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Studies on Antitumor Agents. VI.[1] Syntheses and Antitumor Activities of Acyl Derivatives of 2′-Deoxy-5-trifluoromethyluridine

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Various *O*-acyl and *N*-acyl derivatives of 2′-deoxy-5-trifluoromethyluridine (F_3Thd) were synthesized; namely 5′-*O*-acyl, 3′,5′-di-*O*-acyl, N^3-acyl, 3′,5′-di-*O*-acetyl-N^3-acyl, 3′,5′-di-*O*-carbamoyl and 3′,5′-di-*O*-ethoxycarbonyl compounds. 5′-*O*-Acyl derivatives of 2′-deoxy-5-trifluoromethylcytidine were also synthesized.

The antitumor activities of these compounds against sarcoma 180 were examined by oral administration to mice. Among the 5′- and 3′,5′-diester compounds with aliphatic acids, the 5′-*O*-hexanoyl compound showed the highest activity. Full protection of the sugar moiety with aroyl or carbamoyl groups considerably decreased the activities, and those of the 3′,5′-di-*O*-*m*-fluorobenzoyl and 3′,5′-di-*O*-butylcarbamoyl compounds were the smallest. N^3-Benzoyl compounds were slightly more effective than F_3Thd but none of them showed higher activity than the effective *O*-acyl compounds. In the case of 5′-*O*-acylates of 2′-deoxy-5-trifluoromethylcytidine, the 5′-*O*-benzoyl compound showed the highest activity.

Keywords——2′-deoxy-5-trifluoromethyluridine; 2′-deoxy-5-trifluoromethylcytidine; acyl derivative; antitumor activity; pyrimidine nucleoside acylation; saponification

2′-Deoxy-5-trifluoromethyluridine (F_3Thd) was first synthesized by Heidelberger and his coworkers in 1962.[2] It has considerable biological activities in a number of systems.[3–7] For example, F_3Thd has been reported to be a potent compound in cancer chemotherapy. F_3Thd effectively inhibits the growth of various mammalian cells in culture.[6] The antitumor activities of F_3Thd against transplanted tumors such as adenocarcinoma 755 and L 1210 leukemia are equal to or higher than that of 2′-deoxy-5-fluorouridine (FUdR).[7]

However, F_3Thd showed unsatisfactory results in clinical cancer chemotherapy.[8] A disadvantage of F_3Thd for practical medicinal use is its short half-life in plasma after injection, mainly because of rapid metabolic degradation by thymidine phosphorylase to biologically inactive 5-trifluoromethyluracil (F_3T).[9] Thus, depot forms of F_3Thd which resist degradation by thymidine phosphorylase would be expected to maintain higher concentrations of F_3Thd in plasma and thus show greater antitumor activity *in vivo*.

If the depot form of F_3Thd is activated slowly after absorption, it is also expected to show low toxicity to the gastro-intestinal tract, minimizing the decrease of body weight,[10] and thus giving a high therapeutic index. Therefore, various acyl compounds of F_3Thd were synthesized and their antitumor activities were evaluated. Although similar attempts to enhance the activity of FUdR by *N*-benzoylation[10] or *O*-acylation[11] have been reported, it should be noted that 5-fluorouracil, the metabolite of FUdR, should itself be active, as well as FUdR. In the present case, F_3Thd is active only in the form of the nucleoside, and F_3T is inactive.[7]

TABLE I. Antitumor Effects of Acyl Derivatives of F_3Thd on Sarcoma 180

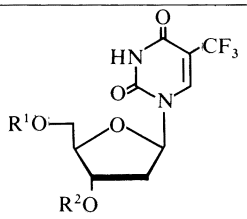
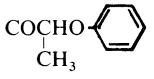
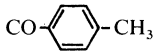
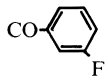
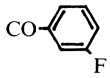
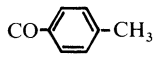
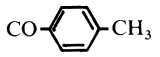
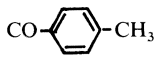
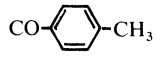
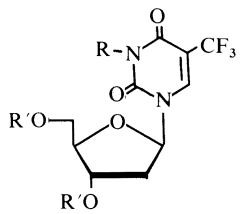
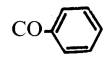
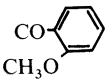
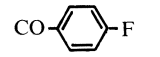
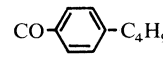
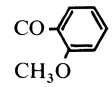
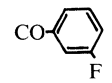
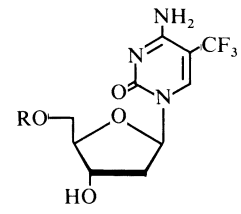
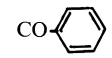
	Compd.	R^1	R^2	ED_{50} (mg/kg/d)
	**1**	$COCH_3$	H	31
	2	$CO(CH_2)_2CH_3$	H	27
	3	$CO(CH_2)_4CH_3$	H	19
	4	$CO(CH_2)_8CH_3$	H	58
	5	$CO(CH_2)_{14}CH_3$	H	40
	6		H	80
	7		H	52
	8	$COCH_3$	$COCH_3$	37
	9	$CO(CH_2)_2CH_3$	$CO(CH_2)_2CH_3$	31
	10	$CO(CH_2)_4CH_3$	$CO(CH_2)_4CH_3$	25
	11	$CO(CH_2)_8CH_3$	$CO(CH_2)_8CH_3$	38
	12	$CO(CH_2)_{14}CH_3$	$CO(CH_2)_{14}CH_3$	40
	13	$CO(CH_2)_{18}CH_3$	$CO(CH_2)_{18}CH_3$	40
	14			80
	15			80
	16	$CO(CH_2)_4CH_3$		80
	17		$CO(CH_2)_4CH_3$	57
	18	$CONH(CH_2)_3CH_3$	$CONH(CH_2)CH_3$	80
	19	$CO_2C_2H_5$	$CO_2C_2H_5$	29
	**20**		H	45
	21		H	38
	22		H	43
	23		H	34
	24		$COCH_3$	40
	25		$COCH_3$	32
	26	$CO(CH_2)_4CH_3$		33
	27	$CO(CH_2)_{14}CH_3$		80
	**28**			24
	F_3Thd			63

TABLE II. Physical Constants and Analytical Data for Acyl Derivatives of F_3Thd

Compound	mp (°C)	Recryst. solvent	Yield (%)	Formula	Analysis (%) Calcd (Found)		
					C	H	N
1	167—168	EtOH	32	$C_{12}H_{13}F_3N_2O_6$	42.61 (42.61)	3.87 3.91	8.28 8.35)
2	159—160	EtOH	35	$C_{14}H_{17}F_3N_2O_6$	45.91 (45.89)	4.68 4.86	7.65 7.58)
3	158—159	EtOH	38	$C_{16}H_{21}F_3N_2O_6$	48.73 (48.60	5.37 5.71	7.10 7.20)
4	158—160	EtOH	46	$C_{20}H_{29}F_3N_2O_6$	53.33 (53.48	6.49 6.79	6.22 6.18)
5	136— (dec.)	EtOH	52	$C_{26}H_{41}F_3N_2O_6$	58.41 (58.90	7.73 7.92	5.24 4.81)
6	Foam		42	$C_{19}H_{19}F_3N_2O_6$	51.35 (51.09	4.30 4.44	6.30 6.37)
7	201—203	EtOH	62	$C_{18}H_{17}F_3N_2O_6$	52.17 (51.96	4.14 4.20	6.76 6.68)
8	139—140.5	EtOH	92	$C_{14}H_{15}F_3N_2O_6$	44.22 (44.30	3.98 3.98	7.37 7.33)
9	Oil		49	$C_{18}H_{23}F_3N_2O_7$	49.54 (49.39	5.31 5.42	6.42 6.20)
10	Oil		64	$C_{22}H_{31}F_3N_2O_7$	53.65 (53.70	6.34 6.42	5.69 5.72)
11	Oil		59	$C_{30}H_{47}F_3N_2O_7$	59.59 (59.39	7.83 7.98	4.63 4.59)
12	Oil		53	$C_{42}H_{71}F_3N_2O_7$	65.26 (65.67	9.26 9.90	3.62 3.55)
13	55—56	EtOH	80	$C_{50}H_{87}F_3N_2O_7$	68.38 (67.86	9.18 9.54	3.19 3.15)
14	196—197	EtOH	72	$C_{24}H_{17}F_3N_2O_7$	53.24 (53.14	3.16 3.06	5.17 5.18)
15	181—182	EtOH	68	$C_{26}H_{23}F_3N_2O_7$	58.65 (58.51	4.35 4.24	5.26 5.33)
16	Foam		66	$C_{24}H_{27}F_3N_2O_7$	56.25 (56.41	5.31 5.44	5.47 5.55)
17	Oil		57	$C_{24}H_{27}F_3N_2O_7$	56.25 (56.39	5.31 5.34	5.47 5.40)
18	184—185	EtOH–ether	20	$C_{20}H_{29}F_3N_2O_7$	48.58 (48.54	5.91 6.13	11.33 11.45)
19	Foam		36	$C_{16}H_{19}F_3N_2O_9$	43.64 (43.26	4.35 4.44	6.36 6.28)
20	155—156	Ether–pet. ether	56	$C_{17}H_{15}F_3N_2O_6$	51.01 (51.27	3.78 3.85	7.00 7.03)
21	Foam		42	$C_{18}H_{17}F_3N_2O_7$	50.24 (50.25	3.98 4.03	6.51 6.54)
22	Foam		46	$C_{17}H_{14}F_4N_2O_6$	48.81 (49.00	3.37 3.49	7.70 7.64)
23	Oil		62	$C_{21}H_{23}F_3N_2O_6$	55.26 (54.98	5.08 5.27	6.14 6.44)
24	Foam		59	$C_{22}H_{21}F_3N_2O_9$	51.36 (51.69	4.11 4.26	5.45 5.45)
25	Foam		45	$C_{21}H_{18}F_4N_2O_8$	50.21 (50.06	3.61 3.76	5.58 5.62)
26	199—202	EtOH	51	$C_{16}H_{22}F_3N_3O_5$	48.85 (48.79	5.64 5.66	10.68 10.93)
27	198—200	EtOH	32	$C_{26}H_{42}F_3N_3O_5$	58.52 (58.74	7.93 8.21	7.87 7.61)
28	202	EtOH	38	$C_{17}H_{16}F_3N_3O_5$	51.13 (51.58	4.04 4.02	10.52 10.31)

This paper describes the antitumor activities against sarcoma 180 of various acyl derivatives of F_3Thd on oral administration to mice.

Materials and Methods

Chemicals——Melting points were determined with a Yanagimoto MP-3 micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained with a JEOL LMN-FX 100 spectrometer (using tetramethylsilane as an internal standard).

5′-*O*-Acyl compounds (**1**—**7**), 3′,5′-di-*O*-acyl compounds (**8**—**17**) and the 3′,5′-di-*O*-ethoxycarbonyl compound (**19**) were synthesized by treatment of F_3Thd with a small excess of the appropriate acyl chloride in pyridine at suitable temperatures. 5′-*O*-Acyl derivatives of 2′-deoxy-5-trifluoromethylcytidine (**26**—**28**) were similarly prepared. 3′,5′-Di-*O*-Acyl compounds (**8**—**15**) can be obtained more easily by the reaction of F_3Thd and acid anhydride in acetonitrile using dimethylaminopyridine as a catalyst.[12] Reaction of F_3Thd and butyl isocyanate in dimethylformamide (DMF) gave 3′,5′-di-*O*-butylcarbamoyl-F_3Thd (**18**).

Reaction of F_3Thd and 1 eq of acyl chloride in the presence of triethylamine in dimethylacetamide (DMA) gave N^3-acyl compounds (**20**—**23**) selectively. The acylation in solvents other than DMA gave 5′-*O*-acyl compound. The procedure for the preparation of N^3-benzoyl-2′-deoxy-5-trifluoromethyluridine (**20**) is described as a typical example. Benzoyl chloride (11.2 g, 0.08 mol) was added to a solution of F_3Thd (24 g, 0.08 mol) and triethylamine (16 ml) in DMA and the mixture was stirred for 12 h at room temperature. The reaction mixture was filtered and the filtrate was concentrated. The residue was dissolved in $CHCl_3$ (150 ml), then water (15—20 ml) was added with stirring to afford a precipitate of **20**. The precipitate was recrystallized from ether–pet. ether, giving 18 g of **20** in 56% yield.

The structures of these compounds were confirmed by the elemental analyses as well as by ^{1}H-NMR measurements. Physical constants are listed in Table II.

Antitumor Test——Mice of the ICR strain (Japan Clea Inc., Tokyo, Japan) were used. Five-week-old male ICR mice were inoculated sobcutaneously in the axillary region with 5×10^6 sarcoma 180 cells and given text compounds orally once a day for 7 consecutive days beginning 24 h after inoculation of the tumor cells. Groups of seven mice were used for each dose and the test compounds were suspended in 0.5% carboxymethylcellulose (CMC) solution containing 0.1% Tween 80. On day 10, the tumors were excised and weighed. The inhibitory effects of test compounds were calculated from the ratio of the tumor weight in the test group to that in the control group.

Inhibitory effects of F_3Thd derivatives on the growth of solid tumor S 180 are shown in terms of the ED_{50} values in Table I.

Results and Discussion

Among the 5′- and 3′,5′-diester compounds with aliphatic acids, the 5′-*O*-hexanoyl compound (**3**) showed the highest activity and the ED_{50} value was one-third of that of F_3Thd. Although the effect of esterification of F_3Thd by aliphatic acids was similar to that in the case of FUdR,[11] the range of optimal chain length was more restricted.

ED_{50} values of other 5′-*O*-acyl compounds with aryl functions, such as the 2-phenoxypropionyl (**6**) or benzoyl (**7**) compound, were equal to that of F_3Thd. The full protection of the sugar moiety by aroyl or carbamoyl groups considerably decreased the activities and the 3′,5′-di-*O*-*m*-fluorobenzoyl (**14**) and 3′,5′-di-*O*-butylcarbamoyl (**18**) compounds showed no activity at the same dose as that of F_3Thd.

The rates of saponification of these inactive compounds to F_3Thd by mouse intestinal homogenate[13] were significantly slower than those of effective *O*-aliphatic acyl compounds. It is therefore assumed that the F_3Thd-releasing ability is the most important factor affecting the activity of *O*-acylated derivatives of F_3Thd. It has been reported that the enzymatic saponification rate of 5′-*O*-acyl FUdR was greater than that of 3′-*O*-acyl FUdR.[13a] This would mean that, in 3′,5′-di-*O*-acyl compounds of F_3Thd, the cleavage of the ester bond at the 3′-position may be the rate-limiting step in activation. In fact, compounds **14**, **15**, **16** and **18**, in which the 3′-position is modified by a benzoyl or carbamoyl group, showed the lowest activities.

N^3-Benzoyl compounds (**20**—**25**) were slightly more effective than F_3Thd but none of them showed higher activity than the effective *O*-acyl compounds. 2′-Deoxy-5-

trifluoromethylcytidine is assumed to be a prodrug of F_3Thd, since it is deaminated to F_3Thd *in vivo*.[14] However, the antitumor activity of the 5′-*O*-benzoyl compound was higher than that of the 5′-*O*-hexanoyl compound, in contrast to the result with F_3Thd. It should be noted that *O*-acyl compounds of 2′-deoxy-5-trifluoromethylcytidine may have different metabolic pathways from the *O*-acyl compounds of F_3Thd.

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References and Notes

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13) Homogenates of various tissues of mice were centrifuged and the supernatants were dialyzed. Enzymatic lysis of the compounds was carried out with these fractions. These results will be reported elsewhere together with other data regarding the concentrations of F_3Thd in tumor tissue and normal tissues. The following papers have described the preparation of the enzyme fractions for lysis. *a*) J. E. Casida, J. L. Engel, and Y. Nishizawa, *Biochem. Pharmacol.*, **15**, 627 (1966); *b*) T. Kawaguchi, Y. Suzuki, N. Nambu, and T. Nagai, *Chem. Pharm. Bull.*, **33**, 2956 (1985).
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