml and 7.XXV (137±1.16) µg/ml were found to be moderately active at higher concentration as compared to ascorbic acid (109±0.7296) µg/ml in 2, 2-diphenyl-1-picryl hydrazide method. *In-vitro* anti-inflammatory by inhibition of protein denaturation method the compounds 7.VIII (64.98±1.154701) µg/ml, 7.XX (67.59±0.8819171) µg/ml, 7.XXII (61.02±1.20185) µg/ml and 7.XXIX (67.5±0.9865) µg/ml were found to be highly active in low concentration and compounds 7.V (137.4±0.881917), 7.VI (119.8±1.527525), 7.X (76.77±0.5773503) µg/ml, 7.XIX (86.48±0.5773503) µg/ml, 7. XXIV (99.56±1.154701) µg/ml, 7.XXV (99.16±0.8819171) µg/ml and 7.XXVIII (100±0.5773503) µg/ml were found to be moderately active at higher concentration as compared to diclofenac sodium (57.08±0.8819) µg/ml.

## 5. Conflict of interest statement

We declare that we have no conflict of interest

## 6. Acknowledgements

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