Studies on the Synthesis of Chemotherapeutics. 10.[1] Synthesis and Antitumor Activity of *N*-Acyl- and *N*-(Alkoxycarbonyl)-5-fluorouracil Derivatives[2]

Tetsuji Kametani,*

Pharmaceutical Institute, Tohoku University, Aobayama, Sendai 980, Japan

Kazuo Kigasawa, Mineharu Hiiragi, Kikuo Wakisaka, Seiji Haga, Yasuo Nagamatsu, Hideo Sugi, Kazunaga Fukawa, Osamu Irino, Teruo Yamamoto, Naoyuki Nishimura, Akira Taguchi,

Research Laboratories, Grelan Pharmaceutical Company Ltd., Sakurashinmachi, Setagayaku, Tokyo 154, Japan

Taiji Okada,

Okada Hospital, Motoujinacho, Hiroshima 732, Japan

and Mitsuru Nakayama

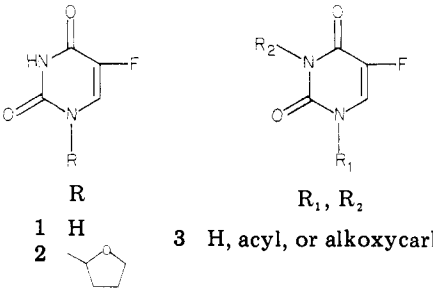
Faculty of Science, Hiroshima University, Higashisendacho, Hiroshima 730, Japan. *Received April 18, 1980*

A number of *N*-acyl and *N*-(alkoxycarbonyl)-5-fluorouracil derivatives possessing, for example, benzoyl, *o*-toluyl, acetyl, propionyl, heptanoyl, ethoxycarbonyl, phenoxycarbonyl, and benzyloxycarbonyl groups as N_1 and/or N_3 substituents were synthesized, and their antitumor activities were evaluated. The synthesis was achieved by a direct and two-step acylation of 5-fluorouracil (1) and by selective N_1-deacetylation of N_1-acetyl-N_3-substituted-5-fluorouracil under appropriate reaction conditions. Several N_3-benzoyl- and N_3-*o*-toluyl-5-fluorouracil derivatives (**11–15**, **24–26**, and **29**) showed significant activity against experimental tumor, and N_1-acetyl-N_3-*o*-toluyl-5-fluorouracil (**12**) was found to be most promising among them. Further investigation revealed **12** to retain higher activity toward various tumors, with lower toxicity and good blood level, than either 1 or FT-207 (**2**), even for oral administration.

It is acknowledged that desirable properties of antitumor chemotherapeutics are low toxicity, high activity against solid tumor, and suitability for oral administration. Although 5-fluorouracil (5-FU, **1**; Chart I)[3] and N_1-(2-tetrahydrofuryl)-5-fluorouracil (FT-207, **2**)[4] have been clinically used as effective antitumor agents, they have been shown to possess high toxicity and poor tumor affinity. In order to minimize theses defects, chemical modification of **1** and **2** has been extensively studied.[5]

In connection with synthetic work on chemotherapeutics, we previously reported a novel synthesis of **2**.[6] The present paper deals with the synthesis and evaluation of the antitumor activity of numerous *N*-acyl- and *N*-(alkoxycarbonyl)-5-fluorouracil derivatives (**3**), which are expected to function as masked forms of **1**, and includes a brief discussion on the SAR of the synthetic compounds (**3**). Of these, N_1-acetyl-N_3-*o*-toluyl-5-fluorouracil (**12**) appeared to be the most promising antitumor agent, even when administered orally.[7]

Chart I

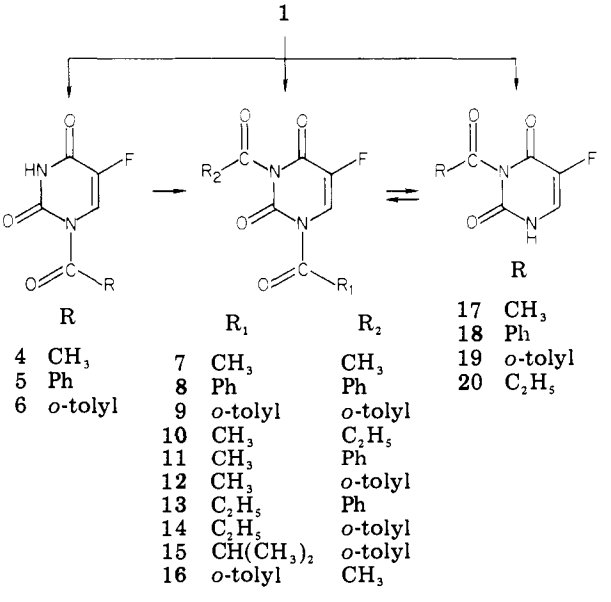


1 R = H

2 R =

3 R_1, R_2 = H, acyl, or alkoxycarbonyl

Scheme I



R	R_1	R_2	R
4 CH_3	7 CH_3	CH_3	17 CH_3
5 Ph	8 Ph	Ph	18 Ph
6 *o*-tolyl	9 *o*-tolyl	*o*-tolyl	19 *o*-tolyl
	10 CH_3	C_2H_5	20 C_2H_5
	11 CH_3	Ph	
	12 CH_3	*o*-tolyl	
	13 C_2H_5	Ph	
	14 C_2H_5	*o*-tolyl	
	15 $CH(CH_3)_2$	*o*-tolyl	
	16 *o*-tolyl	CH_3	

(1) Part IX: T. Kametani, K. Kigasawa, M. Hiiragi, K. Wakisaka, H. Sugi, and K. Tanigawa, *Chem. Pharm. Bull.*, **28**, 1196 (1980).

(2) This constitutes part 859 of "Studies on the Syntheses of Heterocyclic Compounds" by T. Kametani.

(3) R. Dushinsky and E. Pleven, *J. Am. Chem. Soc.*, **79**, 4559 (1957).

(4) (a) S. A. Hiller, R. A. Zhuk, and M. Yu. Lidak, *Dokl. Acad. Nauk SSSR*, **176**, 332 (1967); *Chem. Abstr.*, **68**, 29664*j* (1968). (b) S. A. Hiller, R. A. Zhuk, M. Yu. Lidak, and A. A. Ziermane, *British Patent*, 1168391 (1969).

(5) (a) C. Heidelberger, *Progr. Nucleic Acid Res. Mol. Biol.*, **4**, 1 (1965). (b) M. Yasumoto, I. Yamawaki, T. Marunaka, and S. Hashimoto, *J. Med. Chem.*, **21**, 738 (1978). (c) W. M. Odijk, M. J. Wanner, G. J. Coomen, and U. K. Pandit, *Heterocycles*, **9**, 1403 (1978). (d) G. J. Coomen, F. Alewijk, D. Blok, and U. K. Pandit, *ibid.*, **12**, 1535 (1979). (e) S. Ozaki, Y. Ike, H. Mizuno, K. Ishikawa, and H. Mori, *Bull. Chem. Soc. Jpn.*, **50**, 2406 (1977). (f) M. Tada, *ibid.*, **48**, 3427 (1975). (g) T. Seita, M. Kinoshita, and M. Imoto, *ibid.*, **46**, 1572 (1973). (h) W. Klotzer and M. Herberg, *Monatsh. Chem.*, **99**, 847 (1968). (i) S. A. Hiller, R. A. Zhuk, and G. Ya. Nashatyr, *Khim. Geterotsikl. Soedin.*, no. **3**, 577 (1968); *Chem. Abstr.*, **69**, 96641*h* (1968). (j) M. Yu. Lidak, R. Paegle, M. Plata, K. Ya. Pets, and Yu. P. Shvachkin, *ibid.*, no. **2**, 379 (1968); *Chem. Abstr.*, **69**, 96650*g* (1968). (k) R. Paegle, M. Yu. Lidak, and Yu. P. Shvachkin, *Khim. Geterotsikl. Soedin., Aka. Nauk Latr. SSR*, no. **2**, 316 (1966). (l) T. T. Sakai, A. L. Pogolotti, and D. V. Santi, *J. Heterocycl. Chem.*, **5**, 849 (1968); (m) M. Tada, *Chem. Lett.*, 129 (1975).

(6) T. Kametani, K. Kigasawa, M. Hiiragi, K. Wakisaka, O. Kusama, H. Sugi, and K. Kawasaki, *Heterocycles*, **6**, 529 (1977); *J. Heterocycl. Chem.*, **14**, 473 (1977).

(7) T. Okada, *Hiroshima J. Med. Sci.*, **28**, 49 (1979).

0022-2623/80/1823-1324$01.00/0

Table I. Reaction of 1 with Acyl Halides[a]

rxn temp:	room temp (15–25 °C)		higher temp (80 °C)	
base:	pyridine	triethylamine	pyridine	triethylamine
acetyl chloride	*b*	$N_{1,3}$; 7 (64.5%)	*b*	*b*
benzoyl chloride	N_3; 17 (85.2%)	$N_{1,3}$; 8 (54.0%)	*b*	$N_{1,3}$; 8 (50.3%)
o-toluyl chloride	N_3; 18 (84.5%)	$N_{1,3}$; 9 (55.0%)	*b*	$N_{1,3}$; 9 (10.9%)

[a] The major products are shown with their isolated yields, and N_1, N_3, and $N_{1,3}$ indicate the positions of acylation. [b] No major product was isolated, a mixture of the corresponding N_1-acyl, N_3- acyl, and N_1,N_3-diacyl compounds was obtained. See ref 10.

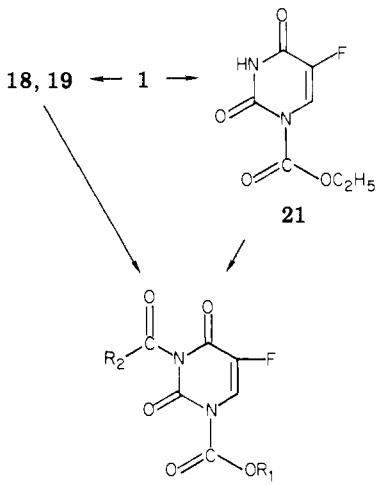
Chemistry. The structures of isolated compounds, most of which were submitted to biological experiments, were determined by spectral (IR and NMR) and elemental analyses. The position (on N_1 or N_3) of an introduced acyl group was easily determined from the NMR spectrum. Thus, the C_6 proton of an N_1-acylated derivative was found to resonate at lower field, by virtue of the anisotropic effect of the neighboring acyl group, than that of the N_3 analogue. The compounds which could not be isolated were detected on TLC by comparison with the authentic samples prepared by us.

(i) Synthesis of *N*-Acyl-5-fluorouracil Derivatives (7–20). Earlier work has shown[5m,8] that acetylation of 1 with acetic anhydride produced only N_1-acetyl-5-fluorouracil (**4**). A few *N*-acyl compounds, e.g., propionyl-, benzoyl-, *p*-nitrobenzoyl-, and 2-furoyl-5-fluorouracils, were reported as the products of acid halide acylation of **1**, but the position of acylation remained ambiguous in some cases.[9]

Since N-acylation of **1** was regarded as the key step in the preparation of our target compounds (**3**), we investigated the reaction of **1** with acyl halides in the presence of base catalyst under various conditions. It was found that the position of acylation was dependent on the reaction temperature employed (room temperature and 80 °C) and on the organic base (pyridine and triethylamine) and acylating agent used (e.g., acetyl, benzoyl, and *o*-toluyl chlorides).

In the presence of pyridine, the reaction of **1** with acetyl chloride produced a mixture of N_1-acetyl, N_3-acetyl, and N_1,N_3-diacetyl derivatives (**4**, **17**, and **7**, respectively)[10] at either room temperature or at 80 °C, whereas acylation using benzoyl and *o*-toluyl chlorides was observed to be temperature dependent (Scheme I). Thus, acylation at room temperature[14] (method A) yielded only the N_3-acyl-5-fluorouracils (**18**[9b,c] and **19**) in high yields, which led to the formation of a complicated mixture of the corresponding N_1-acyl, N_3-acyl, and N_1,N_3-diacyl derivatives[10] when the reaction temperature was increased to 80 °C (Table I).

Scheme II



	R_1	R_2		R_1	R_2
22	C_2H_5	Ph	**28**	cholesteryl	*o*-tolyl
23	Ph	Ph	**29**	Ph	*o*-tolyl
24	CH_3	*o*-tolyl	**30**	C_2H_5	CH_3
25	C_2H_5	*o*-tolyl	**31**	C_2H_5	C_2H_5
26	$(CH_2)_5CH_3$	*o*-tolyl	**32**	C_2H_5	$(CH_2)_5CH_3$
27	CH_2Ph	*o*-tolyl			

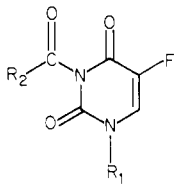
In the presence of triethylamine, the reaction of acetyl, benzoyl, and *o*-toluyl chlorides with **1** at room temperature[14] (method B), as well as at 80 °C (method C), gave the corresponding N_1,N_3-diacyl-5-fluorouracils (**7**, **8**,[9b] and **9**), with acetyl chloride reaction at 80 °C being an exception in which a mixture of the N_1-acyl, N_3-acyl, and N_1,N_3-diacyl compounds[10] was obtained (Table I).

Two procedures, N_3-acylation of the N_1-acyl-5-fluorouracils (**4** and **6**) and N_1-acylation of the N_3 analogues (**18** and **19**), were investigated for the preparation for N_1,N_3-diacyl-5-fluorouracils with different acyl groups at the N_1 and N_3 positions. In a preliminary experiment, pyridine-catalyzed toluylation of **4** was accompanied by loss of the N_1-acetyl group to furnish **19** and **6**, depending on the reaction temperature. However, the first procedure, using triethylamine in place of pyridine, was successfully employed for the preparation of **7**,[13] **10–12**, and **16**. Yields were generally improved by employing lower reaction temperatures and by using dioxane as solvent (method D). According to the second procedure, the requisite diacyl derivatives (**11**[13] and **13–15**) were readily obtained by acid halide acylation of **18** and **19** in the presence of pyridine or triethylamine, in which loss of the N_3-acyl group was negligible (method E). Acid anhydrides[7] have also been successfully employed in this method (Table II).

Selective removal of the N_1-acetyl group of the N_1,N_3-diacyl compounds (**7** and **10**) was also examined. Although hydrolysis of **7** and **10** by the usual inorganic acid or base did not show any selectivity in deacylation, their treatment with protic solvents (e.g., methanol and acetic acid) led to formation of the required monoacyl derivatives

(8) Hoffman La Roche Inc., *Japan Pat.*, 37-8284 (1962).
(9) (a) Mitsui Toatsu, *Japanese Patent*, 53-24952 (1978); (b) Asahi Kasei, *Japanese Kokai Patent*, 51-86479-86480 (1976); (c) Mitsui Toatsu, *ibid.*, 52-91880 (1977).
(10) These products were detected on TLC by comparison with authentic samples prepared by an alternative method described in this paper for compounds **6–9** and **17–19** and the literature[5m] for compounds **4** and **5**. The TLC plates used throughout were E. Merck precoated silica gel 60 F_{254}.
(11) It is interesting to compare this result with the selective hydrolysis of N_1,N_3-bis(2-tetrahydrofuryl)-5-fluorouracil to produce FT-207 (**2**).[5b,6]
(12) (a) Ono Yakuhin, *Japanese Kokai Patent*, 53-31674 (1978); (b) Mitsui Toatsu, *ibid.*, 53-2483 (1978).
(13) This compounds showed an identical melting point and IR and NMR spectra to those of an authentic sample prepared by an alternative method already described.
(14) Room temperature is in the range of 15–25 °C in this paper.

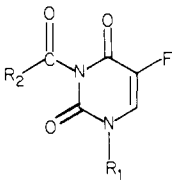
Table II. *N*-Acyl-5-fluorouracil Derivatives (7-20)[a]



no.	R_1	R_2	method	yield, %	mp, °C (recrystn solvent)	formula[b]
7	CH_3CO	CH_3	B	64.5	111-113 (ether)	$C_8H_7FN_2O_4$
			D	55.6		
8	Ph	Ph	B	54.0	169-171 (ethanol)[c]	$C_{18}H_{11}FN_2O_4$
			C	50.3		
9	*o*-toluyl	*o*-tolyl	B	55.0	158-162 (ether)	$C_{20}H_{15}FN_2O_4$
			C	10.9		
10	CH_3CO	C_2H_5	D	66.3	86-89 (ether)	$C_9H_9FN_2O_4$
11	CH_3CO	Ph	D	36.2	128-130 (ether)	$C_{13}H_9FN_2O_4$
			E	86.9		
12	CH_3CO	*o*-tolyl	D	83.4	141-143 (ether)	$C_{14}H_{11}FN_2O_4$
13	C_2H_5CO	Ph	E	84.7	124-126 (ether)	$C_{14}H_{11}FN_2O_4$
14	C_2H_5CO	*o*-tolyl	E	89.7	134-136 (ether)	$C_{15}H_{13}FN_2O_4$
15	$(CH_3)_2CHCO$	*o*-tolyl	E	91.6	126-127 (ether)	$C_{16}H_{15}FN_2O_4$
16	*o*-toluyl	CH_3	D	69.8	112-114 (ether)	$C_{14}H_{11}FN_2O_4$
17	H	CH_3	F	62.1	114-117 (chloroform)	$C_6H_5FN_2O_3$
18	H	Ph	A	85.2	170-172[d] (chloroform)	$C_{11}H_7FN_2O_3$
19	H	*o*-tolyl	A	84.5	165-166 (chloroform)	$C_{12}H_9FN_2O_3$
20	H	C_2H_5	F	60.4	99-102 (chloroform)	$C_7H_7FN_2O_3$

[a] All compounds were obtained as colorless crystals. [b] Analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values. [c] Lit.[9b] mp 195 °C. [d] Lit.[9b] mp 170 °C; lit.[9c] mp 170-171 °C.

Table III. *N*-(Alkoxycarbonyl)-5-fluorouracil Derivatives (22-32 and 35-38)[a]



no.	R_1	R_2	method	yield, %	mp, °C (recrystn solvent)[b]	formula[c]
22	$COOC_2H_5$	Ph	G	87.6	105-107	$C_{14}H_{11}FN_2O_5$
			H	66.0		
23	COOPh	Ph	G	91.9	154-157	$C_{18}H_{11}FN_2O_5$
24	$COOCH_3$	*o*-tolyl	G	85.9	125	$C_{14}H_{11}FN_2O_5$
25	$COOC_2H_5$	*o*-tolyl	G	69.5	115-116	$C_{15}H_{13}FN_2O_5$
			H	77.2		
26	$COO(CH_2)_5CH_3$	*o*-tolyl	G	73.9	96-97	$C_{19}H_{21}FN_2O_5$
27	$COOCH_2Ph$	*o*-tolyl	G	77.3	135-138	$C_{20}H_{15}FN_2O_5$
28	COO-cholesteryl	*o*-tolyl	G	67.3	174-175	$C_{40}H_{53}FN_2O_5$
29	COOPh	*o*-tolyl	G	85.9	129-130	$C_{19}H_{13}FN_2O_5$
30	$COOC_2H_5$	CH_3	H	63.4	62-63	$C_9H_9FN_2O_5$
31	$COOC_2H_5$	C_2H_5	H	52.1	97-98	$C_{10}H_{11}FN_2O_5$
32	$COOC_2H_5$	$(CH_2)_5CH_3$	H	84.9	67-68	$C_{14}H_{19}FN_2O_5$
35	$COCH_3$	OCH_2Ph	I	56.6	118-119 (ether)	$C_{14}H_{11}FN_2O_5$
36	H	OC_2H_5	J	10.1[d]	128-130 (chloroform)	$C_7H_7FN_2O_4$
37	H	OPh	J	16.3[d]	159-162 (chloroform)	$C_{11}H_7FN_2O_4$
38	H	OCH_2Ph	J	54.0	159-160 (chloroform)	$C_{12}H_9FN_2O_4$

[a] All compounds were isolated as colorless crystals. [b] Benzene-ether unless otherwise specified. [c] Analyzed for C, H, and N; analytical results were within ±0.4% of the theoretical values. [d] Based on the starting 4.

17 and **20**, respectively (method F). This result suggested that the N_1-acyl group is more labile toward solvolysis than is the N_3-acyl group.[11]

(ii) Synthesis of *N*-(Alkoxycarbonyl)-5-fluorouracil Derivatives (22-38). The reaction of **1** with alkyl chlorocarbonates is known to produce only the N_1-(alkoxycarbonyl) compounds (**21** and its homologues).[12] Two procedures, N_1-alkoxycarbonylation of **18** and **19** and N_3-acylation of **21**, were used for the preparation of the title compounds (**22-32**) (Scheme II). Thus, **18** and **19** were treated with various alkyl chlorocarbonates in the presence of triethylamine (method G) to afford **22-29** in fair yields, and acid halide acylation of **21** (method H) produced **22**, **25**, and **30-32** in good yields. The use of acetone as solvent led to higher yields in both methods (Table III).

Since N_3-(alkoxycarbonyl)-5-fluorouracils can not be prepared directly from **1**,[12] solvolytic removal of the N_1-acetyl group of the N_1-acetyl-N_3-(alkoxycarbonyl)-5-fluorouracils (**33-35**) was investigated. The intermediate compounds (**33-35**) were prepared by N_3-alkoxycarbonylation of **4** using alkyl chlorocarbonates (method

Table IV. Antitumor Activity against P-388 Leukemia[a]

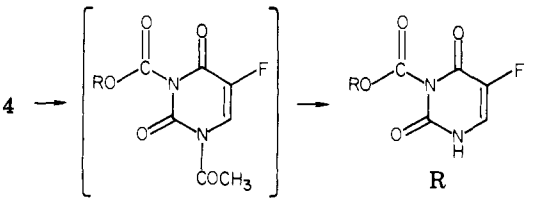
	% T/C				
dose, mg/kg:	200	100	50	25	12.5
5-FU (1)			76[b,c]	135[d]	127[e]
FT-207 (2)	106[b]	136	104	115	111
7		86[b]	103[b]	112	103
11	99[b]	144	116	108	
12	121[b]	151	111	121	
13	90[b]	130	119	110	
14	138[b]	136	112	103	
15	169[b]	116	112	110	
16		108	114	97	111
19		103[b,f]	127[g]	124[h]	
22	83[b]	123	116	103	
23	107	119	112	99	
24	142[b]	138	112	101	
25	164[b]	127	125	112	
26	147	96	106	100	
27	110	125	108	108	
28	112	100	98		
29	135	129	106	100	
30		59[b]	115[b]	103	94
31	52[b]	82[b]	112	105	
32	74[b]	118	119	110	
35	112	116	105		
37		83[b]	101[b]	112	97
38	96	110	101		

[a] T/C ≥ 130% is the criterion for activity; see Experimental Section. [b] Decrease of body weight was observed. [c-h] Exact dosages were: *c*, 40; *d*, 20; *e*, 10; *f*, 120; *g*, 60, and *h*, 30 mg/kg.

I) (Scheme III). Various solvolysis procedures were investigated and reactions were monitored by TLC analysis in each case. In aprotic solvents (e.g., acetone or acetonitrile), negligible loss of the N_1-acetyl group from **33–35** was observed. Reaction in protic solvents (e.g., methanol and ethanol) proceeded slowly at room temperature,[14] with the starting material being almost consumed after several hours. The addition of an organic base (e.g., triethylamine) to the protic medium (method J) greatly accelerated N_1-deacetylation, with the required derivatives (**36–38**) being produced after a few minutes at room temperature. Subsequent workup, however, resulted in low yields of products.

The above results, in parts (i) and (ii), are summarized by the following statements: (a) In the acylation of **1**, higher reaction temperature causes a lowering of the overall yield of acylation and a reduction in selectivity regarding the position of acylation. (b) Triethylamine seems to be a far more effective base catalyst than pyridine for N_1,N_3-diacylation of **1**. (c) With regard to the acylating agents, a difference was found in the reactivity of the acid anhydride[7,8] and the acid halide. The former reagent is suitable for selective N_1-acylation, while the latter can be effectively employed for acylation of vacant N_1 and/or N_3 position. (d) The N_1-acyl group on a diacyl derivative of

Scheme III



R

33 C_2H_5
34 Ph
35 CH_2Ph

36 C_2H_5
37 Ph
38 CH_2Ph

1 possesses a greater lability[9c] toward solvolysis and can, thus, be selectively removed.

Biological Results and Discussion

Antitumor Activity against P-388 (Table IV). In vivo screening of the synthesized compounds (**7**, **11–16**, **19**, **22–32**, **35**, **37**, and **38**) was carried out against the P-388 leukemia system in female BDF_1 mice. The available results, together with those for 5-FU (**1**) and FT-207 (**2**), are summarized in Table IV. Inspection of Table IV reveals that several of the compounds possessing benzoyl and *o*-toluyl groups on the N_3 position showed an activity surpassing the criterion of T/C > 130 and comparable to those of the 5-FU and FT-207. None of the other compounds displayed any significant activity. The N_3-*o*-toluyl compounds were slightly more inhibitory than the N_3-benzoyl analogues, which seemed rather toxic at higher doses. When the comparison is limited to the N_3-*o*-toluyl homologues themselves, it is observed that N_1-acetyl-N_3-*o*-toluyl-5-fluorouracil (**12**) showed the highest activity without toxicity against normal body weight growth of mice, as well as the highest activity at lower dose. Elongation of the alkyl chain of acyl and alkoxycarbonyl groups resulted in decreased activity, and a higher dose of such compounds was required in order to produce significant activities. Thus, derivative **12** was selected for further screening.

Antitumor Activity of Compound 12 against Various Tumors (Table V). Compound **12** was compared with FT-207 (**2**), a masked form of 5-FU (**1**), for activity against various tumors, and the results are summarized in Table V.

Against P-388 tumor, both were most active at a dose of 100 mg/kg, but **12** was more active than FT-207. At a dose of 200 mg/kg, **12** was less active, while FT-207 was toxic. Moreover, both substances produced a decrease in body weight at this dosage.

Against L-1210 tumor, **12** and FT-207 showed approximately the same level of activity at a dose of 200 mg/kg. **12** was most active at a dose of 300 mg/kg, though body weight loss was observed. On the contrary, FT-207 was

Table V. Antitumor Activity of Compound 12 and FT-207 against P-388, L-1210, Meth A, and MH 134 Tumors[a]

			% T/C			
tumor	compd	dose, mg/kg: 300	200	100	50	25
P-388[c]	12		121[b]	151	111	121
	FT-207		106[b]	136	104	115
L-1210[c]	12	167[b]	136	128	110	
	FT-207	102[b]	143	124	110	
Meth A[d]	12		32	58	71	
	FT-207		19	53	82	
MH 134[d]	12		25	43	61	
	FT-207		57[b]	58	70	

[a] See Experimental Section. [b] Decrease of body weight was observed. [c] Survival time. [d] Tumor weight.

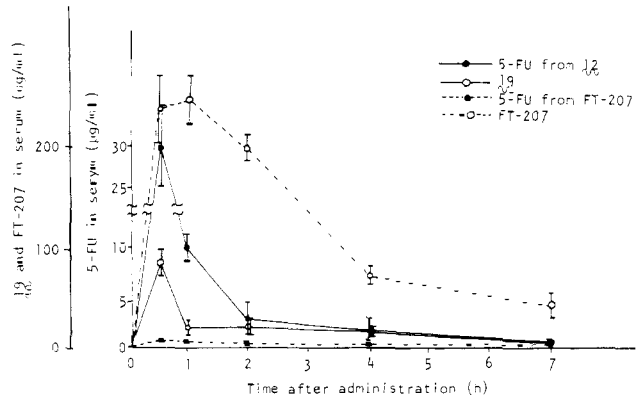


Figure 1. Serum concentration of metabolites in mice after oral administration of compound 12 and FT-207.

inactive at this dosage accompanying a decrease in body weight.

Against Meth A, both compounds showed almost the same activity at a dose of 100 mg/kg, while FT-207 was more effective than **12** at a dose of 200 mg/kg.

Against MH-134, **12** was more effective than FT-207 at a dose of 100 mg/kg and much more effective at a dose of 200 mg/kg. At a dose of 200 mg/kg, a decrease in body weight was observed for mice treated with FT-207 but not for those treated with compound **12**. The above results suggest that compound **12** is more active than FT-207 against P-388, L-1210, and MH 134 tumors.

Acute Toxicity of Compound 12 and FT-207. The LD_{50} value for FT-207 was found to be 1750 mg/kg. With compound **12**, only one of six mice died following the highest dose of 2400 mg/kg, on the 6th day after administration. Spontaneous motor activity was suppressed in mice treated with compound **12** where the dose was greater than 1600 mg/kg, while the corresponding dosage for treatment with FT-207 is over 800 mg/kg. Furthermore, both compounds produced piloerection and tremor at doses of over 1200 mg/kg and convulsion at doses of over 2400 mg/kg.

Serum Concentration of Metabolites of Compound 12 and FT-207. The activity of compound **12** and FT-207 is assumed to be due to their biotransformation to 5-FU in serum and tissue. In the case of oral administration of **12**, the N_1-deacetylated compound **19**, rather than **12** itself, was detected in the serum. The results of an investigation of serum metabolite concentrations are recorded in Figure 1. The serum concentration of 5-FU in mice treated with FT-207 was comparatively low (0.4 μg/mL at maximum), although the concentration of FT-207 itself was high (34.8 μg/mL at maximum). For mice treated with compound **12**, the serum concentration of 5-FU reached a maximum of 29,5 μg/mL at 0.5 h and decreased rapidly to 1 μg/mL at 4 h and 0.4 μg/mL at 7 h. Although the FT-207 concentration at any time was three or more times the concentration of compound **19**, the level of 5-FU in mice treated with compound **12** was at all times higher than the level in mice treated with FT-207. This leads us to the conclusion that metabolite **19** is biotransformed to 5-FU much more effectively than is FT-207.

Experimental Section

All melting points were taken on a Yanagimoto micro melting point apparatus and are uncorrected. NMR spectra were recorded on a JEOL PMX-60 spectrometer using tetramethylsilane as internal standard. Where microanalyses are indicated only by symbols for the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

Acylation of 1 in the Presence of Pyridine (Table I). (a) Reaction at Room Temperature (Method A). A solution of 1.3 g (0.01 mol) of **1** in 24 mL of pyridine was added dropwise over 10 min to a stirred mixture of 0.03 mol of acyl chloride and 6 mL of pyridine. The reaction mixture was stirred for 1 h at room temperature,[14] poured into cold water, and extracted with benzene. The extract was washed (water), dried (Na_2SO_4), and evaporated. The resulting oily residue was thoroughly washed with *n*-hexane and crystallized from ether. Using benzoyl and *o*-toluyl chlorides, this method was used to prepare **18**[9b,c] and **19**, respectively. In the case of acetyl chloride, an unseparable mixture of **4**, **7**, and **17** was obtained. **18**: NMR (acetone-d_6) δ 7.72 (1 H, d, J = 6 Hz, C_6H). **19**: NMR (Me_2SO-d_6) δ 2.60 (3 H, s, CH_3), 8.02 (1 H, d, J = 6.5 Hz, C_6H).

(b) Reaction at 80 °C. Employing the same procedure as for method A, while maintaining the reaction temperature at 80 °C, the above reactions were carried out to yield unseparable mixtures of the corresponding N_1-acyl, N_3-acyl, and N_1,N_3-diacyl compounds.[10]

Acylation of 1 in the Presence of Triethylamine (Table I). (a) Reaction at Room Temperature[14] (Method B). To a mixture of 1.3 g (0.01 mol) of **1**, 2.0 g (0.2 mol) of triethylamine, and 10 mL of dioxane was added over 10 min, with stirring, 0.03 mol of acyl chloride. After the mixture was stirred for an additional 1 h, dioxane and excess reagents were removed by evaporation. The residue was admixed with water and extracted with benzene. Evaporation of the extract gave a semisolid, which was purified by recrystallization. This method, using acetyl, benzoyl, and *o*-toluyl chloride, was used to prepare **7**, **8**,[9b] and **9**, respectively. **7**: NMR ($CDCl_3$) δ 2.60 and 2.73 (each 3 H, s, CH_3), 8.25 (1 H, d, J = 7 Hz, C_6H). **8**: NMR ($CDCl_3$) δ 8.00 (1 H, d, J = 7 Hz, C_6H). **9**: NMR ($CDCl_3$) δ 2.42 and 2.62 (each 3 H, s, CH_3), 8.20 (1 H, d, J = 7 Hz, C_6H).

(b) Reaction at 80 °C (Method C). The above reactions were carried out at 80 °C and worked up as described for method B. **8** and **9**[13] were obtained from the corresponding acyl chloride. The use of acetyl chloride yielded an unseparable mixture of N_1-acetyl, N_3-acetyl, and N_1,N_3-diacetyl compounds (**4**, **7**, and **17**).[10]

Acylation of 4 in the Presence of Pyridine. To a mixture of 2.3 g (0.013 mol) of **4**, 10 mL of pyridine, and 50 mL of dioxane was added 6 g (0.039 mol) of *o*-toluyl chloride. The mixture was stirred for 2 h at room temperature and evaporated. The oily residue was washed with *n*-hexane and crystallized from ether. Recrystallization from chloroform gave 2.6 g (78.4%) of **19**[13] as colorless crystals.

From the reaction carried out at 80 °C, 0.45 g (20.3%) of **6** was obtained and trace amounts of **19** and **12** were detected by TLC analysis[10] of the remaining product. **6**: colorless crystals from chloroform; mp 173–175 °C; NMR (Me_2SO-d_6) δ 2.43 (3 H, s, CH_3), 8.27 (1 H, d, J = 7 Hz, C_6H). Anal. ($C_{12}H_9FN_2O_3$) C, H, N.

N_1,N_3-Diacyl-5-fluorouracil Derivatives (10–16; Table II). (a) Acylation of 1. See methods B and C.

(b) Acylation of 4 and 6 in the Presence of Triethylamine (Method D). To a stirred mixture of 0.173 mol of **4** and **6**, 300 mL of dioxane, and 21 g (0.2 mol) of triethylamine cooled in an ice bath was added 0.208 mol of acyl chloride portionwise over 30 min. The mixture was stirred for 1.5 h at room temperature. The precipitated triethylamine hydrochloride was filtered off, and the filtrate evaporated. The yellowish oily product solidified on addition of ether and was recrystallized. This method, using the corresponding acyl chlorides, provided **7**, **10–12**, and **16**. **10**: NMR ($CDCl_3$) δ 1.26 (3 H, t, J = 7 Hz, CH_2CH_3), 2.72 (3 H, s, $ArCH_3$), 2.88 (2 H, q, J = 7 Hz, CH_2CH_3), 8.20 (1 H, d, J = 7 Hz, C_6H). **11**: NMR ($CDCl_3$) δ 2.65 (3 H, s, CH_3), 8.44 (1 H, d, J = 7 Hz, C_6H). **12**: NMR ($CDCl_3$) δ 2.72 (6 H, s, $COCH_3$ and $ArCH_3$), 8.35 (1 H, d, J = 7 Hz, C_6H). **16**: NMR ($CDCl_3$) δ 2.40 and 2.47 (each 3 H, s, 2 CH_3), 8.07 (1 H, d, J = 6.0 Hz, C_6H).

(c) Acylation of 18 and 19 (Method E). To a stirred mixture of 0.1 mol of **18** or **19**, 21 g (0.4 mol) of triethylamine, and 100 mL of acetone, cooled in ice–water, was added portionwise 0.2 mol of acyl chloride. The mixture was stirred for a further 2 h and then concentrated below 60 °C. Benzene was added to the concentrate and the mixture filtered. The filtrate was evaporated and the resulting solid recrystallized from benzene–ether. Using the corresponding acyl chlorides, this method was employed for the preparation of **11**[13] and **13–15**. **13**: NMR (acetone-d_6) δ 1.15 (3 H, t, J = 7 Hz, CH_2CH_3), 3.09 (2 H, q, J = 7 Hz, CH_2CH_3), 8.45 (1 H, d, J = 7 Hz, C_6H). **14**: NMR (acetone-d_6) δ 1.15 (3

H, t, J = 7 Hz, CH_2CH_3), 2.69 (3 H, s, $ArCH_3$), 3.12 (2 H, q, J = 7 Hz, CH_2CH_3), 8.47 (1 H, d, J = 7 Hz, C_6H). **15**: NMR (acetone-d_6) δ 1.22 [6 H, d, J = 7 Hz, $CH(CH_3)_2$], 2.67 (3 H, s, $ArCH_3$), 8.40 (1 H, d, J = 7 Hz, C_6H).

N_3-Acyl-5-fluorouracil Derivatives (17–20; Table II). (a) Acylation of 1. See method A.

(b) Solvolysis of 7 and 10 (Method F). A solution of 1.0 g of **7** or **10** in 20 mL of ethanol was allowed to stand at room temperature for 3 days. The mixture was then evaporated to give a viscous oil. The solidified product (on trituration with ether) was recrystallized from chloroform. This method was used to prepare **17** and **20**. **17**: NMR ($CDCl_3$) δ 2.60 (3 H, s, CH_3), 7.30 (1 H, d, J = 7 Hz, C_6H). **20**: NMR ($CDCl_3$) δ 1.26 (3 H, t, J = 7 Hz, CH_2CH_3), 2.89 (2 H, q, J = 7 Hz, CH_2CH_3), 7.26 (1 H, d, J = 7 Hz, C_6H).

N_1-(Alkoxycarbonyl)-N_3-acyl-5-fluorouracil Derivatives (22–32; Table III). (a) Reaction of Alkyl Chlorocarbonates with 18 and 19 (Method G). To an ice–water cooled and stirred mixture of 0.01 mol of **18** and **19**, 1.0 g (0.01 mol) of triethylamine, and 100 mL of acetone was slowly added 0.02 mol of alkyl chlorocarbonate. After the addition was complete, the mixture was stirred for 2 h and evaporated below 60 °C. Extraction of the resulting residue with benzene, followed by evaporation of the extract, gave a solid which was recrystallized from benzene–ether. Using the corresponding alkyl chlorocarbonates, this method was employed to prepare compounds **22–29**. **22**: NMR (acetone-d_6) δ 1.34 (3 H, t, J = 7 Hz, CH_2CH_3), 4.44 (2 H, q, J = 7 Hz, CH_2CH_3), 8.36 (1 H, d, J = 7 Hz, C_6H). **23**: NMR (acetone-d_6) δ 8.52 (1 H, d, J = 7 Hz, C_6H). **24**: NMR (acetone-d_6) δ 2.64 (3 H, s, $ArCH_3$), 3.95 (3 H, s, OCH_3), 8.27 (1 H, d, J = 7 Hz, C_6H). **25**: NMR (acetone-d_6) δ 1.33 (3 H, t, J = 7 Hz, CH_2CH_3), 2.65 (3 H, s, $ArCH_3$), 4.43 (2 H, q, J = 7 Hz, CH_2CH_3), 8.33 (1 H, d, J = 7 Hz, C_6H). **26**: NMR (acetone-d_6) δ 0.86 (3 H, distd t, CH_2CH_3), 2.70 (3 H, s, $ArCH_3$), 4.38 (2 H, t, J = 6 Hz, OCH_2), 8.33 (1 H, d, J = 7 Hz, C_6H). **27**: NMR (acetone-d_6) δ 2.66 (3 H, s, $ArCH_3$), 5.41 (2 H, s, OCH_2), 8.27 (1 H, d, J = 7 Hz, C_6H). **28**: NMR (acetone-d_6) δ 8.32 (1 H, d, J = 7 Hz, C_6H). **29**: NMR (acetone-d_6) δ 2.67 (3 H, s, $ArCH_3$), 8.45 (1 H, d, J = 7 Hz, C_6H).

(b) Reaction of Acyl Chloride with 21[12] (Method H). To a stirred mixture of 2 g (0.01 mol) of **21**, 1.1 g (0.01 mol) of triethylamine, and 50 mL of acetone, cooled in ice–water, was slowly added 0.02 mol of acyl chloride. The mixture was stirred for a further 2 h and worked up as in method G. Using the corresponding acyl chlorides, this method provided **22**,[13] **25**,[13] and **30–32**. **30**: NMR (acetone-d_6) δ 1.37 (3 H, t, J = 7 Hz, CH_2CH_3), 2.60 (3 H, s, $COCH_3$), 4.50 (2H, q, J = 7 Hz, CH_2CH_3), 8.28 (1 H, d, J = 7 Hz, C_6H). **31**: NMR (acetone-d_6) δ 1.20 (3 H, t, J = 7 Hz, $COCH_2CH_3$), 1.37 (3 H, t, J = 7 Hz, $COOCH_2CH_3$), 2.87 (2 H, q, J = 7 Hz, $COCH_2CH_3$), 4.46 (2 H, q, J = 7 Hz, $COOCH_2CH_3$), 8.23 (1 H, d, J = 7 Hz, C_6H). **32**: NMR (acetone-d_6) δ 0.90 [3 H, distd t, $(CH_2)_5CH_3$], 2.87 [2 H, distd q, J = 7 Hz, $COCH_2(CH_2)_4$], 4.44 (2 H, q, J = 7 Hz, $COOCH_2CH_3$), 8.20 (1 H, d, J = 7 Hz, C_6H).

N_1-Acetyl-N_3-(alkoxycarbonyl)-5-fluorouracil Derivatives (33–35; Method I). To an ice-cooled mixture of 3 g (0.017 mol) of **4** and 20 mL of dioxane was added, with stirring, 0.026 mol of alkyl chlorocarbonate, followed by 2.6 g (0.026 mol) of triethylamine. The mixture was stirred for 3 h and then filtered. Evaporation of the filtrate gave crude **33–35** as oils. **35** was obtained as colorless crystals, while **33** and **34** remained noncrystalline. **35**: NMR ($CDCl_3$) δ 2.72 (3 H, s, CH_3), 5.10 (2 H, s, CH_2), 7.1–7.6 (5 H, m, ArH), 8.20 (1 H, d, J = 7 Hz, C_6H).

N_3-(Alkoxycarbonyl)-5-fluorouracil Derivatives (36–38; Method J). Each of the above compounds **33–35** was dissolved in methanol–triethylamine (20:1, v/v). The solution was allowed to stand at room temperature for 3–6 min and then evaporated. The resulting oily product solidified on trituration with ether and was purified by recrystallization to afford **36–38**. **36**: NMR ($CDCl_3$) δ 1.25 (3 H, t, J = 7 Hz, CH_2CH_3), 4.03 (2 H, q, J = 7 Hz, CH_2CH_3), 7.23 (1 H, d, J = 7 Hz, C_6H). **37**: NMR ($CDCl_3$) δ 7.0–7.6 (6 H, m, ArH and C_6H). **38**: NMR ($CDCl_3$) δ 5.10 (2 H, s, CH_2Ph), 7.10–7.70 (6 H, m, ArH and C_6H).

Antitumor Activity against P-388. Female BDF_1 mice (Charles River, Japan) weighing 17–21 g were used. Five mice for each test group were implanted intraperitoneally with 10^6 cells of P-388.[15] The test compounds were dissolved or suspended in 0.3% sodium carboxymethylcellulose (CMC-Na) and administered orally once daily for 9 days, starting 1 day after implantation. Antitumor activity of the test compounds was evaluated by the ratio of the median survival time of the treated mice (T) to that of the control mice (C).

Antitumor Activity of Compound 12 against Various Experimental Tumors. The following four tumors were used: P-388 (tested in BDF_1 mice), L-1210 (BDF_1), Meth A (Balb/c), and MH 134 (C3H). Five female mice for each group, weighing 17–23 g, were implanted with one of the tumors: 10^6 cells of P-388 or 10^5 cells of L-1210 were implanted intraperitoneally in the mouse; 5×10^6 cells of Meth A or 10^6 cells of MH 134 were implanted subcutaneously in the side of the abdominal region of the mouse. Test compounds were dissolved or suspended in 0.3% CMC-Na and administered orally once daily for 9 days (P = 383), 5 days (L-1210), or 13 days (Meth A and MH 134), starting 1 day after implantation. Antitumor activity of the compounds was evaluated by the ratio of the median survival time in P-388 and L-1210 and by the ratio of the median weight of tumors in Meth A (mice killed 16 days after implantation) and MH 134 (mice killed 20 days after implanation).

Acute Toxicity of Compound 12. Six female ICR mice for each group, weighing 23–30 g, were used. Test compounds were dissolved or suspended in 0.3% CMC-Na and administered orally. The LD_{50} value was calculated by the Litchfield and Wilcoxon method[16] from the mortality of mice 7 days after administration. Moreover, the general behavior of the mice was observed after administration of compound **12**.

Serum Concentration of Metabolites. Female BDF_1 mice weighing 22–26 g (Charles Rever, Japan) were subjected to this study. Test compounds were suspended or dissolved in 0.3% CMC-Na and administered orally at a dose of 1 mmol/kg (compound **12**, 290 mg/kg; FT-207, 200 mg/kg). At 0.5, 1, 2.4, and 7 h after the administration, the mice were killed by bleeding under ether anesthesia. The blood was centrifuged and the sera were decanted into tubes. The sera were diluted with saline, and the solution was acidified and extracted with chloroform. In this way, 5-FU (aqueous layer) was separated from **19** or FT-207 (organic layer). 5-FU and FT-207 concentration in the sera was determined biologically by the antibacterial activity against *Staphylococcus aureus* P-209,[17] while the concentration of compound **19** was determined by high-pressure liquid chromatography (Waters Associates, Inc.).

Acknowledgment. We thank President A. Yanagisawa and Director O. Takagi, Grelan Pharmaceutical Co. Ltd., for their encouragement. We also thank Dr. K. Fukumoto for his helpful suggestions and the Pharmaceutical Institute, Tohoku University, for the preparation of the manuscript and for microanalyses.

(15) R. I. Geran, H. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 8 (1972).
(16) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).
(17) Y. Yasuda, T. Togo, N. Unemi, S. Watanabe, K. Harima, and T. Suzue, *J. Jpn. Soc. Cancer Ther.*, 21, 1171 (1973).