Synthesis & Analgesic Activity of Mannich & Schiff Bases of 1,5-Benzodiazepines

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ABSTRACT: Benzodiazepines and their polycyclic derivatives are a very important class of bioactive compounds. They are finding numerous new applications and are widely used as anticonvulsant, anti-inflammatory, analgesic, hypnotic, sedative, and antidepressive agents. Hence 1,5-benzodiazepines were synthesized by condensation of o-phenylenediamine and ketones e.g., cyclohexanone in presence of Sulfated Zirconia (catalyst). Mannich bases were synthesized with acetophenone, p-nitroacetophenone, p-chloroacetophenone and formaldehyde. Schiff bases were synthesized using Mannich base of fused ring 1,5-benzodiazepines with p-chloroaniline and p-chlorophenylsemicarbazide in presence of glacial acetic acid. All the synthesized compounds were characterized by ¹H NMR and IR spectral analysis. Synthesized compounds were screened Analgesic (both central and peripheral activity). Compounds NBZD-1, NBZD-3, NBZD-6, NBZD-11, were found to have good peripheral analgesic activity and NBZD-6, NBZD-8, NBZD-9, with good central analgesic activity. © 2011 IGJPS. All rights reserved.

KEYWORDS: 1,5-Benzodiazepines; Mannich Bases; Schiff Bases.

INTRODUCTION

Pain is an unpleasant sensation varying in severity in a local part of the body or several parts of body resulting from injury, disease or emotional disorder. Millions of people suffer from chronic or intractable pain. Pain and its much manifestation may be poorly treated and seriously underestimated. Inappropriate treated pain seriously compromises the patient’s quality of life [1].

Severe chronic pain affects both the pediatric and adult population and often leads to mood disorders, including depression. To treat pain disorders novel heterocycles are discovered.

Indomethacin (Fig. 1) causes redness, pain or oozing at the injection site. Severe allergic reactions (rash, hives, itching, difficulty in breathing, swelling of the mouth, face, lips or tongue): blood in vomit, stool or urine: decreased urination, slow heart beat, unusual bruising or bleeding, unusual weight gain [2].

Piroxicam (Fig. 2) causes diarrhea; dizziness; gas; headache; heartburn; nausea; stomach upset. Severe allergic reactions (rash; hives; itching; trouble breathing; tightness in the chest; swelling of the mouth, face, lips, or tongue); bloody or black, tarry stools; change in the amount of urine produced; chest pain; confusion; dark urine; depression; fainting; fast or irregular heartbeat;
fever, chills, or persistent sore throat; mental or mood changes; numbness of an arm or leg; one-sided weakness; red, swollen, blistered, or peeling skin; ringing in the ears; seizures; severe headache or dizziness; severe or persistent stomach pain or nausea; severe vomiting; shortness of breath; sudden or unexplained weight gain; swelling of the hands, legs, or feet; unusual bruising or bleeding; unusual joint or muscle pain; unusual tiredness or weakness; vision or speech changes; vomit that looks like coffee grounds; yellowing of the skin or eyes [3].

There are no. of heterocyclic derivatives which shows analgesic activity. Celecoxib, a pyrazole derivative used as a analgesic, have a 5 member heterocyclic ring [4].

Cyclobenzaprine (Fig. 3) is compound having 6 member ring system which is used as muscle relaxant and used in acute pain. But they also cause side effects like drowsiness, dizziness [5].

Treatment with the benzodiazepines may reduce complaints of pain, but this seems to be an indirect effect related to their psychotropic properties, such as alleviation of anxiety and, in selected cases, depression. In the absence of definitive data, clinical experience suggests a potential role for treatment with benzodiazepines for acute muscle spasm, concomitant chronic pain and anxiety, and lancinating neropathic pain. They should probably not be considered as first-line choices even for the above indications, since potential benefits must be considered in the context of potential for the development of cognitive impairment, physical and psychological dependence, worsening depression, overdose, and other side effects [6].

1,5-benzodiazepines are biologically important molecules and are extensively used clinically as analgesic[7]. Beside this, 1,5-benzodiazepines show antifungal, antibacterial, antifeedant, anti-inflammatory, analgesic and anticonvulsant activities. The fusion of a heterocyclic system to the benzodiazepine ring appears quite promising for the synthesis of derivatives with greater activity and specificity [8].

Hence in our research work we have concentrated on further enlargement of the heterocyclic ring system and screened the synthesized compounds for analgesic activity and found positive results.
Starting material and reagents were procured from commercial chemical suppliers. All the chemicals and solvents used were of laboratory grade. Melting points were determined in open capillary tubes and are uncorrected. IR spectra (KBr, cm$^{-1}$) were recorded on Perkin Elmer Spectrometer, 1H NMR( $\delta$, ppm) spectra was recorded on a Brucker 300 MHz NMR spectrometer using TMS as an internal standard. The purity of compounds and progress of the reaction was checked by TLC using silica gel-G as adsorbent.

1) Synthesis of fused ring benzodiazepine nucleus.

1.a). Synthesis of fused ring benzodiazepine nucleus: Synthesis of fused ring benzodiazepines in presence of Sulphated Zirconia involves 2 steps which are as follows[9]:

Preparation of catalyst: 25 gm of Zirconium Oxychloride was dissolved in doubly distilled water (pH-2). Dilute aq. Ammonia was then added drop wise from a burette with vigorous (pH= 8). Precipitate was washed with distilled water several times and dried for 24 h. Sample was ground to fine powder and immersed in a 0.5 M H$_2$SO$_4$ solution (30 ml) for 30 min. Excess water was evaporated on water bath and the resulting sample was oven dried.

Synthesis of benzodiazepines: 1:2.5 mole ratio mixture of o-phenylenediamine and ketone (Cyclohexanone (scheme 1), with catalytic amount of sulfated zirconia were taken in RBF with stirring at ambient condition for 2-3 h. 10 ml of CH$_2$Cl$_2$ was added to reaction mixture and Catalyst was recovered by filtration.

![Scheme 1: Synthesis of fused ring benzodiazepine](image)

2) Procedure for preparation of mannich base derivatives:

2.a). Synthesis of various Mannich base derivatives of fused ring benzodiazepine (Scheme 3).

Equimolar quantity of fused ring benzodiazepine (NBZD-1, 0.01M), formaldehyde, and various acetophenone (i.e., acetophenone, p-nitroacetophenone, p-chloroacetophenone) were taken in RBF and mixture was refluxed for 2.30 h. Completion of reaction was monitored by TLC analysis for several times. Then reaction mixture was evaporated on water bath and dried. Melting point, RF value, and % yield were noted. Various Mannich base derivatives are shown in Scheme 3.
3) Procedure for preparation of Schiff base derivatives:

3.a). Synthesis of various Schiff base derivatives of fused ring benzodiazepines

Equimolar quantities of Mannich base derivatives (0.01M, NBZD-3, NBZD-4, NBZD-5) in individual reactions, were dissolved in glacial acetic acid and added with p-chloroaniline (Scheme 5) or p-chlorophenylsemicarbazide (Scheme 6) were taken in RBF and mixture was refluxed for 3 h respectively. Completion of reaction was monitored by TLC analysis for several times in chloroform: ethanol (1:1). Then reaction mixture was evaporated on water bath and dried. Melting point, Rf value, and % yield were noted.

4.(a) Acetic acid induced writhing test:

PROCEDURE:
- Mice of either sex with a weight between 20 and 25 g were used. 0.1 ml of a 0.6% solution of acetic acid was injected i.p to mice. A aliquot of 0.25 ml of this suspension was injected i.p. Groups of 6 animals were used for control and treated mice. Test animals were administered the drug or the standard at various pretreatment times prior to acetic acid injection. The mice were then observed for a period of 10 min and the number of writhes were recorded for each animal.

- % Analgesic activity was calculated.
- DOSE: Test Drug: 20mg/Kg bw i.p
  Standard(diclofenac): 20mg/kg i.p

4.(b) Hot plate method:
The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. Groups of 11 mice of either sex with an initial weight of 18 to 22 g were used for each dose. The hot plate, which is commercially available, consists of a electrically heated surface. The temperature controlled for 55° to 56 °C. The animals were placed on the hot plate and the time of either licking or jumping occurs was recorded by a stopwatch. The latency was recorded before and after 30, 60 and 90 min following.

DOSE: Test drug: 5mg/Kg bw s.c
Standard drug (Morphine sulphate): 5 mg/kg bw s.c

RESULTS

All the various synthesized compounds were characterized with Physicochemical data (shown in Table-1), and spectral analysis with respect to $^1$H NMR spectra and IR spectra.

Synthesized compounds were also screened for analgesic activity. Compounds were screened for peripheral analgesic activity by acetic acid induced writhing test (shown in Table -2), and central analgesic activity by hot plate method (shown in Table 3).

1. PHYSICOCHEMICAL CHERACTERIZATION

<table>
<thead>
<tr>
<th>Compound Code</th>
<th>Molecular Formula</th>
<th>Molecular weight</th>
<th>Melting Point (°C)</th>
<th>Reaction Time(h)</th>
<th>% yield</th>
<th>Rf Value</th>
<th>Log P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBZD-1</td>
<td>C$<em>{18}$H$</em>{24}$N</td>
<td>268.19</td>
<td>110</td>
<td>3</td>
<td>76.48</td>
<td>0.689</td>
<td>4.25</td>
</tr>
<tr>
<td>NBZD-3</td>
<td>C$<em>{27}$H$</em>{32}$N$_2$O</td>
<td>400.25</td>
<td>92</td>
<td>2.30</td>
<td>85.7</td>
<td>0.78</td>
<td>6.25</td>
</tr>
<tr>
<td>NBZD-4</td>
<td>C$<em>{33}$H$</em>{36}$N$_3$Cl</td>
<td>510.12</td>
<td>132</td>
<td>3</td>
<td>83.3</td>
<td>0.73</td>
<td>6.15</td>
</tr>
<tr>
<td>NBZD-5</td>
<td>C$<em>{34}$H$</em>{31}$ClN$_2$O</td>
<td>568.15</td>
<td>140</td>
<td>2.30</td>
<td>72</td>
<td>0.83</td>
<td>6.81</td>
</tr>
<tr>
<td>NBZD-6</td>
<td>C$<em>{27}$H$</em>{31}$N$_3$O$_3$</td>
<td>445.55</td>
<td>98</td>
<td>3</td>
<td>84.3</td>
<td>0.63</td>
<td>8.93</td>
</tr>
<tr>
<td>NBZD-7</td>
<td>C$<em>{25}$H$</em>{31}$ClN$_2$O</td>
<td>435.00</td>
<td>98</td>
<td>3</td>
<td>79.2</td>
<td>0.82</td>
<td>6.70</td>
</tr>
</tbody>
</table>
2 PHARMACOLOGICAL ACTIVITY

2 (a). Analgesic activity

Table 2: Analgesic activity by acetic acid induced writhing test.

<table>
<thead>
<tr>
<th>COMPOUND CODE</th>
<th>AVG.± SEM</th>
<th>% ANALGESIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>NBZD-1</td>
<td>14.84± 0.24</td>
<td>77.1%</td>
</tr>
<tr>
<td>NBZD-3</td>
<td>15.66±0.30</td>
<td>75.14%</td>
</tr>
<tr>
<td>NBZD-4</td>
<td>20.33±0.192</td>
<td>67.7%</td>
</tr>
<tr>
<td>NBZD-5</td>
<td>21.66±0.192</td>
<td>65.6%</td>
</tr>
<tr>
<td>NBZD-6</td>
<td>18.33±0.23</td>
<td>70.9%</td>
</tr>
<tr>
<td>NBZD-7</td>
<td>26.00±0.304</td>
<td>58.7%</td>
</tr>
<tr>
<td>NBZD-8</td>
<td>23.00±0.33</td>
<td>63.8%</td>
</tr>
<tr>
<td>NBZD-9</td>
<td>25.8±0.28</td>
<td>59.0%</td>
</tr>
<tr>
<td>NBZD-10</td>
<td>16.66±0.192</td>
<td>73.30%</td>
</tr>
<tr>
<td>NBZD-11</td>
<td>28.66±0.304</td>
<td>54.50%</td>
</tr>
<tr>
<td>STANDARD</td>
<td>6.3±0.19</td>
<td>90%*</td>
</tr>
</tbody>
</table>

Note: Each value represents the mean ±SEM (n=6). Significant levels *p<0.01 as compared with respective control.

Table 3: Analgesic Activity by Hot Plate Method

<table>
<thead>
<tr>
<th>COMPOUND CODE</th>
<th>AVG. ±SEM (before drug)</th>
<th>AVG. ±SEM (After 30 min.)</th>
<th>AVG. ±SEM (After 60 min.)</th>
<th>AVG. ±SEM (after 90 min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>3.8±0.28*</td>
<td>3.8±0.28*</td>
<td>4.5±0.31*</td>
<td>3.6±0.19*</td>
</tr>
<tr>
<td>NBZD-1</td>
<td>4.5±0.204</td>
<td>8.66±0.304**</td>
<td>12.5±0.204**</td>
<td>7.5±0.204**</td>
</tr>
<tr>
<td>NBZD-3</td>
<td>3.5±0.311†</td>
<td>9.33±0.192**</td>
<td>11.66±0.192**</td>
<td>5.5±0.204**</td>
</tr>
<tr>
<td>NBZD-4</td>
<td>4.5±0.204†</td>
<td>8.5±0.204**</td>
<td>12.33±0.192**</td>
<td>6.3±0.192**</td>
</tr>
<tr>
<td>NBZD-5</td>
<td>3.5±0.20†</td>
<td>6.3±0.311**</td>
<td>7.66±0.192**</td>
<td>4.5±0.204†</td>
</tr>
<tr>
<td>NBZD-6</td>
<td>4.66±0.192**</td>
<td>9.83±0.280**</td>
<td>11.66±0.192**</td>
<td>6.6±0.192**</td>
</tr>
<tr>
<td>NBZD-7</td>
<td>3.5±0.20†</td>
<td>6.3±0.311**</td>
<td>7.3±0.192**</td>
<td>4.3±0.192‡</td>
</tr>
<tr>
<td>NBZD-8</td>
<td>4.5±0.204</td>
<td>9.8±0.152**</td>
<td>10±0.235**</td>
<td>7.5±0.204**</td>
</tr>
<tr>
<td>NBZD-9</td>
<td>4.5±0.204</td>
<td>8.66±0.192**</td>
<td>11.0±0.235**</td>
<td>8.3±0.192**</td>
</tr>
<tr>
<td>NBZD-10</td>
<td>3.33±0.192†</td>
<td>6.66±0.192**</td>
<td>8.1±0.280**</td>
<td>5.3±0.192**</td>
</tr>
<tr>
<td>NBZD-11</td>
<td>3.5±0.20†</td>
<td>8.66±0.192**</td>
<td>7.3±0.192**</td>
<td>5.8±0.152**</td>
</tr>
</tbody>
</table>

Note: Rf values were determined in solvent system chloroform: ethanol (1:1).
<table>
<thead>
<tr>
<th>Standard (Morphine)</th>
<th>3.6±0.192*</th>
<th>3.8±0.28*</th>
<th>4.5±0.31*</th>
<th>3.6±.19*</th>
</tr>
</thead>
</table>

Values represent the mean ± SEM of six animals for each group. *significant at p<0.05, **significant at p<0.01 (Dunnett’s test).

3.3 REPRESENTATIVE SPECTRAL ANALYSIS:

1. 10-Spirocyclohexane-1,2,3,9,10,10a hexahyrdro benzo[b]cyclohexane [e] [1,4] diazepine (NBZD-1):

   \(^1\text{H NMR (300 MHz, } \delta)\) : CH (m, 6.4-7.0, 4H, phenyl), NH (s, 4.1, 1H), CH\(_2\) (m, 1.2-1.6, 18H, Cyclohexane), CH (s, 2.7, 1H, Diazepine ring).

   \(\text{IR (KBr): }\) NH (Ar, 3030 cm\(^{-1}\), str), CH (Ar, 3180 cm\(^{-1}\), str), CH (Ar, 800 cm\(^{-1}\), bend), C=N (1618 cm\(^{-1}\), Str), CH\(_2\) (1490 cm\(^{-1}\), str), C-C (Ar, 1600 cm\(^{-1}\), C=C (Ar, 1410,1500,1580 cm\(^{-1}\)).

2. 1-Phenyl-3-(10-Spirocyclohexane-1,2,3,9,10,10a-hexahydrobenzo[b]cyclohexane [e][1,4]diazepine-1-yl) propan-1-one (NBZD-3):

   \(^1\text{H NMR (300 MHz, } \delta)\) : CH (m, 7.3-7.9, 5H, Acetophenone), CH\(_2\) (s, 2.8, 2H, -COCH\(_2\)), CH\(_2\) (s, 3.5, 2H, -NHCH\(_2\)) (m, 6.6-7.1, 4H, phenyl), CH\(_2\) (m, 1.2-1.5, 18H, Cyclohexane), CH (s, 2.5, 1H, Diazepine ring).

   \(\text{IR (KBr): }\) C=O (1700 cm\(^{-1}\), str), CH (Ar, 3180 cm\(^{-1}\), str), CH (Ar, 810 cm\(^{-1}\), bend), C=N (1618 cm\(^{-1}\), Str), CH\(_2\) (1490 cm\(^{-1}\), str), C-C (Ar, 1600 cm\(^{-1}\), C=C (Ar, 1410,1500,1580 cm\(^{-1}\)).

3. 1-(4-chlorophenyl)-3-(10-Spirocyclohexane-1,2,3,9,10,10a-hexahydrobenzo[b]cyclohexane[e][1,4]diazepine-1-yl) propan-1-one (NBZD-6):

   \(^1\text{H NMR (300 MHz, } \delta)\) : CH (m, 7.3-7.4, 5H, Acetophenone), CH (m, 7.2-7.3, 4H, p-chloroaniline), CH\(_2\) (s, 1.6, 2H, -COCH\(_2\)), CH\(_2\) (s, 3.4, 2H, -NHCH\(_2\)) (m, 6.6-7.1, 4H, phenyl), CH\(_2\) (m, 1.3-1.6, 18H, Cyclohexane), CH (s, 2.4, 1H, Diazepine ring).

   \(\text{IR (KBr): }\) C=N (1569 cm\(^{-1}\), str), C-Cl (727 cm\(^{-1}\), str), C-H (2975 cm\(^{-1}\), str assim), CH (1535.9 cm\(^{-1}\), def sym.), C-H (Ar, 3062 cm\(^{-1}\), str), CH (Ar, 3180 cm\(^{-1}\), str), CH (Ar, 800 cm\(^{-1}\), bend), CH\(_2\) (1490 cm\(^{-1}\), str), C-C (Ar, 1600 cm\(^{-1}\), C=C (Ar, 1410,1500,1580 cm\(^{-1}\)).

4. (4-Chloro-phenyl)-[1-Nitrophenyl-3-10-Spirocyclohexane-1,2,3,9,10,10a-hexahydro benzo[b]cyclohexane[e][1,4]diazepine-1-yl]-propylidene-amine (NBZD-8):

   \(^1\text{H NMR (300 MHz, } \delta)\) : CH (m, 7.30-7.6, 4H, p-chloroacetophenone), CH (m, 7.2-7.3, 4H, p-chloroaniline), CH\(_2\) (s, 1.6, 2H, -COCH\(_2\)), CH\(_2\) (s, 3.4, 2H, -NHCH\(_2\)) (m, 6.6-7.1, 4H, phenyl), CH\(_2\) (m, 1.3-1.6, 18H, Cyclohexane), CH (s, 2.7, 1H, Diazepine ring).

   \(\text{IR (KBr): }\) C=N (1569 cm\(^{-1}\), str), C-Cl (727 cm\(^{-1}\), str), C-H (2975 cm\(^{-1}\), str assim), CH (1535.9 cm\(^{-1}\), def sym.), C-H (Ar, 3062 cm\(^{-1}\), str), CH (Ar, 3180 cm\(^{-1}\), str), CH (Ar, 800 cm\(^{-1}\), bend), CH\(_2\) (1490 cm\(^{-1}\), str), C-C (Ar, 1600 cm\(^{-1}\), C=C (Ar, 1410,1500,1580 cm\(^{-1}\)).

5. (4-Chlorophenylhyrazinecarboxamide)[1-Nitrophenyl-3-(10-Spirocyclohexane-1,2,3,9,10,10a-hexahydro benzo [b] cyclohexane [e][1,4] diazepine-1-yl)-propylidene]-amine (NBZD-11):

   \(^1\text{H NMR (300 MHz, } \delta)\) : CH (m, 7.6-7.6, 4H, p-chloroacetophenone), NH (s, 9.0,1H, =NNH, p-chlorophenyl semicarbazide), NH (s, 6.0, 1H, -NH\(_4\)Cl, p-chlorophenyl semicarbazide), CH (m, 7.2-7.6,4H, p-chlorophenylsemicarbazide), CH (m, 6.6-7.1, 4H, phenyl), CH\(_2\) (m, 1.3-1.6, 18H, Cyclohexane), CH (s, 1.7, 1H, Diazepine ring), CH\(_2\) (s, 1.6, 2H, -COCH\(_2\)), CH\(_2\) (s, 3.4, 2H, -NHCH\(_2\)).
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IR (KBr): C=N (1559 cm\(^{-1}\), str), C-Cl (787 cm\(^{-1}\), str), C-Cl (769 cm\(^{-1}\)), C-H (2975 cm\(^{-1}\), str assym), CH (Ar, 3062 cm\(^{-1}\), str), CH (Ar, 3180 cm\(^{-1}\), str), CH (Ar, 880 cm\(^{-1}\), bend), C=N (1638 cm\(^{-1}\), str) CH\(_2\) (1490 cm\(^{-1}\), str), C-C (Ar, 1600 cm\(^{-1}\)), C=C (Ar, 1410, 1500, 1580 cm\(^{-1}\)).

From the present research we reached to this conclusion that various Mannich and Schiff base derivatives of 1,5-benzodiazepines were found to possess Analgesic (Central and Peripheral). The synthesized derivatives of Benzodiazepine ring were screened for their analgesic activity using Writhing test. The compounds NBZD-1, NBZD-3, NBZD-6, NBZD-10, were found to be good analgesic when compared with reference drug i.e., Diclofenac using peripheral analgesic assay. The compound NBZD-3 was found to be most active among all the screened compounds using acetic acid induced writhing test. The evaluation of all the synthesized derivatives against hot-plate test revealed that the compounds NBZD-6, NBZD-8, NBZD-9, were active as central analgesics & compound NBZD-9 was the most active among all the derivatives tested for the central analgesic activity. Hence this may lead for the future research to synthesize newer Benzodiazepine derivatives through substitution by different groups.

**STRUCTURE ACTIVITY RELATIONSHIP**

Centrally Analgesic active compounds:

I. Highly Active: NBZD-6, 3, 9.
II. Moderately Active: NBZD-8.
III. Less Active: NBZD-1, 4, 5, 7, 10, 11.

Peripheral Analgesic active compounds:

I. Highly Active: NBZD-1, 3, 6, 10.
II. Moderately Active: NBZD-4, 5, 8.
III. Less Active: NBZD-7, 9, 11.

1. The active compound such as NBZD-9, 10, 13, 14, 20 revealed that chlorosubstituted aryl rings alongside Benzodiazepine nucleus possess potent analgesic activity.
2. Mannich base derivatives of 1,5- Benzodiazepines with acetophenone were found to be most active among all the synthesized Mannich base.
3. Schiff bases with p-chloroaniline and p-chlorophenylsemicarbazide were found to be potent analgesics.
4. On the basis of log P value, compounds with more LogP value were found to be more active. E.g, NBZD-6, 8, 9, 10 (Log P value 9.56, 9.49, 8.07, 8.93 respectively).
5. The synthesized derivatives having a p-chloro substituted heteroaryl ring at their one end were also having the resemblance with heteroaryl acetic acid such as Diclofenac containing a heteroaryl ring with chloro substitution at ortho position of the ring. The results showed that NBZD-6, 3 are active compounds against both Peripheral & Central analgesic assays.
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REFERENCES