Synthesis and Central Nervous System Depressant Effects of N^3-Substituted 2′,3′-*O*-Isopropylideneuridines

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Received July 5, 1999; accepted September 1, 1999

N^3-Substituted derivatives of 2′,3′-*O*-isopropylideneuridine (1) were synthesized and their pharmacological effects on the central nervous system (CNS) examined using mice. Methyl (2), ethyl (3), propyl (4), butyl (5), allyl (6), benzyl (7), *o*-, *m*-, *p*-xylyls (8, 9, 10), and α-phenylethyl (11) derivatives of 1 were administered to mice by intracerebroventricular (i.c.v.) injection for evaluating hypnotic activity, pentobarbital-induced sleep prolongation, and spontaneous activity as indices. Only 3 possessed hypnotic activity by i.c.v. injection at the dose of 2.0 μmol/mouse. Compounds 3, 4, and 10 significantly showed synergism with a barbiturate, indicating that the derivatives have some CNS depressant effects. Moreover, 3 and 4 caused decrease in the spontaneous activity of mice, even at low doses. The present study indicated that substitution by ethyl, propyl, and *p*-xylyl groups at the N^3-position of 2′,3′-*O*-isopropylideneuridine imparted the CNS depressant effects.

Key words N^3-substituted nucleoside; 2′,3′-*O*-isopropylideneuridine; hypnotic activity; sleep prolongation; spontaneous activity; central nervous system depressant

In structure–activity relationship studies of N^3-substituted oxopyrimidine nucleosides for sedative and hypnotic activities, we have found for the first time that N^3-benzyl substituted uridine exerted a hypnotic action on mice by intracerebroventricular (i.c.v.) administration.[2)] Among the uridine derivatives previously reported[3—8)] (uridine, 6-azauridine, thymidine, 2′-deoxyuridine derivatives), N^3-phenacyluridine displayed the most potent hypnotic activity. Moreover, it was thought that the hydroxy group on the sugar (ribose) moiety plays an important role for the central nervous system (CNS) depressant effects. Since 2′,3′-*O*-isopropylideneuridine is protected at the 2′,3′-position hydroxy groups in the ribose moiety, it seems a useful lead compound to determine whether the sugar moiety in oxopyrimidine nucleoside derivatives imparts the CNS depressant effects. We thus synthesized various N^3-substituted 2′,3′-*O*-isopropylideneuridine (**1**) derivatives and examined their CNS depressant effects.

Experimental

Animals Male std-ddY mice weighing 22 to 28 g were obtained from Sankyo Laboratories (Toyama, Japan). They were kept in an air-conditioned room (24±2 °C) with controlled lighting (8:00 to 20:00 light period) and given food and water *ad libitum*. Experiments on hypnotic activity and pentobarbital-induced prolongation effects were carried out from 10:00.

Chemicals Sodium pentobarbital and halogenated alkyl compounds were purchased from Tokyo Kasei Kogyo Co., Ltd. (Tokyo, Japan).

Syntheses of N^3-Substituted derivatives of 1 N^3-Substituted derivatives of 2′,3′-*O*-isopropylideneuridine (**1**) were synthesized by the methods described previously.[2—9)] Briefly, **1** (1 mmol) dissolved in dimethylsulfoxide (DMSO) (3 ml) and acetone (3 ml) was refluxed at 80—90 °C for 3 h with halogenated alkyls (1.0 mmol) in the presence of a base (K_2CO_3 1.6 mmol). The product was purified by silica gel column chromatography with a solvent system of chloroform-ethyl acetate-methanol (5 : 4 : 1) and recrystallized except for oily substances.

N^3-Methyl-2′,3′-*O*-isopropylideneuridine (**2**): mp 110—111 °C (from Me_2CO–*n*-hexane), yield 80 %, ^{1}H-NMR (DMSO-d_6) δ: 1.40 (3H, s, CH_3), 1.57 (3H, s,CH_3), 3.33 (3H, s, N–CH_3), 3.50—377 (2H, t, 5′-H_2), 4.03—4.30 (1H, m, –OH), 4.70—5.25 (3H, m, 2′-H, 3′-H, 4′-H), 5.86 (1H, d, J=8 Hz, 5-H), 5.90—6.03 (1H, d, J=3 Hz, 1′-H), 7.89 (1H, d, J=8 Hz, 6-H). MS: m/z=298 (M^+). *Anal*. Calcd for $C_{13}H_{18}N_2O_6$: C, 52.35; H, 6.04; N, 9.40. Found: C, 52.41; H, 5.99; N, 9.40.

N^3-Ethyl-2′,3′-*O*-isopropylideneuridine (**3**): mp 93—94 °C (from Et_2O–*n*-hexane), yield 90%, ^{1}H-NMR (DMSO-d_6) δ: 0.90—1.23 (3H, t, CH_3), 1.27 (3H, s, CH_3), 1.52 (3H, s, CH_3), 3.50—3.75 (2H, t, 5′-H_2), 3.78—3.98 (2H, m, N–CH_2), 4.00—4.20 (1H, m, –OH), 4.65—5.25 (3H, m, 2′-H, 3′-H, 4′-H), 5.79 (1H, d, J=8 Hz, 5-H), 5.94 (1H, d, J=3 Hz, 1′-H), 7.77—8.02 (1H, d, J=8 Hz, 6-H). MS: m/z=312 (M^+). *Anal*. Calcd for $C_{14}H_{20}N_2O_6$: C, 53.85; H, 6.41; N, 8.97. Found: C, 53.72; H, 6.41; N, 8.96.

N^3-Propyl-2′,3′-*O*-isopropylideneuridine (**4**): mp 69—70 °C (from Et_2O–*n*-hexane), yield 79%, ^{1}H-NMR (DMSO-d_6) δ: 0.83 (3H, t, CH_3), 1.30 (3H, s, CH_3), 1.50 (3H, s, CH_3), 3.27 (2H, m, –CH_2–), 3.60 (2H, m, 5′-H_2), 3.67 (2H, m, N–CH_2), 4.13 (1H, m, –OH), 4.67—5.20 (3H, m, 2′-H, 3′-H, 4′-H), 5.80 (1H, d, J=8 Hz, 5-H), 5.90 (1H, d, J=3 Hz, 1′-H), 7.90 (1H, d, J=8 Hz, 6-H). MS: m/z=326 (M^+). *Anal*. Calcd for $C_{15}H_{22}N_2O_6$: C, 55.21; H, 6.75; N, 8.59. Found: C, 55.14; H, 6.74; N, 8.47.

N^3-Butyl-2′,3′-*O*-isopropylideneuridine (**5**): Oil, yield 84%, ^{1}H-NMR (DMSO-d_6) δ: 0.90 (4H, m, –CH_2–CH_2–), 1.40—1.60 (9H, m, CH_3×3), 3.50—4.10 (5H, m, –OH, 5′-H_2, N–CH_2–), 4.41 (1H, s, 4′-H), 4.91 (2H, m, 2′-H, 3′-H), 5.70 (1H, d, J=5 Hz, 5-H), 5.80 (1H, d, J=3 Hz, 1′-H), 7.65 (1H, d, J=8 Hz, 6-H). MS: m/z=340 (M^+). *Anal*. Calcd for $C_{16}H_{24}N_2O_6$: C, 56.47; H, 7.06; N, 8.23. Found: C, 54.93; H, 6.92; N, 7.91.

N^3-Allyl-2′,3′-*O*-isopropylideneuridine (**6**): mp 88—89 °C (from Me_2CO–*n*-hexane), yield 80%, ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, s, CH_3), 1.46 (3H, s, CH_3), 3.50—3.75 (2H, t, 5′-H_2), 4.00—4.20 (1H, m, –OH), 4.29—4.52 (2H, m, N–CH_2), 4.65—5.33 (5H, m, 2′-H, 3′-H, 4′-H, CH_2=), 5.55—5.70 (1H, m, –CH=), 5.76 (1H, d, J=8 Hz, 5-H), 5.92 (1H, d, J=4 Hz, 1′-H), 7.92 (1H, d, J=8 Hz, 6-H). MS: m/z=324 (M^+). *Anal*. Calcd for $C_{15}H_{20}N_2O_6$: C, 55.56; H, 6.17; N, 8.64. Found: C, 55.54; H, 6.24; N, 8.61.

N^3-Benzyl-2′,3′-*O*-isopropylideneuridine (**7**): mp 45—48 °C (from Me_2CO–*n*-hexane), yield 83%, ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, s, CH_3), 1.46 (3H, s, CH_3), 3.43—3.77 (2H, t, 5′-H_2), 4.00—4.20 (1H, m, –OH), 4.62—5.23 (5H, m, N–CH_2, 2′-H, 3′-H, 4′-H), 5.71—6.00 (2H, m, 5-H, 1′-H), 7.28 (5H, s, –C_6H5–), 7.93 (1H, d, J=8 Hz, 6-H). MS: m/z=374 (M^+). *Anal*. Calcd for $C_{19}H_{22}N_2O_6$: C, 60.96; H, 5.88; N, 7.49. Found: C, 60.58; H, 5.88; N, 7.40.

N^3-*o*-Xylyl-2′,3′-*O*-isopropylideneuridine (**8**): mp 159—160 °C (from Me_2CO–*n*-hexane), yield 63%, ^{1}H-NMR (DMSO-d_6) δ: 1.30 (3H, s, CH_3), 1.43 (3H, s, CH_3), 2.40 (3H, s, –CH_3), 3.48—3.75 (2H, t, 5′-H_2), 4.00—4.35 (1H, m, –OH), 4.77—5.25 (5H, m, N–CH_2, 2′-H, 3′-H, 4′-H), 5.77 (1H, s, 5-H), 5.92 (1H, d, J=3 Hz, 1′-H), 6.70—7.30 (4H, m, –C_6H_4–), 8.00 (1H, d, J=8 Hz, 6-H). MS: m/z=388 (M+). *Anal*. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.86; H, 6.19; N, 7.22. Found: C, 61.33; H, 6.24; N, 7.11.

N^3-*m*-Xylyl-2′,3′-*O*-isopropylideneuridine (**9**): mp 37—41 °C (from Me_2CO–*n*-hexane), yield 90%, ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, s, CH_3),

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1.43 (3H, s, CH_3), 2.27 (3H, s, $-CH_3$), 3.44—3.72 (2H, t, 5′-H_2), 4.00—4.24 (1H, m, –OH), 4.63—5.21 (5H, m, N–CH_2, 2′-H, 3′-H, 4′-H), 5.72 (1H, s, 5-H), 5.87 (1H, d, J=8 Hz, 1′-H), 6.93—7.30 (4H, m, $-C_6H_4-$), 7.93 (1H, d, J=8 Hz, 6-H). MS: m/z=388 (M^+). Anal. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.86; H, 6.19; N, 7.22. Found: C, 61.58; H, 6.51; N, 7.04.

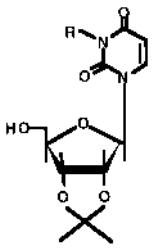
N^3-*p*-Xylyl-2′,3′-*O*-isopropylideneuridine (**10**): mp 111—112 °C (Me_2CO–*n*-hexane), yield 86%, ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, s, CH_3), 1.48 (3H, s, CH_3), 2.25 (3H, s, $-CH_3$), 3.48—3.75 (2H, t, 5′-H_2), 4.00—4.30 (1H, m, –OH), 4.64—5.20 (5H, m, N–CH_2, 2′-H, 3′-H, 4′-H), 5.73 (1H, s, 5-H), 5.86 (1H, d, J=8 Hz, 1′-H), 6.92—7.40 (4H, m, $-C_6H_4-$), 7.92 (1H, d, J=8 Hz, 6-H). MS: m/z=388 (M^+). *Anal*. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.86; H, 6.19; N, 7.22. Found: C, 61.50; H, 6.41; N, 7.16.

N^3-α-Phenylethyl-2′,3′-*O*-isopropylideneuridine (**11**): mp 111—112 °C (from EtOH–*n*-hexane), yield 86%, ^{1}H-NMR (DMSO-d_6) δ: 1.46 (3H, s, CH_3), 1.60 (3H, s, CH_3), 2.00 (3H, d, J=8 Hz, $-CH_3$), 3.86—4.61 (5H, m, 5′-H_2, 2′-H, 3′-H, 4′-H), 5.50—5.92 (1H, m, N–CH<), 6.40 (1H, s, 5-H), 5.92 (1H, d, J=8 Hz, 1′-H), 7.26—7.60 (6H, m, C_6H_5-, 6-H). MS: m/z=388 (M^+). *Anal*. Calcd for $C_{20}H_{24}N_2O_6$: C, 61.86; H, 6.19; N, 7.22. Found: C, 61.48; H, 6.25; N, 7.07.

Drug Administration Compounds (**1**—**11**) were suspended in saline containing 3% Tween 80 and injected i.c.v.[10] Control mice were injected i.c.v. with 3% Tween-80 as a vehicle. Sodium pentobarbital (40 mg/kg) dissolved in saline was administered intraperitoneally (i.p.).

Pharmacological Experiments Compounds tested were administered to mice by i.c.v. injection. Sleeping time was measured as the period between the loss and recovery of the righting reflex. The prolongation effects of N^3-substituted 2′,3′-*O*-isopropylideneuridine on pentobarbital-induced sleep were assessed by the injection of sodium pentobarbital (40 mg/kg, i.p.) 15 min after administration of the test compounds. Spontaneous activity of mice was determined with an animal behavior analyzer equipped with an NEC PC-9801-RX microcomputer (Muromachi Ind., Tokyo) as described previously.[8] The statistical significance of difference between the control and test groups was performed by use of a one-way analysis of variance Student *t*-test.

Table 1. CNS Depressant Activities of N^3-Substituted 2,3′-*O*-Isopropylideneuridines



No. **1**—**11**

R	No.	Hypnotic activity[a] (min)	Ratio of sleeping mice[b]
H	**1**	None	0/7
CH_3	**2**	None	0/8
CH_3CH_2	**3**	11[c]	2/7
$CH_3CH_2CH_2$	**4**	None	0/8
$CH_3CH_2CH_2CH_2$	**5**	None	0/8
CH_2=$CHCH_2$	**6**	None	0/8
$C_6H_5CH_2$	**7**	None	0/8
o-$CH_3C_6H_4CH_2$	**8**	None	0/8
m-$CH_3C_6H_4CH_2$	**9**	None	0/8
p-$CH_3C_6H_4CH_2$	**10**	None	0/8
$C_6H_5(CH_3)$ CH	**11**	None	0/7

Compounds tested were administered by i.c.v. injection at a dose of 2.0 μmol/mouse. *a*) Hypnotic activity is expressed as mean sleeping time (min). "None" indicates no hypnotic activity. *b*) Number of sleeping mice/number of mice used. *c*) Average of 2 sleeping mice.

Results and Discussion

Hypnotic activities of **1** and its N^3-substituted derivatives are summarized in Table 1. Compound **1**, N^3-methyl (**2**), propyl (**4**), butyl (**5**), allyl (**6**), benzyl (**7**), *o*-, *m*-, *p*-xylyls (**8**, **9**, **10**), and α-phenethyl (**11**) did not possess any hypnotic activity, as assessed by loss of righting reflex in mice, at the dose of 2.0 μmol/mouse by i.c.v. injection, whereas N^3-ethyl (**3**) derivative possessed slight hypnotic activity (11 min) in mice at the same dose. Our previous work has shown that N^3-alkyl substituted uridine, 6-azauridine, thymidine, and 2′-deoxyuridine do not exhibit any hypnotic activity in mice.[2—8] However, it was found that only **3** had CNS depressant effects among N^3-alkyl substituted oxopyrimidine nucleosides tested. Amongst uridine derivatives, N^3-alkyl substituted uridines did not exhibit any hypnotic activity, even at a high dose of 3.8 μmol/mouse by i.c.v. injection, while N^3-benzyl, *o*-, *m*-, and *p*-xylyls, and α-phenylethyl substituted uridines displayed 36, 72, 56, 36, and 7 min of hypnotic activity at 2.0 μmol/mouse (i.c.v.), respectively.[4] In the present study, N^3-benzyl, xylyls, and α-phenylethyl derivatives of **1** (**7**—**11**) did not have hypnotic activity, indicating that these derivatives display a different structure-activity relationship from the other N^3-substituted oxopyrimidine nucleosides, presumably due to the lack of free hydroxy groups at the 2′- and 3′-positions of the sugar.

The effects of the derivatives of **1** on pentobarbital-induced sleep were also evaluated (Fig. 1). Compounds **1**, **2**, **5**—**9**, and **11** (2.0 μmol/mouse, i.c.v.) did not prolong pentobarbital-induced sleeping time, however, **3**, **4**, and **10** showed 167, 145, and 149% of control sleeping time (56±2 min) (Fig. 1), respectively. The present results are not the same as

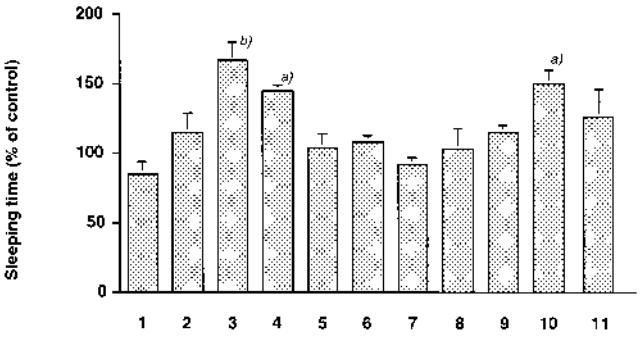


Fig. 1. Effects of N^3-Substituted 2′,3′-*O*-Isopropylideneuridine on Pentobarbital-Induced Sleep by I.c.v. Injection

Pentobarbital (PB)-induced sleep prolonging effect was expressed as the mean % of control sleeping time±S.E.M. (control sleeping time: 56±2 min). *a*) and *b*) indicate significant difference from control at the level of $p<0.05$ and $p<0.01$, respectively.

found in N^3-substituted oxopyrimidine nucleosides such as uridine, 6-azauridine, thymidine, and 2′-deoxyuridine which are not protected at the hydroxy groups of the sugar moiety.[2—8]

The potent compounds **3** and **4** were also evaluated for spontaneous activity. Compounds **3** and **4** significantly decreased spontaneous activity to 6 and 20% of control levels (4505±560 cm/h) at the dose of 2.0 μmol/mouse, respectively. These results support the report by Krooth *et al*.[11] that oxopyrimidine nucleosides decreased mouse locomotor activity.

We previously reported that N^3-benzyl- and benzyl-related derivatives of uridine, 6-azauridine, thymidine, and 2′-deoxyuridine possessed hypnotic activity in mice, suggesting

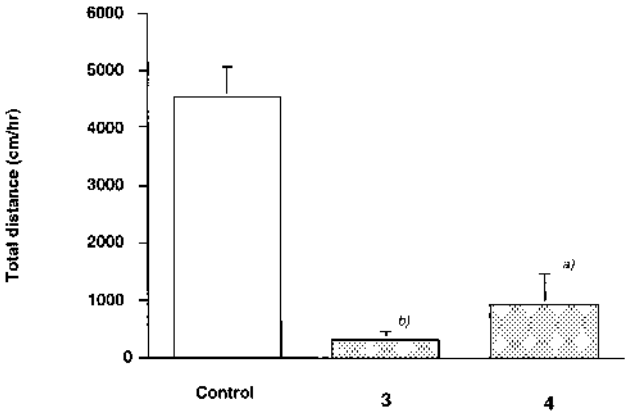


Fig. 2. Spontaneous Activities of **3** and **4** in Mice by I.c.v. Injection

Compounds were administered by i.c.v. injection at a dose of 2.0 μmol/mouse. Results are expressed as the mean total distance (cm) ±S.E.M. within 1 h after administration. *a*) and *b*) indicate significant difference from the control value (4505±560 cm/h) with $p<0.05$ and $p<0.01$, respectively.

that hydroxy groups on the sugar moiety of oxopyrimidine nucleosides play an important role in the CNS depressant effects.[2—8] In the present study, **7**—**11** did not exhibit hypnotic activity despite having benzyl, xylyl, or α-phenethyl groups at the N^3 position. The structure–activity relationships of **1** and its derivatives were different from series of oxopyrimidines previously reported.[4—8]

These results indicate that hydroxy groups at the 2′ and 3′ positions of uridine may be important sites for CNS depressant effects of oxopyrimidine nucleosides. In addition, N^3-ethyl and propyl-substitution of **1** caused CNS depressant effects. For this reason, it was hypothesized that the 2′,3′-*O*-isopropylidene moiety of **1** might stereospecifically interact with the N^3-substituent.

It is known that uridine is one of the pyrimidine nucleosides having a sleep-promoting effect in rats.[12,13] Although **1** was not identified as a sleep substance, it appeared that **3**, **4**, and **10** exert CNS depressant activity. Thus, oxopyrimidines might play an important role in CNS regulation. However, the mechanism of the CNS depressant effects of oxopyrimidine nucleosides is still unknown. It is known that uridine interacts with γ-aminobutyric acid (GABA) binding sites in the cerebellar membranes of the rat,[14] and we have recently reported that N^3-phenacyluridine interacts with the benzodiazepine and uridine receptors.[15] The present results suggest that active and/or inactive derivatives of **1** may be useful as tools for study of the mechanism of CNS depressant effects of the pyrimidine nucleosides, as agonists and antagonists of these receptor ligands, respectively.

In conclusion, substitution with functional groups at the N^3-position of the pyrimidine base and at the hydroxy groups of the sugar moiety could be an important factor for producing CNS depressant effects in oxopyrimidine nucleoside derivatives.

Acknowledgments This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan, and by the Special Research Fund of Hokuriku University.

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