Synthesis and Pharmacological Screening of Some Benzoxazole Derivatives as Anti-inflammatory Agents

Mohammed Rageeb Mohammed Usman*, Rana Sohil D¹, Md. Abullais Md. Usman¹, Shaikh Tanver Y², Sayad Imran wahab³

¹Assistant Professor, Department of Pharmacognosy, Smt. S. S. Patil College of Pharmacy, Chopda, Maharashtra, India
²Assistant Professor, Department of Pharmacognosy, Smt. S. S. Patil College of Pharmacy, Chopda, Maharashtra, India
³Lecturer, Department of Pharmaceutical Chemistry, Y. B. Chavan College of Pharmacy, Aurangabad, Maharashtra, India

ABSTRACT
A series of some new 5-substituted benzoxazoles were synthesized and were characterized by different methods like IR, ¹H NMR and MASS spectra. After conformation of structure assigned, these compounds were screened for its anti-inflammatory activity.

Keywords: Benzoxazole, Anti-inflammatory, Pharmacological, Derivatives, Phytochemical.

INTRODUCTION
Inflammation evidence of many diseases is major concern for physicians throughout the world. The single most important event in this process is accumulation of large number of phagocytic cells of the site of the inflammation. Tissue injury caused by introduction of a foreign antigen, trauma, or local exposure to certain chemicals triggers complex processes of inflammation. This may consists of a fluid stasis as well as the accumulation of several cellular and no cellular elements of the immune response [1-6].

In most of these cases, it has been proved that the 5-substituted benzoxazole [7], substituted sulfonyl derivatives [8] and carbohydrazides [9], have promising anti-inflammatory activity. Also benzoxazole at its 5th position [10], is more prone for its lipophilic action and therefore we go the substitution at 5th position of benzoxazole. Hence, it was planned to synthesize the N-[substituted sulfonyl]-1,3-benzoxazole-5-carboxylic acid methyl esters (IVa and IVb).

In the present investigation, series of N-[substituted sulfonyl]-1,3-benzoxazole-5-carboxylic acid methyl esters (IVa-VIh) was synthesized using appropriate synthetic route (Scheme: I page no.3) and were screened for its anti-inflammatory activity (VIA-VIh).

4-Hydroxy-3-nitro-benzoic acid methyl ester (II) was synthesized in an excellent yield by electrophilic substitution, nitration on p-hydroxy methyl benzoate (I) by concentrated nitric acid and concentrated sulfuric acid. Compound (II) on reduction with the help of reducing agent like sodium dithionate [11], with alcohol afforded 3-amino-4-hydroxy-benzoic acid methyl ester (III). Reaction of compound (III) with two appropriate aliphatic acids such as, formic acid and acetic acid gives corresponding 2-substituted benzoxazole-5-carboxylic acid methyl esters (IVA and IVb). The reaction of compounds (IV) with hydrazine hydrate in ethanol on refluxing gives the corresponding 2-substituted benzoxazole-5-carboxylic acid hydrazides (VA and VB) is the nucleophilic substitution type reaction. On further reaction of compounds (V) with the different nucleophilic substitution of substituted sulfonyl chloride derivatives afforded the corresponding eight N-[substituted sulfonyl]-1,3-benzoxazole-5-carboxylic acid methyl esters (VIA-VIh).

The purity and homogeneity of compounds synthesized were determined by their sharp melting points, TLC, IR spectra. Preliminary pharmacological screening was performed, which includes approximate toxicity testing (LD₅₀) [13] and antiinflammatory activity [14]. The LD₅₀ of the test compounds performed on the rats as per the OECD 423 guidelines for selection of dose.

Figure 1: N-[substituted sulfonyl]-1,3-benzoxazole-5-carboxylic acid methyl esters (VIIa-VIIh)
Table 1: Different substituted compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>R1</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIa</td>
<td>H</td>
<td>4-methyl phenyl</td>
</tr>
<tr>
<td>VIb</td>
<td>CH₃</td>
<td>4-methyl phenyl</td>
</tr>
<tr>
<td>VIc</td>
<td>H</td>
<td>4-aceta amido phenyl</td>
</tr>
<tr>
<td>VId</td>
<td>CH₃</td>
<td>4-aceta amido phenyl</td>
</tr>
<tr>
<td>VIe</td>
<td>H</td>
<td>4-chloro phenyl</td>
</tr>
<tr>
<td>VIf</td>
<td>CH₃</td>
<td>4-chloro phenyl</td>
</tr>
<tr>
<td>VIg</td>
<td>H</td>
<td>Benzene</td>
</tr>
<tr>
<td>VIh</td>
<td>CH₃</td>
<td>Benzene</td>
</tr>
</tbody>
</table>

Scheme I:

MATERIAL AND METHODS
All chemicals were used as purchased pure from Hi-Media, E-Merck. p-hydroxy methyl benzoate (I) was used as starting material undergoes electrophilic substitution reaction, nitration by using concentrated nitric acid and concentrated sulphuric acid gives 4-Hydroxy-3-nitro-benzoic acid methyl ester (II) this reaction is carried out at 0-10°C and recrystallised by methanol. Compound (II) undergoes reduction by using sodium dithionate as reducing agent in mixture with methanol gives good yield of 3-amino-4-hydroxy-benzoic acid methyl ester (III). This was recrystallised by using methanol. On further reaction of compound (III) with aliphatic acid like formic and acetic acid gives corresponding compounds 2-substituted benzoxazole-5-carboxylic acid methyl ester (IVA-IVb). Both these products were recrystallised from alcohol. Compounds (IVA-IVb) on reaction with hydrazine hydrate and mixture with ethanol gives corresponding 2-substituted benzoxazole-5-carboxylic acid hydrazides (VA-Vb) also both these products was recrystallised from alcohol. And finally compounds (VA-Vb) on further reaction with substituted sulfonyl chlorides by using pyridine as catalyst which traps HCl gas in compounds gives corresponding N-[substituted sulfonyl]-1,3-benzoxazole-5-carbohydrazide (VIa-VIh) compounds. Finally these eight compounds were recrystallised by ethanol give pure compounds.

The melting points of the compounds were determined in open capillary method which was uncorrected. Porous silica gel plates activated at 110°C for 30 min. were used for thin layer chromatography (TLC) and were developed with iodine vapours. IR spectra of compounds were recorded using KBr pellets on FTIR. "H-NMR spectra (solvents) were recorded on Varian EM 390 spectra (chemicals shift in ppm). Mass Spectra of the synthesized compounds were recorded on (FAB-MS) [11-12].

**RESULT AND DISCUSSION**

![Figure 2: N-[substituted sulfonyl]-1,3-benzoxazole-5-carbohydrazide (VI)](image)

<table>
<thead>
<tr>
<th>Compound No.</th>
<th>R₂</th>
<th>Molecular Formula</th>
<th>Mp (°C) uncorrected</th>
<th>Yield (%)</th>
<th>Rf Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VIa</td>
<td>Tosyl</td>
<td>C₁₅H₁₃N₅O₄S</td>
<td>72-74</td>
<td>38%</td>
<td>0.63</td>
</tr>
<tr>
<td>VIb</td>
<td>Tosyl</td>
<td>C₁₆H₁₅N₃O₄S</td>
<td>78-80</td>
<td>57%</td>
<td>0.60</td>
</tr>
<tr>
<td>VIc</td>
<td>p-aceta amido</td>
<td>C₁₆H₁₄N₄O₃S</td>
<td>92-94</td>
<td>72%</td>
<td>0.57</td>
</tr>
<tr>
<td>VID</td>
<td>p-aceta amido</td>
<td>C₁₇H₁₆N₄O₃S</td>
<td>88-90</td>
<td>40%</td>
<td>0.54</td>
</tr>
<tr>
<td>VIe</td>
<td>p-chloro</td>
<td>C₁₄H₁₀ClN₃O₄S</td>
<td>110-112</td>
<td>75%</td>
<td>0.53</td>
</tr>
<tr>
<td>VIf</td>
<td>p-chloro</td>
<td>C₁₃H₁₂ClN₃O₄S</td>
<td>102-104</td>
<td>72%</td>
<td>0.64</td>
</tr>
<tr>
<td>VIG</td>
<td>Benzene</td>
<td>C₁₄H₁₁N₃O₄S</td>
<td>69-70</td>
<td>30%</td>
<td>0.62</td>
</tr>
<tr>
<td>VIH</td>
<td>Benzene</td>
<td>C₁₅H₁₃N₃O₄S</td>
<td>66-68</td>
<td>40%</td>
<td>0.72</td>
</tr>
</tbody>
</table>

Elemental analysis of the given compounds is summarized below.
All products were isolated and characterized by spectrometric methods (\(^1\)H NMR, IR and mass spectra). A detailed IR, NMR and mass analysis is explained below:

**Compound VIa (C\(_{18}\)H\(_{13}\)N\(_2\)O\(_2\)S)**

IR (KBr) cm\(^{-1}\): 3390 (-NH-str.), 1315 and 1398 (-S-O str.), 1730 (-CO-str.), 1625 (C-N str.), 3085 (Ar–H str.), 835 (C=C bending), 1165 cm\(^{-1}\) (ether group in ring);

\(^1\)H NMR: (CDCl\(_3\)) \(\delta 7.34-7.95\) (m, Ar-H, 4Hx2), \(\delta 8.0\) (s, NH, 2H), \(\delta 2.35\) (d, CH\(_3\), 3H).

FAB-MS: (m/z, 100%): 331 ([M\(^+\)], 100%)

Elemental analysis (%): Calculated: C: 52.9, H: 4.04, N: 13.08,

**Compound VIb (C\(_{19}\)H\(_{12}\)N\(_2\)O\(_2\)S)**

IR (KBr) cm\(^{-1}\): 3319 (-NH- str.), 1315 (-S-O str.), 1730 (-CO- str.), 1625 (C=N str.), 3010 (Ar–H str.), 2230 (C-C str.)

\(^1\)H NMR: (CDCl\(_3\)) \(\delta 7.34-7.95\) (m, Ar-H, 4Hx2), \(\delta 8.0\) (s, NH, 2H), \(\delta 2.35\) (d, CH\(_3\), 3H).

FAB-MS: (m/z, 100%): 345 ([M\(^+\)], 100%)

Elemental analysis (%): Calculated: C: 55.6, H: 4.34, N: 12.07,
Found: C: 55.5, H: 4.38, N: 12.10.

**Compound VIc (C\(_{19}\)H\(_{14}\)N\(_2\)O\(_3\)S)**

IR (KBr) cm\(^{-1}\): 3327 (-NH- str.), 1352 (-S-O str.), 1730 (-CO- str.), 1116 and1172 cm\(^{-1}\) (CONH str.), 3010 cm\(^{-1}\) (Ar–H str.).

\(^1\)H NMR: (CDCl\(_3\)) \(\delta 7.44-7.95\) (m, Ar-H, 4Hx2), \(\delta 8.0\) (s, NH, 3H), \(\delta 2.02\) (d, CH\(_3\), 3H).

FAB-MS: (m/z, 100%): 374 ([M\(^+\)], 100%)

Elemental analysis (%): Calculated: C: 53.63, H: 3.91, N: 15.64,
Found: C: 53.65, H: 3.87, N: 15.76.

**Compound VId (C\(_{19}\)H\(_{16}\)N\(_2\)O\(_2\)S)**

IR (KBr) cm\(^{-1}\): 3324 (-NH- str.), 1322 (-S-O str.), 1730 (-CO- str.), 1629 (C=N str.), 3110 (Ar–H str.), 3180 (CONH str.).

\(^1\)H NMR: (CDCl\(_3\)) \(\delta 7.44-7.95\) (m, Ar-H, 4Hx2), \(\delta 8.0\) (s, NH, 3H), \(\delta 2.02\) and 2.35 (d, CH\(_3\), 3Hx2).

FAB-MS: (m/z, 100%): 388 ([M\(^+\)], 100%)

Elemental analysis (%): Calculated: C: 52.57, H: 4.12, N: 14.43,
Found: C: 52.56, H: 4.16, N: 14.47.

**Compound VVe (C\(_{19}\)H\(_{10}\)Cl\(_2\)N\(_2\)O\(_2\)S)**

IR (KBr) cm\(^{-1}\): 3216 (-NH- str.), 1339 (-S-O str.), 3090 (Ar–H str.), 772 (C-Cl str.)

\(^1\)H NMR: (CDCl\(_3\)) \(\delta 7.44-7.95\) (m, Ar-H, 4Hx2), \(\delta 8.0\) (s, NH, 2H).

FAB-MS: (m/z, 100%): 351.50 ([M\(^+\)], 100%)

Elemental analysis (%): Calculated: C: 45.94, H: 2.94, N: 12.37,
Found: C: 45.97, H: 2.98, N: 12.40.

**Compound VIf (C\(_{19}\)H\(_{12}\)Cl\(_2\)N\(_2\)O\(_2\)S)**

IR (KBr) cm\(^{-1}\): 3204 (-NH- str.), 1354 and 1329 (-S-O str.), 1730 (-CO- str.), 3180 (CONH str.), 3097 (Ar–H str.), 767 (C-Cl str.).

\(^1\)H NMR: (CDCl\(_3\)) \(\delta 7.44-7.95\) (m, Ar-H, 4Hx2), \(\delta 8.0\) (s, NH, 2H), \(\delta 2.35\) (d, CH\(_3\), 3H).

FAB-MS: (m/z, 100%): 317.50 ([M\(^+\)], 100%)

Elemental analysis (%): Calculated: C: 47.81, H: 3.73, N: 11.81,
Found: C: 47.78, H: 3.75, N: 11.84.

**Compound VIg (C\(_{19}\)H\(_{11}\)N\(_2\)O\(_2\)S)**

IR (KBr) cm\(^{-1}\): 3350 (-NH- str.), 1329 (-S-O str.), 3174 (CONH str.), 3097 (Ar–H str.), 674 (C-C bending)

\(^1\)H NMR: (CDCl\(_3\)) \(\delta 7.03-7.95\) (m, Ar-H, 5Hx4), \(\delta 8.0\) (s, NH, 2H).

FAB-MS: (m/z, 100%): 317 ([M\(^+\)], 100%)

Elemental analysis (%): Calculated: C: 51.31, H: 3.28, N: 13.81,

**Compound VIIh (C\(_{19}\)H\(_{12}\)N\(_2\)O\(_2\)S)**

IR (KBr) cm\(^{-1}\): 3204 (-NH- str.), 1346 (-S-O str.), 1730 (-CO- str.), 1625 (C=N str.), 3085 (Ar–H str.), 2990 (C-C str.).

\(^1\)H NMR: (CDCl\(_3\)) \(\delta 7.03-7.95\) (m, Ar-H, 4Hx2), \(\delta 8.0\) (s, NH, 2H), \(\delta 2.35\) (d, CH\(_3\), 3H).

FAB-MS: (m/z, 100%): 331 ([M\(^+\)], 100%)

Elemental analysis (%): Calculated: C: 53.31, H: 3.75, N: 13.12,
Found: C: 53.34, H: 3.77, N: 13.16.
Pharmacological Screening
LD50
LD50 of test compounds was performed in National Toxicological Center, Pune and determined on mice as per the OECD Guidelines 423. 2000 mg/kg dose was considered as LD50. 1/10th of the LD50 was considered as an effective dose i.e. 200 mg/kg.

Anti-Inflammatory Activity
The anti-inflammatory activities of these compounds were done by using carrageenan induced rat paw edema method described by Winter et al (1962) [10].

Table 4: Anti-inflammatory activity of Some Benzoxazole Derivatives

<table>
<thead>
<tr>
<th>Group</th>
<th>Test Material (dose)</th>
<th>Mean increase in paw volume and % inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 hr.</td>
</tr>
<tr>
<td>1.</td>
<td>Control</td>
<td>1.29 ± 0.152</td>
</tr>
<tr>
<td>2.</td>
<td>Standard(Ibuprofen 50mg/kg)</td>
<td>0.95 ± 0.158</td>
</tr>
<tr>
<td>3.</td>
<td>V1a 200 mg/kg</td>
<td>1.06 ± 0.116</td>
</tr>
<tr>
<td>4.</td>
<td>V1b 200 mg/kg</td>
<td>1.13 ± 0.212</td>
</tr>
<tr>
<td>5.</td>
<td>V1c 200 mg/kg</td>
<td>1.19 ± 0.364</td>
</tr>
<tr>
<td>6.</td>
<td>V1d 200 mg/kg</td>
<td>1.22 ± 0.0740</td>
</tr>
<tr>
<td>7.</td>
<td>V1e 200 mg/kg</td>
<td>1.28 ± 0.98</td>
</tr>
<tr>
<td>8.</td>
<td>V1f 200 mg/kg</td>
<td>1.09 ± 0.0659</td>
</tr>
<tr>
<td>9.</td>
<td>V1g 200 mg/kg</td>
<td>1.47 ± 0.285</td>
</tr>
<tr>
<td>10.</td>
<td>V1h 200 mg/kg</td>
<td>1.10 ± 0.0815</td>
</tr>
</tbody>
</table>
CONCLUSION
5-substituted benzoxazole has proved to be a promising moiety for antiinflammatory activity. The test compounds (VIa-VIIh) showed significant antiinflammatory activity compared with the standard drug Ibuprofen. Among these compounds VId and VIe possesses good and compound VIf possesses moderate antiinflammatory activity. All the significant compounds also possess antiinflammatory activity with reduced toxicity.

The compounds can be further exploited for testing other pharmacological activities. QSAR parameters can be added in the present study shall help to ascertain proposed and observed activity.

REFERENCES
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