SYNTHESIS, CHARACTERIZATION AND ANTI-INFLAMMATORY ACTIVITY OF 5-SUBSTITUTED PHENYL-N-(6-(PROPYLTHIO)-1H-BENZO[D]IMIDAZOL-2-YL)-1, 3, 4-OXADIAZOL-2-AMINES

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ABSTRACT

A series of 5-substituted phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines were prepared by treating substituted 2-benzylidene-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl) hydrazine carboxamides with Chloramine T. The newly synthesized derivatives were screened against anti-inflammatory activity using COX kit. The compounds showed dose dependent activity. The compounds which showed better in-vitro activity tested for in-vivo activity using Carrageenan rat paw edema method.

KEY WORDS

benzimidazole, oxadiazole, Carrageenan, rat paw edema method.

INTRODUCTION

Numerous compounds bearing oxadiazole ring are known to possess important pharmacological activities such as antimicrobial1-3, antifungal4-5, antitubercular6, anti-inflammatory7-9 agents. Our group has been working on development of new series of oxadiazole moieties with anti-inflammatory activity. This manuscript reports the synthesis and anti-inflammatory activity of aforementioned compounds by COX activity by TMMD assay method and rat paw edema method.

MATERIALS AND METHODS

Melting points (mp) were determined in open capillaries, using Toshniwal melting point apparatus, expressed in ⁰C and are uncorrected. The IR spectra of the compounds were recorded on thermo Nicolet Nexus 670S series, FT-IR spectrometer using KBr disc. ¹H NMR was scanned on Avance-400 MHz instrument. Chemical shifts are expressed in d (ppm) relative to TMS as an internal standard using DMSO-d6 as solvent. Mass spectra were recorded on a LC-MSD-Trap-SL. The purity of the compounds was checked on silica gel-coated aluminum sheets (Merck, 1.00554, silica gel HF254–361, Type 60, 0.25 mm, Darmstadt, Germany) by thin-layer chromatography (TLC). TLC was performed on silica gel G for TLC (Merck) and spots were visualized by iodine vapor or by irradiation with ultraviolet light (short wave length, 254 nm). Column chromatography was performed by using sisco’s silica gel for column chromatography (60–120 mesh).
EXPERIMENTAL METHODS

1. **Synthesis of N-((6-(propylthio)-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (II):**

Methyl (6-(propylthio)-1H-benzo[d]imidazol-2-yl)carbamate(I)(0.01mol) was refluxed with 0.1mol of chloramine-T (0.01mol) in absolute ethanol (15ml), in the presence of catalytic amount of glacial acetic acid (3 drops) and the reaction mixture was refluxed for six (6) hours. The solvent was evaporated and poured onto crushed ice. The precipitated compound was filtered and washed with water and recrystallized from absolute ethanol.

2. **Synthesis of 2-benzylidene-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (III):**

A mixture N-((6-(propylthio)-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide(II) (0.01mol) and appropriate aromatic aldehyde (0.01mol) in absolute ethanol (15ml), in the presence of catalytic amount of glacial acetic acid (3 drops) and the reaction mixture was refluxed for six (6) hours. The reaction mixture was allowed to cool to room temperature and then poured onto crushed ice. The precipitated compound was filtered and washed with water and recrystallized from absolute ethanol.

3. **Synthesis of 5-substituted phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amine (IV):**

2-benzylidene-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl) hydrazine carboxamide (0.01mol) was refluxed with 0.1mol of Chloramine-T in absolute ethanol for about one hour. It was allowed to cool to room temperature. That resultant was extracted with ethyl acetate. The product was recrystallized with ethyl acetate.

**Characterization:**

- IR (KBr, cm⁻¹): 2970.21 (Ar-CH), 3346.77(NH), 2960.0 (Ar C-H), C=O (1637.19).
- Mass spectrum of the compound exhibited molecular ion (M+1) peak at m/z 354.

1H NMR (DMSO-d300MHz) δ: 0.9 (t,3H,CH3), 1.5(m,2H,-CH2), 2.9(t,2H,-CH2), Ar-6.8(d, 2H), 7.5 (t,3H), 7.6(d,2H), 7.8 (s,1H), 12 (s, NH).

**Anti-inflammatory activity:**

**In vitro Anti-inflammatory activity:**

The synthesized compounds were evaluated for their in vitro anti-inflammatory activity by TMPD assay method. This assay is based on chromogenic assay based on oxidation of N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) during the reduction of prostaglandinH₂ by COX-2 enzyme. This measures the peroxides component of cyclooxygenases. The peroxide activity is assayed calorimetrically by monitoring the appearance of oxidized N, N, N', N'-tetramethyl-p-phenylenediamine (TMPD) at 590nm. The final volume of the assay was 220µl. All the wells Background wells contain 160µl of assay buffer and 10µl of heme and 10µl of enzyme. The inhibitor wells contain 150µl of assay buffer and 10µl of heme, 10µl of enzyme and 10µl of inhibitor. The plate was shaken for a few seconds and incubated for five minutes at 25ºC. Then 20µl of colorimetric substance, 20µl of arachidonic acid were added. The plate was again shaken for a few seconds and incubated for five minutes at 25ºC. Then the absorbance was noted at 590nm using plate reader.

**In vivo Anti-inflammatory activity:**

Anti-inflammatory activity was assessed by the method described by Winter et al. Rats were divided into three groups (control, test compounds and standard drug) of six animals each. The standardDiclofenac sodium (100mg/kg dose) and synthesized compounds under study (IVB, C, E, F and H) were administered orally to all rats. After 30minutes a freshly prepared suspension of carrageenan (1% in 0.9%, saline 0.5ml) was injected under the sub planter tissues of the right hind paw of each rat. The edema volumes of the injected paw were
measured at 1st, 2nd, 3rd and 4th hour. The difference between the paw volumes of treated animals were compared with that of the control group and the mean oedema volume was calculated. From the data obtained mean volume of oedema ±SEM (Standard Error Mean) and percentage reduction in oedema were calculated. The Percentage reduction or inhibition in oedema volume was calculated by using the formula.

\[
\text{Percentage of inhibition of oedema} = 1 - \frac{V_t}{V_c} \times 100
\]

Where \( V_t \) and \( V_c \) are volumes of oedema in test compound/standard drug treated and control group respectively.

### RESULTS AND DISCUSSION

5-substituted phenyl-N-(6-propylthio)-1H-benzo[d]imidazol-2-yl)-1,3,4-oxadiazol-2-amines (VI a-j) on COX-1 and COX-2 was presented in the table 2 and figure Among the compounds of the series, IVC(R=4-Cl) was active with an IC\(_{50}\) value 2.011 for COX-1 and compounds IVB(R=4-OH) and IVF(R=2-furfuryl) were next in order. Among them, IVC (R=4-Cl) was active on Cox-1 enzyme. IVE(R=N(CH\(_3\))\(_2\)) was active on COX-2 enzyme. Among the compounds of the series, no compound showed significant action on both the enzymes. The compounds IVC(R=4-Cl),IVB(R=4-OH),IVF(R=2-furfuryl), IVH(R=4-OC\(_3\)) and IVE (R=N(CH\(_3\))\(_3\)) were tested for their in-vivo anti-inflammatory activity using Carrageenan induced rat paw edema method and represented in table 3.Among the five compounds, IVC(R=4-Cl) showed good activity.

### TABLE 1: Physical data of 5-substituted phenyl-N-(6-propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines:

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Compound</th>
<th>R</th>
<th>Molecular formula</th>
<th>Molecular weight</th>
<th>Melting point (°C)</th>
<th>Percentage yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IVa</td>
<td>H</td>
<td>C(<em>{18})H(</em>{19})N(_5)O(_5)S</td>
<td>353.44</td>
<td>142-143</td>
<td>72</td>
</tr>
<tr>
<td>2</td>
<td>IVb</td>
<td>4-OH</td>
<td>C(<em>{18})H(</em>{19})N(_5)O(_5)S</td>
<td>369.44</td>
<td>150-151</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>IVc</td>
<td>4-Cl</td>
<td>C(<em>{18})H(</em>{18})Cl(_3)N(_5)O(_5)S</td>
<td>387.89</td>
<td>153-154</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>IVd</td>
<td>4-No(_2)</td>
<td>C(<em>{18})H(</em>{18})N(_5)O(_5)S</td>
<td>382.44</td>
<td>158-160</td>
<td>72</td>
</tr>
<tr>
<td>5</td>
<td>IVe</td>
<td>N(CH(_3))(_2)</td>
<td>C(<em>{20})H(</em>{22})N(_5)O(_5)S</td>
<td>396.51</td>
<td>162-163</td>
<td>73</td>
</tr>
<tr>
<td>6</td>
<td>IVf</td>
<td>2-furfuryl</td>
<td>C(<em>{23})H(</em>{27})N(_5)O(_5)S</td>
<td>421.56</td>
<td>163-164</td>
<td>75</td>
</tr>
<tr>
<td>7</td>
<td>IVg</td>
<td>4-F</td>
<td>C(<em>{18})H(</em>{18})F(_2)N(_5)O(_5)S</td>
<td>371.43</td>
<td>145-146</td>
<td>76</td>
</tr>
<tr>
<td>8</td>
<td>IVh</td>
<td>4-OC(_3)</td>
<td>C(<em>{19})H(</em>{21})N(_5)O(_5)S</td>
<td>383.47</td>
<td>149-151</td>
<td>74</td>
</tr>
<tr>
<td>9</td>
<td>IVi</td>
<td>2-OH</td>
<td>C(<em>{18})H(</em>{19})N(_5)O(_5)S</td>
<td>369.44</td>
<td>151-152</td>
<td>75</td>
</tr>
</tbody>
</table>
TABLE 2: Anti-inflammatory activity data of 5-substituted phenyl-N-(6-(propylthio)-1H benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines:

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Compound</th>
<th>R</th>
<th>IC₅₀(µg/ml)</th>
<th>COX-1</th>
<th>COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IVₐ</td>
<td>H</td>
<td>3.67</td>
<td>95.36</td>
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<tr>
<td>2</td>
<td>IVₐ</td>
<td>4-OH</td>
<td>3.23</td>
<td>96.32</td>
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</tr>
<tr>
<td>3</td>
<td>IVₐ</td>
<td>4-Cl</td>
<td>2.01</td>
<td>86.84</td>
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</tr>
<tr>
<td>4</td>
<td>IVₐ</td>
<td>4-NO₂</td>
<td>4.29</td>
<td>91.88</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>IVₐ</td>
<td>4-N(CH₃)₂</td>
<td>5.01</td>
<td>20.85</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>IVₐ</td>
<td>2-furfuryl</td>
<td>3.35</td>
<td>89.72</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>IVₐ</td>
<td>4-F</td>
<td>3.59</td>
<td>91.25</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>IVₐ</td>
<td>4-OCH₃</td>
<td>4.29</td>
<td>24.23</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>IVₐ</td>
<td>2-OH</td>
<td>6.55</td>
<td>76.61</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>IVₐ</td>
<td>2-CHCHCHO</td>
<td>5.234</td>
<td>96.35</td>
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</tr>
</tbody>
</table>

Table 3: In-vivo anti-inflammatory activity data of 5-phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amine (IV a-j):

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>S.No</th>
<th>Compound</th>
<th>R</th>
<th>Percentage inhibition of rat paw edema</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>1hr</td>
</tr>
<tr>
<td>1</td>
<td>IVE</td>
<td>N(CH₃)₂</td>
<td>0.375±0.055902</td>
</tr>
<tr>
<td>2</td>
<td>IVB</td>
<td>4-OH</td>
<td>0.275±0.055902</td>
</tr>
<tr>
<td>3</td>
<td>IVH</td>
<td>4-OCH₃</td>
<td>0.375±0.055902</td>
</tr>
<tr>
<td>4</td>
<td>IVC</td>
<td>4-Cl</td>
<td>0.025±0.50</td>
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</tbody>
</table>
Figure-1: Anti-inflammatory activity data of 5-substituted phenyl-N-(6-(propylthio)-1H benz[d]imidazol-2-yl)-1, 3, 4-oxadiazol-2-amines (IVa-j):

<p>| | | | | | |</p>
<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>IVF</td>
<td>2-furfuryl</td>
<td>0.225±0.018028</td>
<td>0.24±0.05244</td>
<td>0.1975±0.9875</td>
</tr>
<tr>
<td>6</td>
<td>Std.</td>
<td>Diclofenac Sodium</td>
<td>0.029±0.036</td>
<td>0.0207±0.090</td>
<td>0.0212±0.069</td>
</tr>
</tbody>
</table>

Invitro anti-inflammatory activity data of 5-phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)1,34oxadiazol-2-amine (IVa-j)

<table>
<thead>
<tr>
<th></th>
<th>COX-1</th>
<th>COX-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVb</td>
<td></td>
<td></td>
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<tr>
<td>IVc</td>
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<td>IVd</td>
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<tr>
<td>IVe</td>
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<td>IVf</td>
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<td>IVg</td>
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<tr>
<td>IVh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVi</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVj</td>
<td></td>
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</tr>
</tbody>
</table>
Synthesis of 5-substituted phenyl-N-(6-(propylthio)-1H-benzo[d]imidazol-2-yl)-1,3,4-oxadiazol-2-aminoses (Iva-j):

R=H, 4-OH, 2-OH, 4-Cl, 4-NO₂, N(CH₃)₂, 2-OCH₃, CH=CHCHO, 2-furfuryl

REFERENCES


5. Weiming Xu, Song Yang, Pinaki Bhadury, Jiag He, Ming He, Lili Gao, Deyu Hu, Baoran Song, Synthesis and bioactivity of novel sulfone derivatives containing 2,4-dichlorophenyl substituted 1,3,4-oxadiazole/thiadiazole moiety as chitinase inhibitors, *Pesticide Biochemistry and Physiology*, (2011), 101, 6-15.


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