Preparation and Pharmacological Evaluation of N^3-Substituted Thymidine Derivatives as Central Deprassants

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Central depressant effects in mice of N^3-substituted thymidines (Td) (1) were examined by intracerebroventricular (i.c.v.) injection. Nine derivatives including the methyl, ethyl, propyl, allyl, benzyl, xylyl and α-phenylethyl derivatives at the N^3-position of 1 were synthesized and their pharmacological effects were evaluated by using hypnotic activity, pentobarbital-induced sleep prolongation and locomotor activity as indices for central depressant effects. At a dose of 2.0 μmol/mouse, the values of mean sleeping time induced by N^3-benzylthymidine (6), N^3-*o*-xylylthymidine (7), N^3-*m*-xylylthymidine (8), N^3-*p*-xylylthymidine (9) and N^3-α-phenylethylthymidine (10) were 61, 30, 48, 45 and 23 min, respectively. These derivatives (2.0 μmol/mouse) prolonged pentobarbital-induced (40 mg/kg, i.p.) sleeping time. None of the alkyl (2—4) or allyl (5) derivatives exerted hypnotic activity, although the derivatives tested (2—10) significantly prolonged the pentobarbital-induced sleeping time. Compound 1 and its xylyl derivatives tested (0.25 μmol/mouse, i.c.v.) decreased locomotor activity. These results indicate that thymidine derivatives have central depressant activity, and the benzyl derivatives but not alkyl derivatives possess a hypnotic activity.

Keywords N^3-substituted nucleoside; thymidine; hypnotic activity; alkyl derivative; sleep prolongation

It has been reported that uridine is one of the sleep-promoting substances isolated from the brainstem of sleep-deprived rats.[1] However, uridine itself does not possess any hypnotic activity as determined by loss of righting reflex in experimental animals. In connection with this finding, we have shown for the first time that N^3-substituted derivatives of uridine and 6-azauridine possess central nervous system (CNS) depressant effects including hypnotic activity.[2-5] Although there is no evidence that thymidine (**1**), a pyrimidine nucleoside like uridine, is a sleep-promoting substance, **1** was reported to decrease locomotor activity of mice.[6] Therefore, we considered that introduction of a substituent at the N^3-position of **1** might lead to CNS depressant effects. Moreover, N^3-benzyl-substituted **1** might produce a stronger hypnotic activity than N^3-benzyluridine and related compounds previously reported.[2-5] The present study describes the structure–activity relationship of N^3-substituted thymidine derivatives having CNS depressant effects 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.

Syntheses of N^3-Substituted 1 N^3-Substituted thymidines (1-β-D-(2-deoxyribofuranosyl)thymine) were prepared by the methods described previously.[3,7] Briefly, **1** (6 mmol) dissolved in dimethylsulfoxide (6 ml) and acetone (6 ml) was reacted with halogenated alkyls (9 mmol) in the presence of a base (NaH (10 mmol) or K_2CO_3 (10 mmol)). The product was purified by silica gel column chromatography with a solvent system of chloroform–ethyl acetate–methanol (5:4:1).

Analytical data of the derivatives prepared were as follows:

N^3-Methylthymidine (3-methyl-1-β-D-(2-deoxyribofuranosyl)thymine) (**2**): Recrystallization solvent, methanol, chloroform and *n*-hexane, yield 90%, mp 133—135 °C (lit. 131—133 °C).[8] ^{1}H-NMR (DMSO-d_6) δ: 1.87 (3H, s, CH_3), 2.14 (2H, t, H-2′), 3.45 (3H, s, NCH_3), 3.55—3.60 (2H, m, H_2-5′), 3.65—4.76 (2H, m, H-3′, H-4′), 6.27 (1H, t, H-1′), 7.83 (1H, s, H-6). *Anal.* Calcd for $C_{11}H_{16}N_2O_5$: C, 51.53; H, 6.29; N, 10.98. Found: C, 51.38; H, 6.69; N, 10.88.

N^3-Ethylthymidine (3-ethyl-1-β-D-(2-deoxyribofuranosyl)thymine) (**3**): Recrystallization solvent, methanol, chloroform and *n*-hexane, yield 83%, mp 110—112 °C. ^{1}H-NMR (D_2O) δ: 1.10 (3H, t, CH_3), 1.84 (3H, s, CH_3), 2.14 (2H, t, H-2′), 2.48—2.60 (2H, m, NCH_2), 3.48—3.66 (2H, m, H_2-5′), 3.70—3.88 (1H, m, H-3′), 4.16—4.32 (1H, m, H-4′), 6.22 (1H, t, H-1′), 7.76 (1H, s, H-6). *Anal.* Calcd for $C_{12}H_{18}N_2O_5$: C, 53.80; H, 6.71; N, 10.41. Found: C, 53.30; H, 6.66; N, 10.22.

N^3-Propylthymidine (3-propyl-1-β-D-(2-deoxyribofuranosyl)thymine) (**4**): Compound **4** was prepared from **5** described below. Compound **5** (1 mmol) and palladium on activated carbon (100 mg) were dissolved in 5 ml of ethanol and hydrogenated with hydrogen gas. The mixture was filtered, and ethanol was removed from the filtrate *in vacuo* to yield **4** (94%) as an oil. ^{1}H-NMR (DMSO-d_6) δ: 0.98 (3H, s, CH_3), 1.42—1.72 (2H, m, H-5′), 1.92 (3H, s, CH_2), 2.45 (3H, t, H-2′), 3.20—3.70 (5H, m, H_2-5′, H-3′, NCH_2), 3.90—4.28 (1H, m, H-4′), 6.21 (1H, t, H-1′), 7.47 (1H, s, H-6). *Anal.* Calcd for $C_{13}H_{20}N_2O_5$: C, 54.89; H, 7.02; N, 9.90. Found: C, 54.77; H, 6.96; N, 9.51.

N^3-Allylthymidine (3-allyl-1-β-D-(2-deoxyribofuranosyl)thymine) (**5**): Recrystallization solvent, ethyl acetate and *n*-hexane, yield 94%, 102—103 °C. ^{1}H-NMR (D_2O) δ: 1.92 (3H, s, CH_3), 2.16 (2H, t, H-2′), 3.66 (2H, t, H_2-5′), 3.78—3.94 (1H, m, H-3′), 4.24—4.44 (3H, m, H-4′, NCH_2), 4.88 (2H, d, J=8 Hz, $=CH_2$), 5.82 (1H, t, H-1′), 7.46 (1H, s, H-6). *Anal.* Calcd for $C_{13}H_{18}N_2O_5$: C, 55.28; H, 6.42; N, 9.92. Found: C, 55.07; H, 6.40; N, 9.81.

N^3-Benzylthymidine (3-benzyl-1-β-D-(2-deoxyribofuranosyl)thymine) (**6**): Recrystallization solvent, *n*-hexane, yield 84%, mp 145—146 °C (lit. 167—168 °C).[9] ^{1}H-NMR (DMSO-d_6) δ: 1.84 (3H, s, CH_3), 2.14 (2H, t, H-2′), 3.52—3.68 (2H, m, H_2-5′), 3.72—3.88 (1H, m, H-3′), 4.16—4.36 (1H, m, H-4′), 4.96 (2H, m, NCH_2), 6.20 (1H, t, H-1′), 7.00—7.30 (5H, m, C_6H_5), 7.84 (1H, s, H-6). *Anal.* Calcd for $C_{17}H_{20}N_2O_5$: C, 61.41; H, 6.06; N, 8.47. Found: C, 61.49; H, 6.30; N, 8.49.

N^3-*o*-Xylylthymidine (3-[(2-methylphenyl)methyl]-1-β-D-(2-deoxyribofuranosyl)thymine) (**7**): Recrystallization solvent, methanol and water, yield 72%, mp 145—146 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.82 (3H, s, CH_3), 2.10 (2H, t, H-2′), 2.32 (3H, s, CH_3), 3.60—3.80 (2H, m, H_2-5′), 3.82—4.00 (1H, m, H-3′), 4.26—4.50 (1H, m, H-4′), 5.14 (2H, m, NCH_2), 6.24 (1H, t, H-1′), 7.42—7.58 (4H, m, C_6H_4), 8.06 (1H, s, H-6). *Anal.* Calcd for $C_{18}H_{22}N_2O_5$: C, 62.39; H, 6.40; N, 8.12. Found: C, 62.36; H, 6.30; N, 8.05.

N^3-*m*-Xylylthymidine (3-[(3-methylphenyl)methyl]-1-β-D-(2-deoxyribofuranosyl)thymine) (**8**): Recrystallization solvent, methanol and water, yield 85%, 142—143 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.84 (3H, s, CH_3), 2.12 (2H, t, H-2′), 2.26 (3H, s, CH_3), 3.50—3.68 (2H, m, H_2-5′), 3.74—3.86

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(1H, m, H-3'), 4.18—4.26 (1H, m, H-4'), 4.96 (2H, m, NCH_2), 6.24 (1H, t, H-1'), 7.00—7.32 (4H, m, C_6H_4), 7.84 (1H, s, H-6). *Anal.* Calcd for $C_{18}H_{22}N_2O_5$: C, 62.39; H, 6.40; N, 8.12. Found: C, 62.48; H, 6.18; N, 8.00.

N^3-*p*-Xylylthymidine (3-[(4-methylphenyl)methyl]-1-β-D-(2-deoxyribofuranosyl)thymine) (**9**): Recrystallization solvent, methanol and water, yield 89%, 130—131 °C, ^{1}H-NMR (DMSO-d_6) δ: 1.84 (3H, s, CH_3), 2.12 (2H, t, H-2'), 2.36 (3H, s, CH_3), 3.54—3.60 (2H, m, H_2-5'), 3.76—3.92 (1H, m, H-3'), 4.16—4.40 (1H, m, H-4'), 4.96 (2H, m, NCH_2), 6.22 (1H, t, H-1'), 6.72—7.22 (4H, m, C_6H_4), 7.84 (1H, s, H-6). *Anal.* Calcd for $C_{18}H_{22}N_2O_5$: C, 62.39; H, 6.40; N, 8.12. Found: C, 62.32; H, 6.14; N, 8.12.

N^3-α-Phenylethylthymidine (3-(1-phenylethyl)-1-β-D-(2-deoxyribofuranosyl)thymine) (**10**): The product obtained was evaporated *in vacuo* to yield **10** (49%) as an oil. ^{1}H-NMR (DMSO-d_6) δ: 1.74 (3H, d, J=8 Hz, CH_3), 1.83 (3H, s, CH_3), 1.96—2.16 (2H, m, H-2'), 3.50—4.00 (3H, m, H_2-5', H-3'), 4.06—4.47 (1H, m, H-4'), 6.00—6.46 (2H, m, NCH, H-1'), 7.10—7.50 (5H, m, C_6H_5), 7.83 (1H, s, H-6). *Anal.* Calcd for $C_{18}H_{22}N_2O_5$: C, 62.39; H, 6.40; N, 8.12. Found: C, 62.50; H, 6.13; N, 8.00.

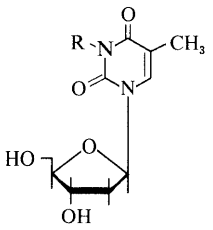
Drug Administration N^3-Substituted thymidines were suspended in saline containing 3% Tween-80 and injected intracerebroventricularly (i.c.v.).[10] Sodium pentobarbital (40 mg/kg) dissolved in saline was administered intraperitoneally (i.p.)

Pharmacological Experiments Sleeping time was measured as the period between loss and recovery of the righting reflex. Prolonging effects of N^3-substituted thymidines on pentobarbital-induced sleep were assessed by the injection of sodium pentobarbital (40 mg/kg, i.p.) 15 min after the administration of test compounds. Locomotor activity of mice was recorded on a Digiscan computer (Omnitech Electronics Inc.) as described previously.[5] In the data on pentobarbital-induced sleep-prolonging effect, the statistical significance of differences between control and test groups was analyzed by use of the Duncan test. The statistical significance of differences in locomotor activity was calculated by means of the Bonferroni test.

Results and Discussion

Effects of **1** and its derivatives on pentobarbital-induced sleep are summarized in Table I. N^3-Alkylated compounds **1** examined (2.0 μmol/mouse, i.c.v.) significantly prolonged pentobarbital-induced sleeping time, and benzyl-related derivatives (benzyl, xylyl and α-phenylethyl derivatives) also significantly prolonged the sleeping time at the same dose.

TABLE I. Central Depressant Effects of Thymidine and Its Derivatives



R		Sleeping time (min)[a]	Pentobarbital-induced sleep prolongation[b]
H	**1**	None	132± 6
CH_3	**2**	None	220±18[c]
CH_2CH_3	**3**	None	249±35[c]
$CH_2CH_2CH_3$	**4**	None	380± 6[c]
$CH_2CH=CH_2$	**5**	None	246± 9[c]
$CH_2C_6H_5$	**6**	61±9	256±25[c]
o-$CH_2C_6H_4CH_3$	**7**	30±3	192±19[c]
m-$CH_2C_6H_4CH_3$	**8**	48±3	264±20[c]
p-$CH_2C_6H_4CH_3$	**9**	45±2	251± 7[c]
$CH(CH_3)C_6H_5$	**10**	23±2	371±16[c]

a) Compounds tested were administered by i.c.v. injection at a dose of 2.0 μmol/mouse. Results are expressed as mean sleeping time (min)±S.E.M. None indicates no hypnotic activity. n=6 to 10. *b*) Compounds tested were administered by i.c.v. injection at a dose of 2.0 μmol/mouse 15 min before pentobarbital challenge (40 mg/kg, i.p.). Results are expressed as mean % of control sleeping time±S.E.M. n=8. *c*) Indicates a significant difference from the control value (p<0.01).

The prolonging effect was in the following order of potency: **4** (% of control, 380), **10** (371) >**8** (264), **6** (256), **9** (251), **3** (249), **5** (246), **2** (220), **7** (192)>**1** (132). These results did not show the same tendency as that found in N^3-substituted derivatives of uridine.[3] Table I summarizes the hypnotic activity of **1** and its derivatives. Five of 10 compounds examined exhibited the hypnotic activity; among them, **6** was the most potent. At 2.0 μmol/mouse, the values of mean sleeping time of **6**, **7**, **8**, **9** and **10**, were 61, 30, 48, 45 and 23 min, respectively. Our previous work[3] demonstrated that the N^3-*o*-xylyluridine had a stronger activity than the benzyl derivative. However, the present study indicates that *o*-xylyl derivative of **1** have a weaker activity than the benzyl derivative. The dose–response relation of the hypnotic activities of xylyl derivatives is shown in Fig. 1. All compounds tested dose-dependently produced the hypnotic activity in mice. At 0.5 μmol/mouse, their hypnotic activities were almost the same, whereas the activity of **6** was 2-fold stronger than that of **7** at a dose of 2.0 μmol/mouse.

Table II summarizes the effects of **1**, **6** and the xylylderivatives on locomotor activity of mice. At the dose of 0.25 μmol/mouse, **6**, **7** and **8** significantly reduced the activity by 42, 36 and 27%, respectively as compared to the Tween-80 saline control. The result demonstrates that the benzyl and xylyl derivatives could decrease the locomoter activity of mice even at low dose.

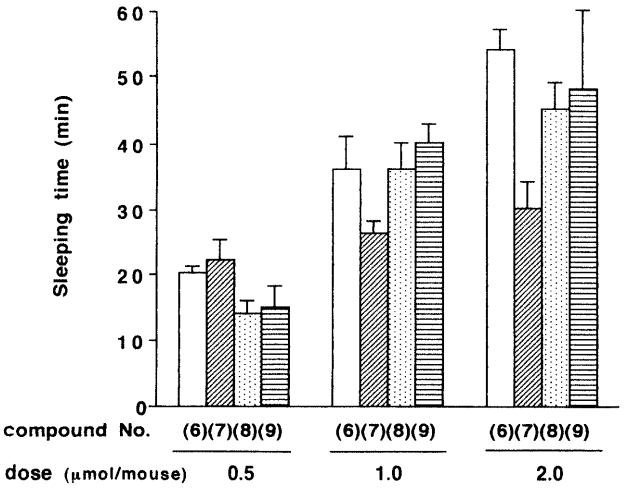


Fig. 1. Hypnotic Activities of N^3-Benzyl- and Xylyl Substituted Thymidines

Results are expressed as the mean sleeping time±S.E.M. of 7 to 8 mice. Each compound tested was injected by i.c.v. at doses of 0.5 to 2.0 μmol/mouse. □, N^3-ByTd (**6**); ▨, N^3-*o*-XyTd (**7**); ▩, N^3-*m*-XyTd (**8**); ⊟, N^3-*p*-XyTd (**9**).

TABLE II. Effects of Thymidine and Its Xylyl Derivatives on Locomotor Activity

Compounds	Dose (μmol/mouse)	Total distance (inches)	% of control
Control	3% Tween-80-saline	3122±484	100
1	0.25	1798±259	58
6	0.25	1315±309[a]	42
7	0.25	1138±197[b]	36
8	0.25	852±279[b]	27
9	0.25	1956±474	63

Compounds tested were administered by i.c.v. injection at a dose of 0.25 μmol/mouse. Results are expressed as mean total distance (inches)±S.E.M. *a*) and *b*) Indicate significant difference from the control value with p<0.05 and p<0.01, respectively.

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Although **1** itself did not exhibit hypnotic activity when given by i.c.v. injection, **1** potentiated pentobarbital-induced sleep and decreased locomotor activity as CNS depressant effects. The results indicate that **1** basically has CNS depressant activity. Therefore, our present results are consistent with the report by Krooth *et al.*[6] that oxopyrimidine nucleosides including uridine and **1** decreased mouse locomotor activity. Chemical modification of the oxopyrimidine nucleosides (such as N^3-substitution) produced strong CNS depressants. We previously reported that N^3-benzyl- or benzyl-related derivatives of uridine and 6-azauridine possess hypnotic activity in mice.[3,4] In the present study, N^3-benzyl- or benzyl-related thymidine derivatives also showed hypnotic activity. The results demonstrate that not only uridine derivatives, but also thymidine derivatives possess hypnotic activity. The structure-activity relationships of thymidine derivatives were different from those of uridine derivatives.[3] In the case of uridine derivatives, N^3-*o*-xylyluridine possessed the strongest CNS depressant activity among the uridine derivatives. Conversely, **6** caused 2-fold longer sleeping time than **7**, and showed the most potent hypnotic activity. This result might be due to the structural difference, *e.g.* 5-methyl group on the pyrimidine ring and/or 2′-hydroxy group on ribose.

Recently, we have shown that N^3-benzyluridine inhibits the binding of a benzodiazepine agonist, flunitrazepam, to the benzodiazepine receptor. The finding suggested that the uridine derivatives might exert a part of their effect through the benzodiazepine receptor.[11] In the present study, it appeared that thymidine derivatives also had CNS depressant activity like the uridine derivatives, so that thymidine derivatives may also interact with the benzodiazepine receptor. In addition, there may be another active site such as a specific receptor for the nucleoside derivatives, since the inhibitory effect of N^3-benzyluridine on the benzodiazepine receptor binding was still weak.[11] However, further studies are necessary to clarify the involvement of the benzodiazepine receptor in the hypnotic activity of the oxopyrimidine nucleosides.

In conclusion, the present study supports our previous findings that N^3-substituted nucleosides such as uridine and 6-azauridine possess hypnotic and sedative activities, and that the introduction of benzyl-related groups at the N^3-position is an important factor in exhibiting the central depressant effects of oxopyrimidine nucleoside derivatives. Although thymidine was not isolated from sleep-deprived rat brainstem as a sleep-promoting substance, it appeared that thymidine derivatives exert CNS depressant activity as do uridine derivatives. However, we were unable to establish fully the structure–activity relationship of oxopyrimidine nucleosides for CNS activity. A further study is in progress with deoxyuridine derivatives.

References

1) Y. Komoda, M. Ishikawa, H. Nagasaki, M. Iriki, K. Honda, S. Inoue, A. Higashi and K. Uchizono, *Biomed. Res.*, **4**, 223 (1983).
2) I. Yamamoto, T. Kimura, Y. Tateoka, K. Watanabe and I. K. Ho, *Chem. Pharm. Bull.*, **33**, 4088 (1985).
3) I. Yamamoto, T. Kimura, Y. Tateoka, K. Watanabe and I. K. Ho, *J. Med. Chem.*, **30**, 2227 (1987).
4) M. Koshigami, K. Watanabe, T. Kimura and I. Yamamoto, *Chem. Pharm. Bull.*, **39**, 2597 (1991).
5) I. Yamamoto, T. Kimura, Y. Tateoka, K. Watanabe and I. K. Ho, *Res. Commun. Chem. Pathol. Pharmacol.*, **52**, 321 (1986).
6) R. S. Krooth, W. L. Hsiao and G. F. M. Lam, *J. Pharmacol. Exp. Ther.*, **207**, 504 (1978).
7) T. Sasaki, K. Minamoto and H. Suzuki, *J. Org. Chem.*, **38**, 598 (1973).
8) O. M. Freidman, G. N. Mahapatra, B. Dash and R. Stevenson. *Biochem. Biophys. Acta*, **103**, 286 (1965).
9) R. T. Markiw and E. C. Canellakis, *J. Org. Chem.*, **34**, 3707 (1969).
10) T. J. Haley and W. G. McCormick, *Br. J. Pharmacol.*, **12**, 12 (1957).
11) I. Yamamoto, T. Kimura, K. Watanabe, Y. Tateoka and I. K. Ho, "Endogenous Sleep Factors," ed. by S. Inoue and I. M. Krueger, SPB Academic Pub. bv, Hage, 1990, pp. 133—142.

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