Synthesis and Hypnotic Activities of 4-Thio Analogues of $N^3$-Substituted Uridines

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Reaction of tri-O-acetyluridine (1) with benzyl bromide or 2-chloroacetoephene in the presence of K$_2$CO$_3$ gave the $N^3$-substituted analogues 2a,2c. Condensation of 1 with ($\pm$)-1-phenylethanol or 3,5-dimethylbenzyl alcohol using the Mitsunobu reaction also gave 2b, d in good yields. These compounds were allowed to react with Lawesson’s reagent and were subsequently treated with ammonia to afford the 4-thiouracil derivatives 5a–d. Compounds 5a–c showed moderate hypnotic activity in mice. However, $N^3$-(3,5-dimethyl)benzyl derivatives 3d, 5d were found to be almost inactive in this assay.

Key words 4-thiouridine; hypnotic activity; central nervous system depressant; Mitsunobu reaction

It has been reported that uridine shows depressant effects toward the central nervous system (CNS). For instance, uridine decreased spontaneous activity in mice and showed protection against seizures caused by penicillin or metrazol. In addition, uridine was extracted from the brainstems of 24 h sleep-deprived rats and caused natural sleep following nocturnal infusion into the rat brain.6) However, uridine itself does not possess any hypnotic activity, as determined by loss of the righting reflex in experimental animals. In 1985, it was demonstrated that $N^3$-benzyluridine exerts hypnotic activity on mice by intracerebroventricular (i.c.v.) administration, and the structure–activity relationship has been further explored.7–9) It became clear that the introduction of methyl-, benzyl, as well as a benzyl group, at $N^3$ of uridine is effective in producing CNS depressant effects.7,8) To modify the sugar showed a diminished effect,7–9) except for $N^3$-substituted thymidine10) and arabinofuranosyluracil.11) It has been proven that $N^3$-phenacyluracil strongly intensifies the sleep induced by pentobarbital (PB) or diazepam.8a,8b) Although $N^1$-allyl-5,6-substituted 2-thiouracil derivatives have been synthesized and evaluated in terms of sedative-hypnotic activity,9) no report concerning $N^3$-substituted 4-thiouridines has appeared. Barbiturates have been in use as a sedative hypnotic since 1903, and 2-thiobarbituric acids (2-thioxohexahydropyrimidine-4,6-dione) continue to be used as short-acting barbiturates. This background prompted us to explore the 4-thio analogues of $N^3$-substituted uridine. In this paper, we wish to report the synthesis and CNS-depressant effect of $N^3$-substituted 4-thiouridines (5a–d).

**Synthesis** Tri-O-acetyl derivatives of $N^3$-benzyluridine (2a) and $N^3$-phenacyluridine (2c) were prepared by the nucleophilic displacement of 2',3',5'-tri-O-acetyluridine (1) with benzyl bromide or 2-chloroacetoephene in the presence of K$_2$CO$_3$ in N,N-dimethylformamide (DMF), according to the published method.7a,7b,8a) In the case of 2d, condensation of 1 with 3,5-dimethylbenzyl alcohol using diisopropyl azodicarboxylate and tributylphosphine was employed since aralkyl halide was not commercially obtainable. Also, ($\pm$)-1-phenylethanol was similarly reacted with 1 to give a 1-phenylethyl derivative (2b) as a mixture of diastereomers. Compound 2d was deprotected by treatment with ammonia in MeOH to give 3d. Next, the synthesis of $N^3$-substituted 4-thiouridines was explored as follows. Methods concerning the thiation of uracil or uridine have been universally reported. However, the introduction of an alkyl group on $N^3$ of 4-thiouridine has been difficult because 4-alkylthio derivatives were inevitably formed.11) Recently, 3-alkyluracils were successfully converted to the 4-thio congener by Lawesson’s reagent.12) We adopted this method for the synthesis of compounds 5a–d. Thus, compound 2a was refluxed with Lawesson’s reagent in benzene to give 4a in 52% yield, and subsequent treatment of the product with ammonia in MeOH gave $N^3$-benzyl-4-thiouridine 5a, which showed an absorption maximum at 330 nm on UV.13) In a similar manner, compounds 5b–d were prepared from the corresponding $N^3$-substituted tri-O-acetyluridine (2b–d). To confirm the site of alkylation, the most biologically active compound, 5c, was subjected to heteronuclear multiple bond connectivity (HMBC) study. As shown in Fig. 1, this compound was demonstrated to be the 3-phenacyl analogue, since correlation between the methylene protons of the phenacyl group and C(4) as well as C(2) was observed.

**Pharmacological Results**

The hypnotic activities of the $N^3$-substituted 4-thiouridines were assayed according to previously established procedures,7f and the results are presented in Table 1 and Fig. 2. By comparison with the earlier result of $N^3$-benzyluridine (3a, 36±2 min),7f 4-thio congener (5a) exhibited better hypnotic activity (84±10 min) when administered to mice by i.c.v. injection at 2.0 μmol/mouse. This is the first demonstration that $N^3$-substituted 4-thiouridine has CNS-depressant activity. In contrast, 5a reduced a prolongation of PB-induced sleep by 24% compared with 3a. In the series of $N^3$-(1-phenylethyl) derivatives, it was reported that a uracil congener (3b) caused only 7 min sleep at the same dosage.7f However, the 4-thiouridine derivative (5b) showed moderate hypnotic activity (58±15 min). The PB-induced sleep-promoting activity of 5b was similar to that of 3b.7f These results indicate that $N^3$-benzyl (5a) or the $N^3$-(1-phenylethyl) derivative (5b) of 4-thiouracil show enhanced hypnotic activity and decreased PB-induced sleep-promoting activity compared to the corresponding uracil congener (3a, b). Although 5c showed the most potent hypnotic activity (96±19 min)
among the 4-thio series, its activity was almost half that of \(3c\) (184 ± 13 min). It is indicated that the oxygen of the 4 position of the uracil ring is important in the hypnotic activity of \(3c\). However, \(5c\) caused a prolongation of PB-induced sleep and its activity was more than 30% stronger compared with the reported data of compounds \(3a\), \(3b\) and \(3c\) is 292, 231 and 230%, respectively. Since \(N^3\)-benzyluridines bearing a methyl group onto the benzene ring, i.e. \(o\)-, \(m\)- and \(p\)-methylbenzyl derivatives, have been proved to be better CNS-depressants than \(N^3\)-benzyluridine \((3a)^{7a,c}\) the activity of \(N^3\)-(3,5-dimethylbenzyl)uridine \((3d)\) and its 4-thio congener \((5d)\) was also examined. However, the hypnotic activity of \(3d\) and \(5d\) was poor, and \(3d\) showed the weakest PB-induced sleep-prolonging activity in this series.

In conclusion, \(N^3\)-benzyl- \((5a)\) or \(N^3\)-(1-phenylethyl)-4-thiouridine \((5b)\) enhanced hypnotic activity compared with the original compounds \(3a\) and \(5a\), respectively. However, the strong hypnotic activity of \(N^3\)-phenacyluridine \((3c)\) was reduced to half by 4-thio modification, as shown in Table 1. This tendency was reversed in the PB-induced sleep-prolonging activity. The PB-induced sleeping time of \(5a\) was shorter than that of \(3a\), and \(5c\) showed more prolongation than \(3c\).

<table>
<thead>
<tr>
<th>Compound (^{a)})</th>
<th>Hypnotic activity (^{b)}) (min)</th>
<th>n/n (^{c)})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>None</td>
<td>0/8</td>
</tr>
<tr>
<td>(3a^{(b)})</td>
<td>36 ± 2</td>
<td>6/8</td>
</tr>
<tr>
<td>(5a^{(b)})</td>
<td>84 ± 10</td>
<td>6/8</td>
</tr>
<tr>
<td>(3b^{(b)})</td>
<td>7</td>
<td>6/7</td>
</tr>
<tr>
<td>(5b)</td>
<td>58 ± 15</td>
<td>5/6</td>
</tr>
<tr>
<td>(3c)</td>
<td>184 ± 13</td>
<td>7/7</td>
</tr>
<tr>
<td>(5c)</td>
<td>96 ± 19</td>
<td>6/7</td>
</tr>
<tr>
<td>(3d)</td>
<td>None</td>
<td>0/8</td>
</tr>
<tr>
<td>(5d)</td>
<td>None</td>
<td>0/8</td>
</tr>
</tbody>
</table>

\(^{a)}\) Compounds were administered by i.c.v. injection at the dose of 2.0 \(\mu\)mol/mouse.  
\(^{b)}\) Results are expressed as the mean sleeping time (min) ± S.E.M. "None" indicates no hypnotic activity.  
\(^{c)}\) Ratio of animals which slept to animals tested.
Experimental

Melting points (mp) were determined using a Yanagimoto micro-melting point apparatus (hot stage type) and are uncorrected. UV spectra were recorded with a Shimadzu UV-190 digital spectrophotometer. Low-resolution mass spectra were obtained on a Shimadzu-LKB 9000B mass spectrometer in the direct-inlet mode. 1H-NMR spectra were recorded on a Varian UNITY 200 MHz (200 MHz) or UNITY 600 (600MHz) in CDCl3, for dimethyl sulfoxide (DMSO)-d6 with tetramethylsilane as an internal standard. Merck Art 5554 plates precoated with Silica gel 60 containing fluorescent indicator F254 were used for thin-layer chromatography, and Silica gel 60 (Merck 7734, 60—200 mesh) was employed for column chromatography.

\[ \text{N-2-Benzyl-2',3'-tri-O-acetyluridine (2a). General Procedure for 2a} \]

To a solution of 2,3',5'-tri-O-triacetyluridine (1, 3.7 g, 10 mmol) in DMF (50 ml) was added potassium carbonate (1.64 g, 7.5 mmol) and benzyl bromide (7.36 g, 40 mmol), and the mixture was stirred at 50 °C for 1 h; then acetic acid (0.9 ml) was added and the mixture was concentrated to a small volume. The residue was partitioned between CHCl3 (50 ml) and water (50 ml). The organic layer was dried over MgSO4, and the residual solution was chromatographed over a column of Silica gel G (3.1×40 cm) using 33—66% AcOEt in hexane (1 l) to give a caramel (247 mg, 52%). 1H-NMR (CDCl3) δ: 7.17 (1H, d, J = 7.7 Hz, H6), 6.50 (1H, d, J = 7.7 Hz, H5), 5.84—6.07 (3H, m, H1', CH3), 5.30—5.45 (2H, m, H2', H3'), 4.38—4.40 (3H, m, H4', H5'), 2.15 (3H, s, Ac), 2.12 (3H, s, Ac), 2.10 (3H, s, Ac). UV λmax (MeOH) nm: 325. HR-MS m/z: 504.1203 (M'+, C17H18N2O6S requires 504.1207).

\[ \text{N-(3,5-Dimethylbenzyl)-2',3'-tri-O-acetyl-4-thiouridine (4d): Compound 4d} \]

was obtained as a caramel (2.17 g, 8%) from 2d (2.50 g, 5.1 mmol). 1H-NMR (CDCl3) δ: 7.17 (1H, d, J = 7.7 Hz, H6), 7.02 (1H, brs, two of C2H5), 6.88 (1H, brs, one of C2H5), 6.66 (1H, d, J = 7.7 Hz, H5), 6.02 (1H, d, J = 4.4 Hz, H1'), 5.63 (2H, dd, J = 13.9, 11.1 Hz, CH3), 5.31—5.38 (2H, m, H1', H2'), 4.36 (3H, m, H3', H4'), 2.28 (6H, s, CH3), 2.13 (3H, s, Ac), 2.11 (3H, s, Ac), 2.05 (3H, s, Ac). UV λmax (MeOH) nm: 329.5. HR-MS m/z: 504.1584 (M'+, C17H18N2O6S requires 504.1587).

References and Notes


