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Studies on Antitumor Agents. V.[1] Syntheses and Antitumor Activities of 5-Fluorouracil Derivatives

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Six types of 5-fluorouracil (5-FU) derivatives were synthesized; namely, 2,4-di-*O*-substituted, 2-*O*-substituted, 4-*O*-substituted, 1,3-disubstituted, 1-substituted and 3-substituted compounds.

After oral administration of these compounds to rats, the blood levels of 5-FU were determined. Among *O*-substituted derivatives, a 4-*O*-substituted derivative was most easily activated to 5-FU and 2-*O*-substituted derivatives were next most easily activated. Among *N*-substituted derivatives, acyl and sulfonyl derivatives showed the highest 5-FU releasing abilities and 1-alkoxymethyl substituted derivatives showed low ability. *N*-Alkyl substituted derivatives were not activated to 5-FU.

Several compounds which gave higher blood levels of 5-FU than that obtained with 1-(tetrahydro-2-furyl)-5-fluorouracil (Thf-FU), as well as same related compounds, were selected and their antitumor activities were examined. The 2-*O*-substituted derivatives, 2-butoxy-5-fluoro-4(1*H*)-pyrimidone (**11**) and 2-benzyloxy-5-fluoro-4(1*H*)-pyrimidone (**19**), were as effective as Thf-FU. The activities of 2,4-di-*O*-substituted derivatives, 2,4-dibutoxy-5-fluoropyrimidine (**1**) and 2,4-dibenzyloxy-5-fluoropyrimidine (**6**), against Ehrlich carcinoma and against sarcoma 180, respectively, were the same as those of Thf-FU. The 1-substituted derivatives, 1-ethoxymethyl-5-fluorouracil (**49**) and 1-(1-ethoxy-1-phenylmethyl)-5-fluorouracil (**50**), were found to be as effective as Thf-FU.

Keywords——1-(tetrahydro-2-furyl)-5-fluorouracil; 5-fluorouracil derivatives; blood concentration; antitumor activities; tegafur

5-Fluorouracil (5-FU) was synthesized by Duschinsky and his coworkers[2] and its antitumor activity was demonstrated by Heidelberger and his coworkers.[3] The therapeutic index of 5-FU is low because of side effects, although it has a strong antitumor effect. Therefore, to obtain a more suitable therapeutic drug, various derivatives of 5-FU have been synthesized and their antitumor activities have been examined.[4]

1-(Tetrahydro-2-furyl)-5-fluorouracil (Thf-FU, tegafur), which was synthesized by Hiller and his coworkers,[5] is a masked derivative of 5-FU. Thf-FU has a high therapeutic index[6] and has been widely used clinically for maintenance therapy after surgical treatment[7,8] because of its low toxicity, especially to the gastro-intestinal tract, and ease of administration (oral or intrarectal).

A disadvantage of this derivative is a low blood concentration of 5-FU,[9] which is an active metabolite of the derivative.[10] It has been reported that when Thf-FU is used clinically by oral administration the blood levels of 5-FU were mostly in the range of 0.09—0.02 μg/ml,[11] which is about equal to the minimum effective blood level of 5-FU (0.05—0.03 μg/ml).[12]

A derivative of 5-FU which maintains a higher blood concentration of 5-FU than Thf-FU is expected to be a more effective prodrug, although it has been reported that in the case of a time-dependent drug such as 5-FU, the retention time is more influential as regards antitumor activity than the concentartion.[13]

Therefore, various other 5-FU derivatives were synthesized and the blood levels of 5-FU after oral administration of these compounds were determined. The derivatives which main-

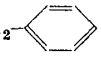
tained higher blood levels of 5-FU than Thf-FU and related compounds were selected and their antitumor activities were examined.

The present paper describes the syntheses of six types of 5-FU derivatives, their 5-FU releasing ability after oral administration and the antiumor activities of selected compounds.

Synthesis

As *O*-substituted 5-FU derivatives, 2,4-di-*O*-substituted compounds (type I), 2-*O*-substi-

TABLE I. Compounds of Type I, Type II (R^2=H), Type III (R^1=H)

Compd. No.	R^1	R^2	bp(°C/mmHg) or mp (°C)	Recryst. solvent	Formula	Analysis (%) Calcd (Found) C	H	N	UV(nm) λ_{max}^{pH2}	λ_{max}^{pH12}	Synthetic method
Type I											
1	$(CH_2)_3CH_3$	R^1	125—126/3—4		$C_{12}H_{19}FN_2O_2$	59.49 (59.69	7.90 8.19	11.56 11.27)	267	272	A
2	$CH(CH_3)CH_2CH_3$	R^1	88—89/2—3		$C_{12}H_{19}FN_2O_2$	59.49 (59.51	7.90 8.10	11.56 11.29)	268	271	A
3	$C(CH_3)_3$	R^1	70—72/2—3		$C_{12}H_{19}FN_2O_2$	59.49 (59.41	7.90 8.12	11.56 11.47)	Unstable		A
4	$(CH_2)_9CH_3$	R^1	214—220/3—4		$C_{24}H_{43}FN_2O_2$	70.20 (70.31	10.56 10.62	6.82 6.46)	Insoluble		A
5	$(CH_2)_{15}CH_3$	R^1	62—63	EtOH	$C_{36}H_{67}FN_2O_2$	74.69 (74.55	11.67 11.72	4.84 4.72)	Insoluble		A
6	CH_2- 	R^1	48.5—49.5	EtOH	$C_{18}H_{15}FN_2O_2$	69.67 (69.94	4.87 5.06	9.03 8.75)	Insoluble		A
7	CH_2- 	R^1	190—192/3—4		$C_{14}H_{19}FN_2O_4$	56.37 (56.64	6.42 6.79	9.39 9.18)	268	269	A
Type II											
8	CH_3	H	196—197.5	EtOH	$C_5H_5FN_2O_2$	41.67 (41.66	3.50 3.31	19.44 19.49)	263	269	B′
9	CH_2CH_3	H	182—183	EtOH	$C_6H_7FN_2O_2$	45.57 (45.53	4.46 4.57	17.71 17.43)	261	270	B′
10	$(CH_2)_2CH_3$	H	154—154.5	EtOH	$C_7H_9FN_2O_2$	48.84 (48.55	5.23 5.19	16.27 6.10)	261	270	B
11	$(CH_2)_3CH_3$	H	127—129	EtOH	$C_8H_{11}FN_2O_2$	51.61 (51.40	5.95 5.80	15.05 15.00)	261	270	B′
12	$CH(CH_3)CH_2CH_3$	H	104—105	EtOH	$C_8H_{11}FN_2O_2$	51.61 (51.43	5.95 5.80	15.05 15.21)	262	271	B′
13	$(CH_2)_4CH_3$	H	117—117.5	EtOH	$C_9H_{13}FN_2O_2$	53.99 (53.91	6.55 6.63	13.99 13.79)	260	270	B
14	$(CH_2)_5CH_3$	H	115—115.5	EtOH	$C_{10}H_{15}FN_2O_2$	56.06 (55.60	7.06 7.54	13.08 12.94)	262	270	B
15	$(CH_2)_9CH_3$	H	100—101	EtOH–H_2O	$C_{14}H_{23}FN_2O_2$	62.20 (62.29	8.58 8.87	10.36 10.21)	Insoluble		B′
16	$CH_2CH=C(CH_3)CH_2$ $(CH_3)_2C=CHCH_2$	H	Oil	Column.[a]	$C_{14}H_{19}FN_2O_2 \cdot 1/4H_2O$	62.09 (62.01	7.26 7.05	10.34 10.21)	Insoluble		B
17	$(CH_2)_{15}CH_3$	H	97—98	EtOH	$C_{20}H_{35}FN_2O_2$	67.76 (67.50	9.95 10.33	7.20 7.41)	Insoluble		B′
18		H	224—225	EtOH	$C_{10}H_7FN_2O_2$	58.26 (58.01	3.42 3.40	13.59 13.41)	264	271	B′
19	CH_2- 	H	146—147	EtOH	$C_{11}H_9FN_2O_2$	60.00 (60.21	4.12 4.08	12.72 12.55)	262	271	B′
20	CH_2- 	H	89.5—90.5	Et_2O	$C_9H_{11}FN_2O_3$	50.47 (50.49	5.18 5.11	13.08 13.22)	260	270	B′
Type III											
21	H	$(CH_2)_3CH_3$	150.5—151	EtOH	$C_8H_{11}FN_2O_2$	51.61 (51.75	5.95 6.12	15.05 14.90)	279	290	C

a) $CHCl_3$: EtOH=10.1 (v/v).

tuted compounds (type II) and a 4-*O*-substituted compound (type III) were synthesized (Table I). Various *N*-substituted 5-FU derivatives, 1,3-disubstituted compounds (type IV), 1-substituted compounds (type V) and 3-substituted compounds (type VI) were also synthesized (Table II).

Synthetic routes to the compounds of types I, II and III are shown in Chart 1. The compounds of type I (vi, **1**—**7**) were synthesized by reactions of 2,4-dichloro-5-fluoropyrimidine (ii)[14] with sodium alkoxide. Purification of compounds **1** to **4** and **7** was accomplished by

Table II. Compounds of Type IV, Type V (R'=H), and Type VI (R=H)

Compd. No.	R	R'	mp (°C)	Recryst. Solvent	Formula	Analysis (%) Calcd (Found) C	H	N	UV (nm) $\lambda_{max}^{pH\,2}$	$\lambda_{max}^{pH\,11}$	Synthetic method
Type IV											
22		CH_3	111	EtOH	$C_9H_{11}FN_2O_3$	50.47 (50.21)	5.18 5.59	13.08 13.11)	270	270	D'
23		CH_2SCH_3	76—76.5	EtOH	$C_{10}H_{13}FN_2O_3S$	46.15 (45.92)	5.03 4.97	10.76 10.56)	274	274	D'
24		$(CH_2)_6CH_3$	63	EtOH	$C_{16}H_{29}FN_2O_3$	63.51 (63.33)	8.59 8.77	8.23 8.23)	281	281	D'
25		CH_2COOCH_3	Oil		$C_{11}H_{13}FN_2O_5 \cdot 1/2H_2O$	46.78 (46.97)	4.96 4.60	9.96 9.72)	272	272	D'
26		CH_2CH_2CN	240—242	EtOH	$C_{11}H_{12}FN_3O_3$	52.17 (52.21)	4.78 4.80	16.59 16.43)	273	271	D'
27			110—112	aq. EtOH	$C_{12}H_{15}FN_2O_4$	53.33 (53.49)	5.94 5.88	10.37 10.49)	275	275	D'
28		CH_3-	72.5—73.5	EtOH	$C_{13}H_{17}FN_2O_4$	54.92 (54.70)	6.03 6.34	9.85 9.95)	272	272	D'
29		CH_3-	77—77.5	EtOH	$C_{15}H_{15}FN_2O_3$	62.06 (62.00)	5.21 5.26	9.65 9.67)	273	273	D'
44	CH_2CH_2CN	R	105—108	H_2O	$C_{10}H_9FN_4O_2$	50.85 (51.15)	3.84 3.79	23.72 23.73)	271	268	D
45	CH_2- 	R	Oil	Column[d]	$C_{14}H_{19}FN_2O_4 \cdot 1/3H_2O$	55.26 (55.21)	6.62 6.61	9.21 9.01)	274	274	D
46		R	Room temp. oil 0°C crystal	Column[e]	$C_{14}H_{19}FN_2O_4 \cdot 1/2H_2O$	54.27 (54.65)	6.56 6.42	9.12 9.12)	271	271	D
47	$COOCH_2CH(CH_3)_2$	R	70.5—71	EtOH	$C_{14}H_{19}FN_2O_6$	50.91 (50.83)	5.80 5.88	8.48 8.43)	Unstable		D
48	$CH_2OC_2H_5$	R	54	EtOH–H_2O	$C_{10}H_{15}FN_2O_4$	48.78 (48.53)	6.14 6.54	11.38 11.21)	268	267	D
Type V											
30	$(CH_2)_3CH_3$	H	124—126	Et_2O–pet. ether	$C_8H_{11}FN_2O_2$	51.61 (51.49)	5.95 6.01	15.05 15.30)	275	273	D
31	CH_3- 	H	171—171.5	EtOH	$C_9H_{11}FN_2O_3$	50.47 (50.40)	5.18 5.26	13.08 12.95)	273	271	D
32		H	174—175	EtOH	$C_9H_{11}FN_2O_3 \cdot 1/2H_2O$	48.43 (48.52)	5.42 5.57	12.55 12.54)	267	267	D
33		H	156—158	Et_2O	$C_{10}H_{13}FN_2O_4$	49.18 (48.93)	5.36 5.28	11.47 11.72)	267	267	D
34	$COOC_2H_5$	H	128—130	Benzene	$C_7H_7FN_2O_4$	41.59 (41.31)	3.49 3.23	13.86 13.57)	Unstable		D
35	$COO(CH_2)_3CH_3$	H	144—146	Benzene	$C_9H_{11}FN_2O_4$	46.96 (46.95)	4.82 4.70	12.17 12.01)	Unstable		D
36	$COOCH_2CH(CH_3)_2$	H	130—132	Benzene	$C_9H_{11}FN_2O_4$	46.96 (46.69)	4.82 4.79	12.17 12.30)	Unstable		D
37	COO- 	H	177—181	EtOH	$C_{10}H_9FN_2O_4$	54.55 (54.78)	3.43 3.36	10.60 10.57)	Unstable		D
38	$SO_2(CH_2)_3CH_3$	H	139—140	aq.EtOH	$C_8H_{11}FN_2O_4S$	38.40 (38.86)	4.43 4.98	11.19 11.19)	253 Shoulder		D
49	$CH_2OC_2H_5$	H	128—129	EtOH	$C_7H_9FN_2O_3$	44.68 (44.64)	4.82 5.02	14.89 14.71)	265	265	D
50	CH-  OC_2H_5	H	128—129	EtOH–H_2O	$C_{13}H_{13}FN_2O_3$	59.09 (59.32)	4.96 4.92	10.60 10.55)	267	267	D
Type VI											
39	H		129—131	EtOH	$C_8H_9FN_2O_3$	48.00 (48.11)	4.53 4.49	13.99 13.89)	269	301	F
40	H	CH_2COOCH_3	131—132	Column[a]	$C_7H_7FN_2O_4$	41.59 (41.94)	3.49 3.56	13.86 13.48)	268	296	F
41	H	$NHCO(CH_2)_2CH_3$	185—187(dec)	EtOH	$C_8H_{10}FN_3O_3$	44.65 (44.59)	4.68 4.78	19.53 19.44)	264	287	
42	H	$NHSO_2$--CH_3	243—245	Column[c]	$C_{11}H_{10}FN_3O_4S$	44.15 (44.01)	3.37 3.23	14.04 13.92)	267	286	
43	H	CO-	148—152	Column[b]	$C_{11}H_7FN_2O_3$	56.42 (56.82)	3.01 3.18	11.96 11.72)	259	292	E

a) $CHCl_3$: EtOH=10:1 (v/v); *b*) $CH_3COOC_2H_5$: $CHCl_3$=5:1 (v/v); *c*) $CHCl_3$; *d*) $CHCl_3$: EtOH=10:1 (v/v); *e*) $CHCl_3$: EtOH=5:1 (v/v).

distillation, while compounds **5** and **6** were recrystallized from ethanol. Treatment of 2-chloro-5-fluoro-4(1*H*)-pyrimidone (iii)[14] with sodium alkoxide gave compounds **10**, **13**, **14** and **16** of type II (vii). Alkaline hydrolysis of compounds of type I (vi) in potassium hydroxide solution gave compounds **8**, **9**, **11**, **12**, **15**, and **17** to **20**. Reaction of 4-chloro-5-fluoro-2(1*H*)-pyrimidone (v)[15] with sodium *n*-butoxide gave compound **21** of type III (viii).

Synthetic routes to the compaunds of types IV, V and VI are shown in Chart 2. Alkylation of Thf-FU with alkyl halide gave compounds **22** to **26**, **28** and **29** (type IV, x'). Reactions of 5-FU with alkyl halide using potassium carbonate as a base gave compounds **44** and **45** (type IV, x) and compounds **30** and **31** (type V). 1,3-Bis(tetrahydro-2-pyranyl)-5-fluorouracil (**46**) and 1,3-bis(tetrahydro-2-furyl)-5-fluorouracil (**27**) were synthesized from 2-*O*, 4-*O*-bis(trimethylsilyl)-5-fluorouracil and 2-acetoxytetrahydropyran or 2-acetoxytetrahydrofuran according to the method reported previously.[4a]

1-(Tetrahydro-2-pyranyl)-5-fluorouracil (**32**) (type V) was synthesized from 2-*O*, 4-*O*-bis(trimethylsilyl)-5-fluorouracil and 2-chlorotetrahydropyran by the method described for the synthesis of Thf-FU.[16] Reaction of 2-*O*, 4-*O*-bis(trimethylsilyl)-5-fluorouracil with 2-

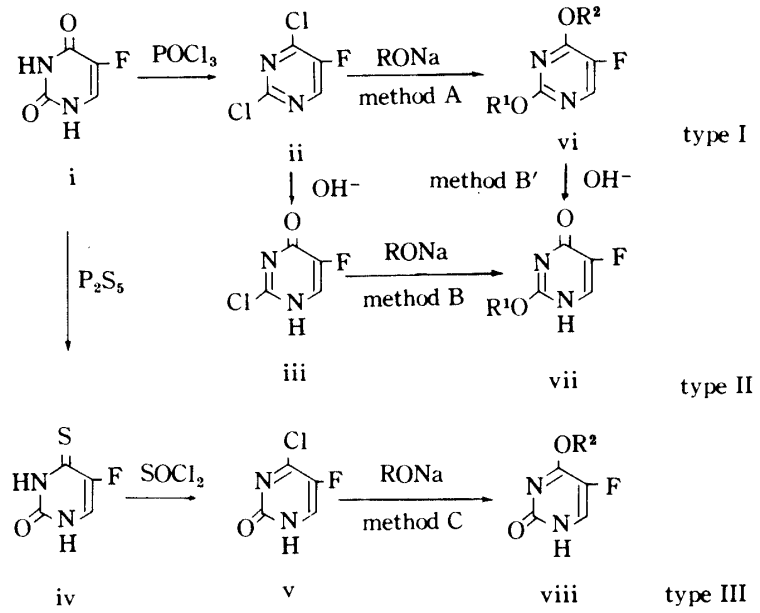


Chart 1. Synthetic Methods for Type I, II and III Compounds

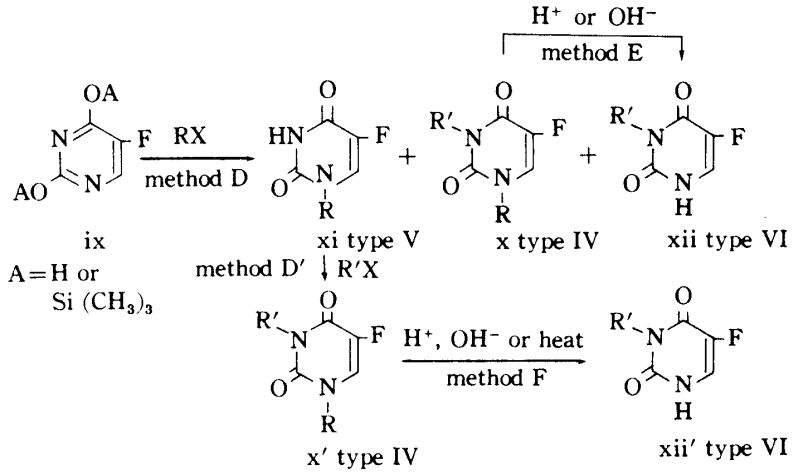


Chart 2. Synthetic Method for Type IV, V and VI Compounds

chloro-6-acetoxymethyltetrahydropyran gave 1-(6-acetoxymethyltetrahydro-2-pyranyl)-5-fluorouracil, which was deacetylated with NH_3-saturated methanol to give 1-(6-hydroxymethyltetrahydro-2-pyranyl)-5-fluorouracil (**33**) (type V).

Treatment of 5-FU with acyl halide in pyridine gave compounds **34** to **38** (type V) and compound **47** (type IV, x). Reaction of 2-*O*, 4-*O*-bis(trimethylsilyl)-5-fluorouracil with acetal using sodium iodide as a catalyst in acetonitrile gave 1-ethoxymethyl-5-fluorouracil (**49**) and 1-(1-ethoxy-1-phenylmethyl)-5-fluorouracil (**50**) (type V) and compound **48** (type IV, x).

3-(Tetrahydro-2-furyl)-5-fluorouracil (type VI, xii) was synthesized from 1-methanesulfonyl-5-fluorouracil according to the method reported previously.[17] Thermal decomposition of 3-methoxycarbonylmethyl-1-(tetrahydro-2-furyl)-5-fluorouracil (**25**) gave 3-methoxycarbonylmethyl-5-fluorouracil (**40**) (type VI, xii'). Reaction of 2-*O*, 4-*O*-bis(trimethylsilyl)-5-fluorouracil with benzoyl chloride in pyridine gave 1,3-dibenzoyl-5-fluorouracil, which was solvolyzed in ethanol to 3-benzoyl-5-fluorouracil (**43**) (type VI, xii).

Treatment of 5-FU with hydroxylamine-*O*-sulfonic acid gave 3-amino-5-fluorouracil.[18] Acylation of 3-amino-5-fluorouracil with *n*-butyric anhydride gave compound **41**. Compound **42** was synthesized similarly from *p*-toluenesulfonyl chloride.

The structures of these compounds were confirmed by elemental analysis, and ultraviolet (UV), nuclear magnetic resonance (NMR) and mass spectroscopy.[19] The results of elemental analysis and the UV spectral data are presented in Table I and II.

Biological Activities

Since metabolism by drug-metabolizing enzymes in liver microsomes, spontaneous hydrolysis and other enzymatic hydrolysis all contribute to the activation process of orally

Table III. Plasma Levels of 5-FU after Oral Administration of the Compounds of Types I—VI

Compd. No.	Released 5-FU (μg/ml) 1	4	8 (h)	Compd. No.	Released 5-FU (μg/ml) 1	4	8 (h)
Type I				**24**	0.02	0.02	<0.02
1	<0.02	0.07	0.04	**25**	<0.02	<0.02	<0.02
2	<0.02	<0.02	0.04	Type IV			
3	6.61	0.78	0.35	**26**	<0.02	<0.02	<0.02
4	0·02	0.04	0.04	**27**	1.47	0.35	0.03
5	0.02	0.08	0.06	**28**	<0.02	<0.02	<0.02
6	2.50	3.60	6.50	**29**	<0.02	<0.02	0.03
7	0.05	0.02	0.03	**45**	<0.02	<0.02	<0.02
Type II				**47**	—	0.95	0.26
8	0.28	0.38	0.24	**48**	0.02	0.07	0.06
9	0.03	0.04	0.04	Type V			
10	0.38	0.87	0.49	**30**	<0.02	<0.02	<0.02
11	0.42	0.33	0.81	**31**	0.03	0.02	<0.02
12	0.19	0.27	0.26	**32**	0.03	0.14	0.10
13	0.84	1.95	1.12	**34**	0.66	0.02	<0.02
14	0.52	0.69	0.54	**36**	0.08	<0.02	<0.02
15	0.06	0.24	0.05	**38**	15.00	3.20	0.26
17	<0.02	0.04	0.04	**49**	0.11	0.14	0.08
18	0.33	0.50	0.28	Thf-FU	0.08	0.20	0.13
19	0.88	2.14	1.93	Type VI			
20	0.02	0.02	0.03	**39**	8.83	2.50	0.51
Type III				**40**	<0.02	0.02	<0.02
21	0.94	0.80	0.33	**41**	<0.02	<0.02	<0.02
Type IV				**42**	<0.02	<0.02	<0.02
22	<0.02	<0.02	<0.02	**43**	4.70	0.11	<0.02
23	<0.02	<0.02	<0.02				

Rats (3 animals/group) weighing about 150 g were deprived of food for 18 h before oral administration of the compounds. Blood samples were collected every 1, 4, 8 h after administration.

administered compounds, the blood levels of 5-FU were determined (Table III). Among the synthesized derivatives, *N*-acyl or *N*-sulfonyl derivatives are most easily activated to 5-FU, but these compounds are not suitable as a prodrug for oral administration because of their lability. Other *N*-acyl or *N*-sulfonyl derivatives have also been reported to be too labile to apply through the oral route.[20]

The order of 5-FU releasing ability in *O*-substituted derivatives was as follows; 4-*O*-substituted derivative>2-*O*-substituted derivatives>2,4-di-*O*-subtituted derivatives. Generally, 2-*O*-substituted derivatives are more easily activated to 5-FU than Thf-FU and substituents do not modify the characteristic. In contrast, among 2,4-*O*-di-*O*-substituted derivatives, a 2,4-di-*tert*-butoxy derivative **3** and a 2,4-dibenzyloxy derivative **6** show higher blood concentrations of 5-FU that than produced by Thf-FU.

N-Alkyl substituted derivatives were not activated to 5-FU, but some 1-alkoxymethyl substituted derivatives show durable blood levels, though these are still low because the activation of the derivatives is mainly by a drug metabolizing enzyme. Thf-FU is known to be metabolized to 5-FU by a drug-metabolizing-enzyme in liver microsomes.[21] 1-Ethoxymethyl-5- fluorouracil (**49**) and 1-(1-ethoxy-1-phenylmethyl)-5-fluorouracil (**50**) were also found to be substrates for the enzyme. These results will be reported elsewhere.

A 1,3-disubstituted derivative, 1,3-bis(tetrahydro-2-furyl)-5-fluorouacil (Thf_2-FU, **27**), has been reported to be activated by both spontaneous hydrolysis and metabolism in liver microsomes, and to maintain a higher blood concentration of 5-FU than Thf-FU.[22] Since Thf_2-FU has been reported to show much greater antitumor activity than Thf-FU,[23] it is expected that derivatives which result in higher blood levels of 5-FU than Thf-FU may be more effective as prodrug.

From among the *O*-substituted derivatives, the 2-butoxy derivative **11**, 2-benzyloxy derivative **19**, 4-butoxy derivative **21** and 2,4-dibenzyloxy derivative **6** were selected and their antitumor activities against Ehrlich carcinoma, sarcoma 180 and AH 130 carcinoma were examined (Table IV). The 2,4-dibutoxy derivative **1** was also tested for comparison.

Table IV. Antitumor Effects of **1**, **6** (Type I), **11**, **19** (Type II) and **21** (Type III) on Ehrlich Carcinoma, Sarcoma 180 and AH 130 Carcinoma[a]

Compd.	Inhibition			
	Dose (mmol/kg)	Ehrlich carcinoma[b]	Sarcoma 180[b]	AH 130 carcinoma[c]
1	0.45	51	15	9
6	0.45	10	58	14
11	0.45	64	62	25
19	0.45	52	70	5
21	0.45	—[d]	5	—[d]
Thf-FU	0.45	39	64	12
5-FU	0.25	61	49	—[d]
	0.10	41	20	—[d]

a) See Experimental. *b*) Mice were used. *c*) Rats were used. *d*) Not tested.

From among the *N*-substituted derivatives of 5-FU, the 1-ethoxymethyl derivative **49**, 1-(1-ethoxy-1-phenylmethyl) derivative **50**, 3-(tetrahydro-2-furyl) derivative **39** and 1,3-bis-(ethoxymethyl) derivative **48** were selected and their antitumor activities were examined (Table V).

Two 2-*O*-substituted derivatives, **11** and **19**, are as effective as Thf-FU against both Ehrlich carcinoma and sarcoma 180. 2,4-Di-*O*-substituted derivatives, **1** and **6**, are as effective as Thf-FU against Ehrlich carcinoma and against sarcoma 180, respectively. The 4-*O*-substituted derivative, **21** shows no activity against sarcoma 180, although it produces a significant

Table V. Antitumor Effects of **48** (type IV), **49**, **50** (type V) and **39** (type VI) on Ehrlich Carcinoma, Sarcoma 180 and AH 130 Carcinoma[a]

Compd.	Dose (mmol/kg)	Inhibition (%) Ehrlich carcinoma[b]	Sarcoma 180[b]	AH 130 carcinoma[c]
48	0.45	13	34	—[d]
49	0.45	38	45	—[d]
50	0.45	49	46	—[d]
39	0.45	—[d]	—[d]	80
	0.15	—[d]	—[d]	45
Thf-FU	0.45	31	43	35
5-FU	0.10	—[d]	—[d]	33

a) See Experimental. *b*) Mice were used. *c*) Rats were used. *d*) Not tested.

blood level of 5-FU.

The activities of the *N*-monosubstituted derivatives, **39**, **49** and **50**, were the same as that of Thf-FU, or greater. The activity of the 1,3-disubstituted derivative, **48**, is rather lower than that of Thf-FU. In view of the greater activity of Thf_2-FU[23] and the above results, among *N*-substituted derivatives, the derivatives which maintain higher blood levels of 5-FU than Thf-FU show greater activities than Thf-FU. In contrast, the antitumor activities of *O*-substituted derivatives were variable. Therefore, the blood concentration of 5-FU cannot always be used as an index for selection.

The oral LD_{50} values were determined in mice by administering the compounds for three weeks. The LD_{50} values were 945 mg/kg for **11**, 894 mg/kg for **19**, 5000 mg/kg for **1** and **6**, and 2664 mg/kg for Thf_2-FU, while they were 800 mg/kg for Thf-FU and 115 mg/kg for 5-FU. Thus, some of the derivatives selected in the present study show higher LD_{50} values than Thf-FU.

Experimental

UV absorption spectra were obtained with a Hitachi 124 spectrometer and elemental analyses were done with a Yanagimoto CHN corder MT-2 type apparatus. Melting points were determined with a Yanagimoto microanalysis apparatus and are reported as uncorrected values.

2,4-Dibutoxy-5-fluoropyrimidine (**1**)——2,4-Dichloro-5-fluoropyrimidine[14] (10 g, 0.06 mol) was dissolved in a solution of *n*-C_4H_9ONa in *n*-C_4H_9OH, prepared by dissolving sodium (4.2 g, 0.18 mol) in *n*-C_4H_9OH (150ml), and the mixture was refluxed for 3 h. The mixture was cooled, then water and benzene were added, and the organic layer was separated. The organic layer was dried over Na_2SO_4, concentrated and distilled. On distillation at 125—126°C/3—4 mmHg, 12 g of **1** was obtained (82.8%). Compounds **2** to **7** were synthesized similarly.

2-Butoxy-5-fluoro-4(1*H*)-pyrimidone (**11**)——A mixture of 2,4-dibutoxy-5-fluoropyrimidine (**1**, 11.2 g, 0.046 mol) in EtOH (50 ml) and 2 N KOH (180 ml) was heated for 18 h at 90—100°C. The mixture was cooled, and extracted with Et_2O. The aqueous layer was separated and the pH was adjusted to 4—5 with HCl. The precipitate was recrystallized from EtOH, giving 7.2 g of **11** in 86.2% yield.

Compounds **8**, **9**, **12**, **15**, **17**, **18**, **19** and **20** were synthesized similarly.

5-Fluoro-2-pentyloxy-4(1*H*)-pyrimidone (**13**)——2-Chloro-5-fluoro-4(1*H*)-pyrimidone[14] (4.0 g, 0.027 mol) was added to a solution of *n*-$C_5H_{11}ONa$ in *n*-$C_5H_{11}OH$, prepared by dissolving sodium (1.6 g, 0.07 mol) in *n*-$C_5H_{11}OH$ (40 ml), and the mixture was heated at 160°C for 9 h in a sealed tube. The solvent was evaporated off, and the residue was dissolved in water. The pH was adjusted to 4—5. The precipitate was recrystallized from EtOH, giving 4.2 g of **13** in 77.8% yield.

Compounds **10**, **14** and **16** were synthesized similarly.

3-Methyl-1-(tetrahydro-2-furyl)-5-fluorouracil (**22**)——A mixture of Thf-FU (10 g, 0.05 mol), dimethyl sulfate (7.1 ml, 0.076 mol) and K_2CO_3 (27.6 g, 0.2 mol) in acetone (250 ml) was stirred for 12 h at room temperature. Then water (200 ml) and saturated K_2CO_3 solution were added with stirring and the mixture was stirred for 0.5 h at room temperature. The acetone was evaporated off and the residue was extracted with $CHCl_3$ (400 ml). The $CHCl_3$ layer was washed with water, dried over Na_2SO_4, and concentrated. The residue was recrystallized from EtOH, giving 9.13 g of **22** in 85.3% yield.

3-Methylthiomethyl-1-(tetrahydro-2-furyl)-5-fluorouracil (23)——Chloromethyl methyl sulfide (3.8 g, 0.04 mol) was added dropwise to a solution of Thf-FU (5.0 g, 0.025 mol) and K_2CO_3 (5.6 g, 0.04 mol) in dimethylsulfoxide (DMSO) (70 ml) below 10°C, and the mixture was stirred for 1 h at the same temperature then for 8 h at room temperature. The solvent was evaporated off. The residue was dissolved in water and extracted with $CHCl_3$. The $CHCl_3$ layer was separated, dried over Na_2SO_4 and concentrated. The residue was recrystallized from EtOH, giving 6.05 g of **23** in 93.9% yield.

Compounds **24**, **25**, **28** and **29** were synthesized similarly.

1-(Tetrahydro-2-furylmethyl)-5-fluorouracil (31)——A mixture of 5-FU (6.5 g, 0.05 mol) and tetrahydrofurfurylbromide (4.2 g, 0.025 mol) in DMSO (100 ml) was heated for 24 h at 80—90°C. The mixture was cooled and water (100 ml) was added. The pH was adjusted to 5.5—6.0 with HCl. The solution was extracted with $CHCl_3$, and the extract was concentrated. The residue was recrystallized from EtOH, giving 4.2 g of **31** in 42.1% yield.

Compound **30** was synthesized similarly.

1-(Tetrahydro-2-pyranyl)-5-fluorouracil (32)——Compound **32** was synthesized from 2-*O*,4-*O*-bis(trimethylsilyl)-5-fluorouracil and 2-chlorotetrahydropyran by the method described for the synthesis of Thf-FU.[16]

1-(6-Hydroxymethyltetrahydro-2-pyranyl)-5-fluorouracil (33)——An Et_2O solution (30 ml) of 2-chloro-6-acetoxymethyltetrahydropyran, prepared from 2-acetoxymethyl-2,3-dihydro-4*H*-pyran[24] (3.9 g 0.025 mol) and HCl, was added dropwise to a solution (10 ml) of 2-*O*,4-*O*-bis(trimethylsilyl)-5-fluorouracil (5.7 g, 0.021 mol) in toluene at −10—0°C, and the mixture was stirred for 2.5 h at the same temperature then for 13 h at room temperature. Then EtOH (10 ml) was added and the mixture was concentrated. The residue was extracted with $CHCl_3$ and the $CHCl_3$ layer was concentrated. The residue was recrystallized from EtOH–pet. ether, giving 4.3 g (75.2%) of 1-(6-acetoxymethyltetrahydro-2-pyranyl)-5-fluorouracil (mp 143.5—144°C). This compound (2.9 g) was dissolved in NH_3-saturated MeOH (300 ml) at 0°C. The solution was left for 70 h at 0°C and then concentrated. The residue was dissolved in water and the pH was adjusted to 3—4 with HCl. The solution was extracted with $CH_3CO_2C_2H_5$. The extract was concentrated and the residue was recrystallized from Et_2O, giving 2.1 g of **33** in 87.5% yield.

1-Ethoxymethyl-5-fluorouracil (34)——Ethyl chloroformate (16.3 g, 0.15 mol) was added to a mixture of 5-FU (6.5 g, 0.05 mol), pyridine (18 ml) and dioxane (400 ml), and the whole was stirred for 1 h at 70—90°C. The volatile materials were evaporated off and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with dil. HCl and water, dried over Na_2SO_4 and concentrated. The residue was recrystallized from benzene, giving 3.4 g of **34** in 31.5% yield.

Compounds **35** to **38** and **47** were synthesized similarly.

3-Methoxycarbonylmethyl-5-fluorouracil (40)——3-Methoxycarbonylmethyl-1-(tetrahydro-2-furyl)-5-fluorouracil (**25**, 3.0 g, 0.011 mol) was heated in a bath at 180°C. The reaction mixture was purified by silica gel column chromatography ($CHCl_3$: EtOH=10: 1 v/v), yielding 0.5 g of **40** (23%).

3-Butyrylamino-5-fluorouracil (41)——Butyric anhydride (4.4 g, 0.028 mol) was added to a solution (20 ml) of 3-amino-5-fluorouracil[18] (1.0 g, 0.007 mol) in dioxane, and the mixture was stirred for 9 h at 70—90°C. The solution was concentrated and the residue was purified by silica gel column chromatography ($CHCl_3$: EtOH=5: 1 v/v). The eluate was concentrated and the residue was recrystallized from EtOH, giving 0.7 g of **41** in 46.7% yield.

Compound **42** was synthesized similarly using *p*-toluenesulfonyl chloride.

3-Benzoyl-5-fluorouracil (43)——2-*O*,4-*O*-Bis(trimethylsilyl)-5-fluorouracil (11.3 g, 0.043 mol) was dissolved in dioxane (50 ml)–pyridine (5 ml), then benzoyl chloride (6.1 g, 0.043 mol) was added dropwise and the mixture was stirred for 5 h at room temperature. It was then poured into ice water, giving as a precipitate 7.7 g of 1,3-dibenzoyl-5-fluorouracil. A part (3.4 g) of the precipitate was dissolved in EtOH (100 ml) containing 0.37% HCl and the solution was refluxed for 0.5 h. The solvent was evaporated off and the residue was washed with Et_2O then extracted with acetone. The acetone was evaporated off and the residue was purified by silica gel column chromatography ($CH_3CO_2C_2H_5$: $CHCl_3$=5: 1 v/v) to yield 0.8 g of **43** (34.2%).

1,3-Bis(tetrahydro-2-pyranyl)-5-fluorouracil (46)——Compound **46** was synthesized from 2-*O*,4-*O*-bis(trimethylsilyl)-5-fluorouracil and 2-acetoxytetrahydrofuran as described for the synthesis of 1,3-bis(tetrahydro-2-furyl)-5-fluorouracil (**27**).[4a]

3-Cyanoethyl-1-(tetrahydro-2-furyl)-5-fluorouracil (26)——Acrylonitrile (1.8 g, 0.033 mol) was added to a solution of Thf-FU (5.0 g, 0.025 mol) and Na_2CO_3 (1.3 g) in 50% aqueous EtOH, and the mixture was kept for 12 h at room temperature. Then the solvent was evaporated off and the residue was recrystallized from aqueous EtOH to yield 3.2 g of **26** (51%).

1,3-Bis(cyanoethyl)-5-fluorouracil (44)——A solution of 5-FU (6.5 g, 0.05 mol) in water (50 ml) was adjusted to pH 9.5—10 with Na_2CO_3. Then a solution of acrylonitrile (8.1 g, 0.15 mol) in dimethylformamide (DMF) (10 ml) was added and the mixture was stirred for 7 h at 35—40°C. The mixture was concentrated and the residue was recrystallized from aq. EtOH to yield 6.8 g of **44** (57.6%).

1,3-Bis(tetrahydro-2-furylmethyl)-5-fluorouracil (45)——5-FU (6.5 g, 0.05 mol), tetrahydrofurfuryl bromide (20 g, 0.12 mol), K_2CO_3 (14 g, 0.1 mol) and NaI (3.8 g, 0.025 mol) were added to DMSO (80 ml) and the mixture was stirred for 20h at 90—95°C. The reaction mixture was cooled, dissolved in water and

extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over Na_2SO_4 and concentrated. The residue was purified by silica gel column chromatography ($CHCl_3$: EtOH=10: 1 v/v) and 11.1 g of oily substance **45** was obtained (73.3%).

4-Butoxy-5-fluoro-2(1*H*)-pyrimidone (**21**)——4-Thio-5-fluorouracil (7.3 g, 0.05 mol) was dissolved in $SOCl_2$ (130 ml) in an ice bath and then stirred for 12 h at room temperature. Excess $SOCl_2$ was evaporated off and the residue was coevaporated with benzene.[15] The residue was dissolved in n-C_4H_9OH (300 ml) and the solution was stirred for 6 h at room temperature. Then the solvent was evaporated off, and water and $CHCl_3$ were added to the residue. The $CHCl_3$ layer was separated and concentrated. The residue was recrystallized from EtOH, giving 7.9 g of **21** in 85% yield.

1-Ethoxymethyl-5-fluorouracil (**49**)——A mixture of 2-*O*,4-*O*-bis(trimethylsilyl)-5-fluorouracil (33 g, 0.12 mol) and NaI (18 g, 0.12 mol) in acetonitrile (180 ml) was stirred for 0.5 h at room temperature. Then diethoxymethane (18.6 g, 0.18 mol) was added and the mixture was refluxed for 5 h. The solvent was evaporated off and the residue was extracted with $CHCl_3$. The $CHCl_3$ layer was washed with water, dried over Na_2SO_4 and concentrated. The residue was recrystallized from EtOH, giving 10 g of **49** in 44% yield.

Compounds **48** and **50** were synthesized similarly.

Determination of Blood Level of 5-FU——Compounds were given orally (1 mmol/kg) to male Wistar strain rats. The plasma (1 ml) was diluted with 1 ml of physiological saline and the pH was adjusted to 2 with 5 N HCl. This solution was extracted twice with 20 ml of chloroform. The aqueous layer was centrifuged after neutralization. The supernatant was used for bioassay by the thin layer-cup method.[25]

Antitumor Test——Mice of the ddY strain were used for tests on Ehrlich carcinoma, ICR strain mice for tests on sarcoma 180 and Donryu strain rats for tests on AH 130 carcinoma. Animals were inoculated subcutaneously in the axillary region with 5×10^6 tumor cells and given test compounds orally or intraperitoneally once a day for 7 consecutive days beginning 24 h after inoculation of tumor cells. 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.

Acute Toxicity——Test compounds were given orally to male ddY strain mice of 5 weeks of age and the LD_{50} values were calculated as described by Litchfield and Wilcoxon.[26]

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