Central Depressant Effects of N3-Substituted 6-Azaauridines in Mice

Makoto Koshigami, Kazuhiro Watanabe, Toshiyuki Kimura and Ikuo Yamamoto*

Faculty of Pharmaceutical Sciences, Hokuriku University, 3-51 Kanazawa-machi, Kanazawa 920-11, Japan. Received April 17, 1991

Central depressant effects in mice of N3-substituted 6-azauridines (6-Azud) (1) were examined by intracerebroventricular (i.c.v.) injection. Eleven derivatives including alkyl-, benzyl-, xylyl- and phenylthio-substitution onto the N3-position of 1 were synthesized and their pharmacological effects were evaluated using hypnic activity, locomotor activity, motor incoordination and pentobarbital-induced sleep prolongation as indexes. Six of 12 compounds showed the hypnotic activity. At a dose of 2 μmol/mouse, the mean sleeping time induced by 1, N3-benzyl-6-Azud (7), N3-o-xylyl-6-Azud (8), N3-m-xylyl-6-Azud (9), N3-p-xylyl-6-Azud (10) and N3-2-phenylthio-6-Azud (11) was 14, 11, 45, 12 and 16 min, respectively. These derivatives and N3-β-phenylthio-6-Azud (12) (1.5 μmol/mouse) significantly prolonged pentobarbital-induced (40 mg/kg, i.p.) sleeping time, whereas none of the N3-alkylated derivatives (methyl-, ethyl-, n-propyl-, n-butyl- and allyl-substitution) exerted the hypnotic activity or pentobarbital-induced sleep prolongation. Nucleoside 1 and its xylyl-derivatives (1.5 μmol/mouse) significantly decreased locomotor activity of mice, their effects paralleled the hypnotic activity. These compounds (1.5 μmol/mouse) also produced motor incoordination and potentiated the effect of diazepam-induced motor incoordination. These results indicate that 1 and its benzyl-related derivatives, but not alkyl-derivatives have a depressant effect on the central nervous system.

Keywords 6-azauridine; alkyl-derivatives; benzyl-derivatives; hypnotic activity; locomotor activity; motor incoordination; sleep prolongation; N3-substituted nucleoside; xylyl-derivatives

Uridine has been identified as one of the sleep-promoting substances isolated from the brainstem of sleep-deprived rats. In connection with this finding, our previous studies have shown that N3-substituted derivatives of uridine and thymidine exert central depressant effects involving hypnotic activity. Studies suggested the importance of a sugar moiety as well as the substitution of benzyl-related groups on the N3-position of uracil or thymine to exhibit the hypnotic activity. 6-Azaauridine (6-Azud) (1), a pyrimidine nucleoside, was reported to have antitumor activity and cytotoxicity. Welch et al reported that 6-azaaracil, a pyrimidine base, showed hypnotic activity at a relatively higher dose of 2.0 to 3.0 g/kg i.p. In connection with our recent study on the central depressant effects of N3-substituted nucleosides, the present paper describes pharmacological effects of 6-Azud derivatives in mice.

Experimental

Animals Male ddN mice weighing 22 to 28 g were used throughout the experiments. Mice were kept in an air-conditioned room (24 ± 2°C) with controlled lighting (8:00 to 20:00 light period). They were given food and water ad libitum.

Chemicals Sodium pentobarbital and halogenated alkyls were purchased from Tokyo Kasei Kogyo Co., Ltd; 1 from Aldrich Chemical Co.; diazepam from Yamamoto Chemicals, Ltd.

Syntheses of N3-Substituted 1 N3-Substituted 6-Azuds were prepared by the methods described previously. Briefly, 1 dissolved in dimethyl sulfoxide (DMSO) and acetone was reacted with halogenated alkyls in the presence of a base (NaOH or Na2CO3). The product was purified by column chromatography with a solvent system of chloroform-ethyl acetate-methanol (5:5:1).

Analytical data of the derivatives prepared were as follows: N3-Methyl-6-Azud (2): Yield 52%, mp 127–128°C. 1H-NMR (DMSO-d6): δ 2.50 (3H, s, N-CH3), 3.26–4.37 (2H, m, 5’-CH2), 4.52–5.37 (7H, m, 2’, 3’, and 4’-H, 5’-CH-N-CH3), 5.96 (1H, d, J = 2 HZ, 1’H), 7.72 (1H, s, 5-H). MS m/z: 260 (M+1)
N3-Ethyl-6-Azud (3): Yield 63%, oil. 1H-NMR (CDCl3-d6): δ 1.23 (3H, t, CH3), 3.47–4.80 (7H, 3H, d, J = 2 Hz, 1’H), 7.62 (1H, s, 5-H). MS m/z: 274 (M+1)
N3-Propyl-6-Azud (4): Yield 64%, oil. 1H-NMR (CDCl3-d6): δ 0.95 (3H, t, CH3), 1.26–2.04 (2H, m, 6’-CH2), 3.20–4.73 (7H, s, m and 2’, 3’, and 4’-H, 5’-CH-N-CH3), 6.20 (1H, d, J = 2 Hz, 1’H). MS m/z: 348 (M+1)

© 1991 Pharmaceutical Society of Japan
the F-test. The data for motor incoordination were analyzed by the χ²-test.

Results and Discussion

Effects of I and its derivatives on pentobarbital-induced sleep are described in Table I. As shown, none of the N²-alkylated derivatives examined (1.5 μmol/mouse, i.e.v.) significantly prolonged pentobarbital-induced sleeping time, while nucleoside I and its benzyl-related derivatives (benzyl-, xylyl- and phenethyl-derivatives) significantly prolonged the sleeping time at the same dose. The prolongation effect was in the following order in potency: I (10% of control, 248), 8 (225), 12 (219), 7 (188), 9 (181), 1 (169) and 10 (165). These results showed the same tendency as the previous finding in N²-substituted derivatives of uridine, the only difference being that I itself showed a significant prolongation on pentobarbital-induced sleep while uridine exerted none. The result indicates that I has some depressant effect on the central nervous system. Table I also summarizes the hypnotic activity of I and its derivatives. Six of the 12 compounds examined exhibited the hypnotic activity; among them, that of 8 was the highest. Nucleoside I and other derivatives, 7, 9, 10 and 11 showed almost the same potency. At a dose of 2.0 μmol/mouse, the mean sleeping time was 9 to 16 min. This is the first case nucleoside itself being reported a hypnotic. Welch et al. reported that 6-azauracil showed hypnotic activity in mice and rats, while I did not produce any hypnotic in mice. The activity of 8 is comparable to that of N²-benzyluridine.

Table II summarizes the effects of I and its xylyl-derivatives on locomotor activity of mice. At a dose of 1.5 μmol/mouse, 1, 8, 9 and 10 reduced activity by 40, 3, 19 and 34%, respectively. The result demonstrates that I could decrease the locomotor activity of mice, and that xylyl-derivatives have a stronger effect than the parent nucleoside, as has been reported for thymidine.

Motor incoordination induced by I and its benzyl and xylyl derivatives is shown in Fig. 1. Nucleoside I induced motor incoordination for 40 min after administration (1.5 μmol/mouse). The effect of 9 and 10 was almost the same as that of I. Compound 8, which exhibited the highest activity in hypnosis and pentobarbital-induced sleep

### Table I. Central Depressant Effects of 6-AzUd and Its Derivatives

| R          | 1min | 2min | 3min | Pentobarbital-induced sleep prolongation
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>H</td>
<td>6±2</td>
<td>14±5</td>
<td>−</td>
<td>169±21 (a)</td>
</tr>
<tr>
<td>CH₃</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>83±4</td>
</tr>
<tr>
<td>C₆H₅</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>111±5</td>
</tr>
<tr>
<td>H₂C₆H₅</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>96±12</td>
</tr>
<tr>
<td>N-C₆H₅H₅</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>108±8</td>
</tr>
<tr>
<td>CH₃CH=CH₂</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>120±9</td>
</tr>
<tr>
<td>CH₃CH=CH₂</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>188±21</td>
</tr>
<tr>
<td>CH₃CH=CH₂</td>
<td>2±1</td>
<td>9±1</td>
<td>12±2</td>
<td>165±8</td>
</tr>
<tr>
<td>CH₃CH=CH₂</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>248±31</td>
</tr>
<tr>
<td>CH₃CH=CH₂</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>219±23</td>
</tr>
</tbody>
</table>

(a) % of control. The mean sleeping time of control mice was 30±2 min. b) Dose (μmol/mouse, i.e.v.) Data are expressed as the mean±S.E. from 6 to 10 mice. c) and d) indicate significant difference from the control value with p<0.05 and p<0.01, respectively.

### Table II. Effects of 6-AzUd and Its Xylyl Derivatives on Locomotor Activity

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Dose (μmol/mouse)</th>
<th>Total distance (inches)</th>
<th>% of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3% Tween 80-saline</td>
<td>1634±245</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>1.5</td>
<td>651±260</td>
<td>40</td>
</tr>
<tr>
<td>8</td>
<td>1.5</td>
<td>44±23</td>
<td>3</td>
</tr>
<tr>
<td>9</td>
<td>1.5</td>
<td>306±144</td>
<td>19</td>
</tr>
<tr>
<td>10</td>
<td>1.5</td>
<td>556±300</td>
<td>34</td>
</tr>
</tbody>
</table>

Data are expressed as the mean±S.E. of 7 to 9 mice. Compounds tested were injected i.e.v. a) and b) indicate significant difference from the control value with p<0.05 and p<0.01, respectively.

![Fig. 1. Motor Incoordination Induced by 6-AzUd and Its Xylyl Derivatives](image)

Each point indicates % of mice (N=6) that fell from the bar within 30s. Each compound tested was injected at a dose of 1.5 μmol/mouse i.e.v. 1-4, control (3% Tween 80-saline); 6-12, 6-AzUd; 6-8, N²-xylyl-6-AzUd; 6-9, N²-xylyl-6-AzUd; 6-10, N²-xylyl-6-AzUd; 6-11, N²-p-xylyl-6-AzUd.

![Fig. 2. Effects of 6-AzUd and Its Xylyl-Derivatives on Diazepam-Induced Motor Incoordination](image)

Each point indicates % of mice (N=12) that fell from the bar within 30s. Each compound tested was injected at a dose of 1.5 μmol/mouse i.e.v. Diazepam (15 mg/kg, i.p.) was administered 15 min after the injection of the test compounds. 1-4, control (3% Tween 80-saline); 5-6, diazepam; 6-6, 6-AzUd + diazepam; 6-8, N²-xylyl-6-AzUd + diazepam; 6-9, N²-xylyl-6-AzUd + diazepam; 6-10, N²-p-xylyl-6-AzUd + diazepam. a) Significantly different from diazepam group (p<0.05). b) Significantly different from diazepam group (p<0.01).
prolongation, also showed the strongest effect in that it took 180 min to recover from motor incoordination. The effects of these compounds were additive to that of diazepam as shown in Fig. 2. The potentiating effect of the compounds tested on diazepam-induced motor incoordination was in the following order: 8, 9, 10 and 1.

We have shown that N3-benzyluridine inhibited the binding of a benzodiazepine agonist, flunitrazepam to the benzodiazepine receptor.13) The potentiation by N3-substituted 1 on the effect of diazepam suggests that these derivatives may exert their effects through the benzodiazepine receptor. In conclusion, the present study strongly supports our previous findings2-4) that N3-substituted nucleosides possess hypnotic and sedative activities, and that the introduction of benzyl-related groups onto the N3-position is an important factor in exhibiting the central depressant effects of nucleoside derivatives.

References