Synthesis, spectral studies and antimicrobial activity of 7-chloro-2-alkyl/aryl-4-alkyl/aryl-3-arylidene-3H,1,5-benzodiazepines

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Condensation of various β-diketones 2 with appropriate araldehydes 1 in the presence of piperidine has afforded 2-arylidene-1,3-diketones 3 which in turn on condensation with 4-chloro-1,2-phenylene diamine in acidic medium give a series of 7-chloro-2-alkyl/aryl-4-alkyl/aryl-3-arylidene-3H,1,5-benzodiazepines 5a-j. All the newly synthesized compounds are characterized on the basis of their spectral (IR, 1H NMR, Mass) and analytical data. Representative compounds have been screened for their antibacterial and antifungal activities. Some of the compounds have been found to exhibit promising antibacterial and antifungal activities.

Keywords: Antimicrobial, benzodiazepines, araldehydes, piperidine, β-diketones

Since the discovery of flurazepam, flunitrazepam, quazepam, halazepam and triflubazam, the chemistry of benzodiazepines and allied compounds continues to draw attention of synthetic organic chemists due to their varied biological activities1,2. A large number of benzodiazepine derivatives have been synthesized as sedative3, tranquillizer3, anticonvulsant1, antidepressant1, anxiolytic4,5 and antihypertensive6,7 drugs. Some benzodiazepines and allied derivatives are known to exhibit muscle relaxant3, anticoagulant8, antiobesity9, antiulcer10, calcium channel blocking11, cholecystokinin antagonists12, thrombopoietin receptor agonist13, endothelin antagonist14, vasopressin receptor antagonist15,16 activity.

From a structure activity standpoint2, generally electron withdrawing substituents at the 7-position of benzodiazepine moiety impart a high degree of activity and a rough rank order is: NO2 ≈ CF3 > Br > Cl ≥ CH3 ≥ F > H.

It has been observed that introduction of Cl at 7-position of benzodiazepine moiety enhances the CNS activity greatly, for example, chlorodiazepoxide, oxazepam, quazepam and fletazepam are well known CNS drugs. Keeping these observations a comprehensive programme of developing some new halogenated benzodiazepine and allied derivatives have been undertaken and as a part of this the synthesis and antimicrobial activity of some 7-fluoroaryl-1H-1,4-diazepine17, 3-arylazo-1H-1,5-benzodiazepine18 and 6-arylazo-2,3-dihydro-1H-1,4-diazepines19 have been already reported.

Now in the present communication the synthesis, spectral studies and antimicrobial activity of 7-chloro-2-alkyl/aryl-4-alkyl/aryl-3-arylidene-3H,1,5-benzodiazepines have been reported.

Results and Discussion

β-Diketones 2 were prepared by condensation of different substituted acetophenones with appropriate ester according to the method of Joshi et al20. β-Diketones 2 were treated with appropriate araldehydes 1 in the presence of piperidine to give 2-arylidene-1,3-diketones 3. Condensation of 2-arylidene-1,3-diketones 3 with 4-chloro-1,2-phenylene diamine in the presence of gl. acetic acid afforded 7-chloro-2-alkyl/aryl-4-alkyl/aryl-3-arylidene-3H,1,5-benzodiazepines 5a-j. The reaction sequence is depicted in Scheme I.

The synthesized compounds were characterized by IR, 1H NMR and mass spectral data. The IR spectra of 3a-j showed absorption bands in the region of 1680-1700 (>C=O str.) and 1640-1655 cm⁻¹ (-CH=C< str.). The IR spectra of 5a-j exhibited absorption band in the region of 1589-1600 cm⁻¹ (>C=N str.). The 1H NMR (CDCl₃) spectra of compounds 5a-j exhibited resonance signal at δ 6.68-8.24 ppm as multiplet for the aromatic protons and at 3.60-4.30 ppm as singlet for the =CH proton. Compounds 5a-e showed singlet at δ 2.35-2.83 ppm for the methyl protons. Compound 5d exhibited a resonance signal at δ 3.89 for the methoxy and =CH proton. Finally, the structure of compounds was confirmed by high resolution mass spectra. Mass spectra of compounds 5a-j showed molecular ion peak M⁺.

The mass fragmentation pathways of compound 5j are given in Scheme II. Compound 5j exhibited a molecular ion peak (M⁺) 1 at m/z 392.8 corresponding to the molecular formula C₂₃H₁₅ClF₂N₂ and M⁺+1 peak
at m/z 393.8 due to isotopic carbon and hydrogen atoms. The molecular ion I further fragmented by two pathways A and B. In pathway-A, molecular ion I eliminated HF molecule to afford cation radical II at m/z 372.8 (8.0%). The successive elimination of HF molecule and hydrogen radical from cation radical II gave a cation III at m/z 351.8 (9.4%) which in turn eliminated phenyl radical to afford cation radical IV at m/z 274.7 (6.5%). Cation radical IV extruded CH₂ moiety followed by scrambling of hydrogen to give cation radical V at m/z 260.6 (18.9%). In pathway-B, molecular ion I eliminated C₂HF₂ radical to give cation VI at m/z 329.7 (20.2%). The loss of C₃H radical from cation VI afforded cation radical VII at m/z 292.7 (100.0%) corresponding to the base peak, which was stabilized by resonance. The elimination of methyl radical from cation VI gave cation radical VIII at m/z 314.7 (5.0%) which in turn lost a C₃H₂ moiety to give cation radical IX at m/z 228.6 (66.1%). Cation radical IX eliminated C₃H₂ moiety to give cation X at m/z 166.6 (71.5%).

Antimicrobial activity

Representative compounds were screened for their antibacterial activity against gram-negative bacteria Escherichia coli and gram-positive bacteria Staphylococcus aureus at 200, 400 and 800 ppm concentration. Antifungal activity was done against Candida albicans and Aspergillus niger at 200, 400 and 800 ppm concentration. Streptomycin and Ketoconazole were used as standard drugs for antibacterial and antifungal evaluations, respectively. The compounds were screened for their biological activity by using inhibition zone technique. The results obtained for antibacterial and antifungal evaluations are recorded in Table I and Table II, respectively. The perusal of our results revealed that compounds possessing o-methoxy and m-fluoro substituents in the arylidene moiety of the synthesized compounds 5c, 5e, p-chloro, p-fluoro substituent in the arylidene moiety in combination with 4-chlorophenyl ring at 2-position of the synthesized compounds 5f, 5g and p-fluoro, 3,4-difluoro substituents in the arylidene moiety in combination with phenyl ring at 2-position of the synthesized compounds 5i, 5j showed higher degree of activity as compared to rest of the compounds against S.aureus at 200,400 and 800 ppm concentration.

The compounds possessing o-methoxy and p-fluoro substituents in the arylidene moiety of the synthesized compounds 5c, 5i and p-chloro, p-fluoro substituent in the arylidene moiety in combination with p-chlorophenyl ring at 2-position of the synthesized...
Scheme II — Mass fragmentation pattern of 7-chloro-2-phenyl-4-methyl-3-(3,4-difluorobenzylidene)-3H-1,5-benzodiazepine, 5j
compound 5f, 5g, 5h showed higher degree of activity as compared to rest of the compounds against E. coli at 200, 400 and 800 ppm concentration. Compound possessing m-fluoro substituent in the arylidene moiety of the synthesized compound 5e showed higher degree of activity as compared to rest of the compounds against C. albicans and the compounds possessing o-methoxy and 3,4-difluoro substituents in the arylidene moiety showed higher degree of activity against A. niger at 200, 400 and 800 ppm concentration.

**Experimental Section**
Melting points were determined in open glass capillary tubes and are uncorrected. The IR spectra (cm\(^{-1}\)) were recorded on a Perkin-Elmer 557 grating infrared spectrophotometer in KBr pellets; \(^1\)H NMR spectra in CDCl\(_3\) (chemical shift in \(\delta\), ppm) on a Bruker spectrometer (200 MHz) using TMS as internal standard and mass spectra on an Esquire 3000 Bruker make spectrometer. The purity of the synthesized compounds was checked by TLC on silica gel G in open glass capillary tubes and are uncorrected.

**Table I** — Antibacterial activity data of compounds 5a-j

<table>
<thead>
<tr>
<th>Compd</th>
<th>Mean value of area of inhibition in mm (800 ppm)</th>
<th>Mean value of area of inhibition in mm (400 ppm)</th>
<th>Mean value of area of inhibition in mm (200 ppm)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IZ (AI)</td>
<td>IZ (AI)</td>
<td>IZ (AI)</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>12.5</td>
<td>12.0</td>
<td>10.3</td>
</tr>
<tr>
<td>5a</td>
<td>8.0 (0.64)</td>
<td>9.5 (0.79)</td>
<td>6.0 (0.58)</td>
</tr>
<tr>
<td>5b</td>
<td>7.5 (0.60)</td>
<td>10.0 (0.83)</td>
<td>5.2 (0.50)</td>
</tr>
<tr>
<td>5c</td>
<td>15.0 (1.20)</td>
<td>12.5 (1.04)</td>
<td>12.0 (1.16)</td>
</tr>
<tr>
<td>5d</td>
<td>8.0 (0.64)</td>
<td>12.0 (1.0)</td>
<td>5.0 (0.48)</td>
</tr>
<tr>
<td>5e</td>
<td>13.0 (1.04)</td>
<td>8.0 (0.67)</td>
<td>10.6 (1.03)</td>
</tr>
<tr>
<td>5f</td>
<td>13.5 (1.08)</td>
<td>13.0 (1.08)</td>
<td>10.9 (1.06)</td>
</tr>
<tr>
<td>5g</td>
<td>14.0 (1.12)</td>
<td>13.2 (1.10)</td>
<td>11.0 (1.07)</td>
</tr>
<tr>
<td>5h</td>
<td>12.5 (1.0)</td>
<td>13.0 (1.08)</td>
<td>10.0 (0.97)</td>
</tr>
<tr>
<td>5i</td>
<td>13.0 (1.04)</td>
<td>14.0 (1.16)</td>
<td>10.7 (1.04)</td>
</tr>
<tr>
<td>5j</td>
<td>13.5 (1.08)</td>
<td>12.0 (1.0)</td>
<td>11.0 (1.07)</td>
</tr>
</tbody>
</table>

IZ = Inhibition area (zone) excluding diameter of disc
AI (Activity Index) = Inhibition area of sample/inhibition area of standard

**Table II** — Antifungal activity data of compounds 5a-j

<table>
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<tr>
<th>Compd</th>
<th>Mean value of area of inhibition in mm (800 ppm)</th>
<th>Mean value of area of inhibition in mm (400 ppm)</th>
<th>Mean value of area of inhibition in mm (200 ppm)</th>
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<td></td>
<td>IZ (AI)</td>
<td>IZ (AI)</td>
<td>IZ (AI)</td>
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<tr>
<td>Ketoconazole</td>
<td>15</td>
<td>12</td>
<td>12.1</td>
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<tr>
<td>5a</td>
<td>12 (0.80)</td>
<td>9 (0.75)</td>
<td>9.2 (0.76)</td>
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<tr>
<td>5b</td>
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<td>09 (0.75)</td>
<td>10.2 (0.84)</td>
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<tr>
<td>5e</td>
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<td>8.0 (0.67)</td>
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<td>6.0 (0.40)</td>
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<td>4.1 (0.34)</td>
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<tr>
<td>5g</td>
<td>7.0 (0.46)</td>
<td>9.2 (0.76)</td>
<td>5.4 (0.45)</td>
</tr>
<tr>
<td>5h</td>
<td>8.2 (0.54)</td>
<td>7.3 (0.61)</td>
<td>6.4 (0.53)</td>
</tr>
<tr>
<td>5i</td>
<td>10.5 (0.70)</td>
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<td>6.8 (0.56)</td>
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<tr>
<td>5j</td>
<td>12.5 (0.83)</td>
<td>13.0 (1.08)</td>
<td>9.1 (0.75)</td>
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</table>

IZ = Inhibition area (zone) excluding diameter of disc
AI (Activity Index) = Inhibition area of sample/inhibition area of standard
various nonaqueous solvent systems. All the compounds gave satisfactory elemental analyses.

**Synthesis of 3-(4-chlorobenzylidene) pentane-2,4-dione 3a:** A homogeneous mixture of 4-chlorobenzaldehyde (2.81 g, 20 mmoles), acetylacetone (2.0 g, 20 mmoles) and piperidine (0.02 mL) in chloroform (20 mL) was stirred at room temperature for about 50 hr. The mixture was diluted with chloroform and the piperidine was removed by washing with dilute hydrochloric acid followed by water. The chloroform layer was dried over MgSO4. The solvent was removed and the solid residue was recrystallized from ethanol. Molecular formula C12H11ClO2, yield 62%, m.p. 170°C; IR (KBr): 3054 (aromatic C-H str.), 2828 (aliphatic C-H str.), 1690 (>C=C str.), 1270 (C-F str.), 727 (C-Cl str.).

**3-(2-Chlorobenzylidene) pentane-2,4-dione 3b:** Mol. formula: C12H11ClO2, yield 61%, m.p. 41°C; IR (KBr, cm−1): 3055 (aromatic C-H str.), 2835 (aliphatic C-H str.), 1692 (>C=O str.), 1640 (>C=C< str.); 1H NMR (CDCl3): δ 2.90 (s, 6H, CH3), 4.35 (s, 1H, CH), 7.21-7.40 (m, 4H, ArH). All other compounds 3b-j were prepared similarly.

**3-(2-Methoxybenzylidene) pentane-2,4-dione 3c:** Mol. formula: C12H11FO2, yield 59%, m.p. 100°C; IR (KBr, cm−1): 3056 (aromatic C-H str.), 2838 (aliphatic C-H str.), 1692 (>C=O str.), 1640 (>C=C< str.).

**3-(4-Chlorobenzylidene) pentane-2,4-dione 3d:** Mol. formula: C18H14Cl2O2, yield 60%, m.p. 90°C; IR (KBr, cm−1): 3055 (aromatic C-H str.), 2828 (aliphatic C-H str.), 1690 (>C=O str.), 1640 (>C=C< str.).

**3-(3-Fluorobenzylidene) pentane-2,4-dione 3e:** Mol. formula: C18H13FO2, yield 64%, m.p. 96°C, IR (KBr, cm−1): 3055 (aromatic C-H str.), 2830 (aliphatic C-H str.), 1686 (>C=O str.), 1640 (>C=C< str.), 1275 (C-F str.).

**3-(2-Methoxybenzylidene) propane-1,3-dione 3f:** Mol. formula: C12H14Cl2O2, yield 55%, m.p. 165°C; IR (KBr, cm−1): 3055 (aromatic C-H str.), 2828 (aliphatic C-H str.), 1685 (>C=O str.), 1640 (>C=C< str.), 727 (C-Cl str.).

**1-Phenyl-2-(4-fluorobenzylidene)butane-1,3-dione 3i:** Mol. formula: C17H12ClFO2, yield 64%, m.p. 96°C, IR (KBr, cm−1): 3055 (aromatic C-H str.), 2830 (aliphatic C-H str.), 1686 (>C=O str.), 1640 (>C=C< str.), 1275 (C-F str.).

**7-Chloro-2,4-dimethyl-3-(4-chlorobenzylidene)-3H-1,5-benzodiazepine 5a:** A mixture of 4-chloro-1,2-phenylene diamine (1.42 g, 10 mmoles), 3-(4-chlorobenzylidene) pentane-2,4-dione (2.22 g, 10 mmoles) and gl. acetic acid (1.0 mL) in ethanol (25 mL) was refluxed for 7 to 8 hr. The reaction mixture was cooled, hydrochloric acid (12N, 10 mL) was added to it and the whole mixture was kept overnight at 0°C. The crystals of benzodiazepinium hydrochloride were filtered off, washed successively with cold ethanol, ether and dried. The crystals were recrystallized from ethanol. Treatment of 50% aqueous ethanolic solution of this hydrochloride with ammonia solution afforded the benzodiazepine free base. Mol. Formula: C19H13ClN2. Yield 45%, m.p. 140°C; IR (KBr): 3050 (aromatic C-H str.), 2838 (aliphatic C-H str.), 1651 (C=C str.), 1592 (>C=N str.), 1492 (aromatic C=C str.), 762 cm⁻¹ (C-Cl str.).

**1-Phenyl-2-(3,4-difluorobenzylidene)butane-1,3-dione 3j:** Mol. formula: C17H12F2O2, yield 61%, m.p. 88°C, IR (KBr, cm−1): 3060 (aromatic C-H str.), 2830 (aliphatic C-H str.), 1685 (>C=O str.), 1650 (>C=C< str.), 1275 (C-F str.).
str.; Anal. Calcd for C_{19}H_{17}ClN_{2}O (324.5): C, 70.26; H, 5.24; N, 8.63. Found: C, 70.14; H, 5.23; N, 8.61%.

7-Chloro-2,4-dimethyl-3-(3,4,5-trimethoxybenzylidene)-3H-1,5-benzodiazepine 5d: Yield 49%, m.p. 160°C; IR (KBr): 3050 (aromatic C-H str.), 2849 (aliphatic C-H str.), 1639 (C=C str.), 1590 (>C=N str.), 1238 (C-O str.), 725 cm\(^{-1}\) (C-Cl str.); \(^1\)H NMR (CDCl\(_3\)): 6.68-8.09 (m, 16H, ArH); Mass (m/z) (%): M\(^+\) 384.8 (15), 351.8 (9.5), 318.5 (34.5), 288.5 (20.2), 257.6 (13.1), 230.4 (100), 195.5 (45.2), 180.5 (19.0), 151.6 (7.1), 63.4 (2.3); Anal. Calcd for C\(_{23}\)H\(_{15}\)ClF\(_2\)N\(_2\) (392.5): C, 70.32; H, 3.82; N, 7.13.

7-Chloro-2,4-dimethyl-3-(3-fluorobenzylidene)-3H-1,5-benzodiazepine 5e: Yield 46%, m.p. 155°C; IR (KBr): 3055 (aromatic C-H str.), 2829 (aliphatic C-H str.), 1638 (C=C str.), 1597 (>C=N str.), 1490 (aromatic C=C str.), 1270 (C-F str.), 757 cm\(^{-1}\) (C-Cl str.); \(^1\)H NMR (CDCl\(_3\)): 6.70-8.06 (m, 16H, ArH); Mass (m/z) (%): M\(^+\) 318.3 (15), 307.5 (63.4), 288.4 (28.3), 230.5 (82.3), 200.5 (100), 166.4 (21.6), 134.5 (24.3), 107.5 (4.0); Anal. Calcd for C\(_{19}\)H\(_{14}\)ClF\(_2\)N\(_2\) (338.6): C, 67.54; H, 4.48; N, 8.96. Found: C, 67.01; H, 4.49; N, 8.94%.

7-Chloro-2-(4-chlorophenyl)-4-phenyl-3-(4-fluorobenzylidene)-3H-1,5-benzodiazepine 5f: Yield 50%, m.p. 150°C (d); IR (KBr, cm\(^{-1}\)): 3061 (aromatic C-H str.), 2830 (aliphatic C-H str.), 1657 (C=C str.), 1600 (>C=N str.), 1448 (aromatic C=C str.), 1275 (C-F str.), 765 (C-Cl str.); \(^1\)H NMR (CDCl\(_3\)): 6.81-8.03 (m, 11H, ArH); Mass (m/z) (%): M\(^+\) 315.6 (21%), 311.6 (8.0%), 299.6 (9.4), 261.6 (18.9), 228.6 (66.1), 166.4 (71.5); Anal. Calcd for C\(_{23}\)H\(_{15}\)ClF\(_2\)N\(_2\) (392.5): C, 70.21; H, 3.82; N, 7.11%.

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References


