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5-Fluorouracil Derivatives. IX.[1] Synthesis and Antitumor Activities of (Alkylthio)carbonyl-5-fluorouracils

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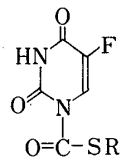
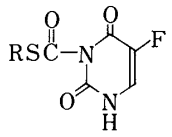
A series of 5-fluorouracil derivatives having (alkylthio)carbonyl groups at the 1-, 3-, and 1,3-positions was synthesized and tested for antitumor activity against L-1210 leukemia in mice. Among them, 1-(octylthio)carbonyl-5-fluorouracil showed promising activity; it gave a greater increase in life span than 1-hexylcarbamoyl-5-fluorouracil (HCFU) and 1-(2-tetrahydrofuryl)-5-fluorouracil (Tegafur), which are both in clinical use.

Keywords——5-fluorouracil; (alkylthio)carbonyl-5-fluorouracil; antitumor agent; 1-(octylthio)carbonyl-5-fluorouracil

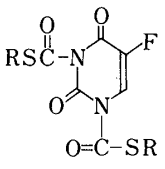
As part of a series of synthetic studies on 5-fluorouracil derivatives aimed at obtaining improved antitumor agents, we have reported the syntheses of 1-carbamoyl-5-fluorouracils[2] and acyloxyalkyl-5-fluorouracils.[3] This time, we wish to report the synthesis and antitumor activity of 1-(alkylthio)carbonyl-5-fluorouracils (**1**), 3-(alkylthio)carbonyl-5-fluorouracils (**2**), and 1,3-bis(alkylthio)carbonyl-5-fluorouracils (**3**).



1a—k



2a—c

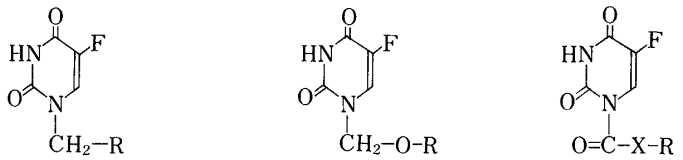


3a—c

a: R=*n*-C_6H_{13}, **b**: R=*n*-C_8H_{17}, **c**: R=$CH_2CO_2C_2H_5$, **d**: R=C_2H_5, **e**: R=*n*-C_4H_9, **f**: R=*sec*-C_4H_9, **g**: R=*tert*-C_4H_9, **h**: R=cyclo-C_6H_{11}, **i**: R=C_6H_5, **j**: R=$CH_2C_6H_5$, **k**: R=CH_2- 

Chart 1

As mentioned in the previous report,[3] many kinds of 5-fluorouracil derivatives have been synthesized. It has been found that alkyl-5-fluorouracils (**4**) have no antitumor activity and the compounds having oxygen at the α-position of the alkyl group had weak or moderate antitumor activity. 1-Tetrahydrofuryl-,[4] 1-ethoxymethyl-,[5] and acyloxyalkyl-5-fluorouracil (**5**)[3,6] belong to this class. Furthermore it was found that compounds having a carbonyl group bound directly to the nitrogen of 5-fluorouracil show very strong antitumor activity. Examples of such compounds are 1-acyl-5-fluorouracils (**6**: RX=alkyl or aryl),[7,8] 1-alkoxycarbonyl-5-fluorouracils (**6**: X=O, R=alkyl)[9,10] and 1-carbamoyl-5-fluorouracils[11] (**6**: X=NH, R=alkyl).



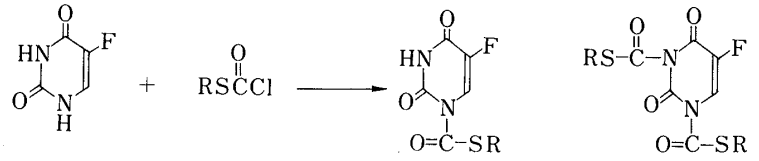
4 5 6

Chart 2

1-Acyl-5-fluorouracils are unstable in water, and decompose readily. 1-Alkoxylcarbonyl-5-fluorouracils are also unstable in water. Therefore these compounds are not suitable for use as drugs. 1-Carbamoyl-5-fluorouracils are stable in acidic media and decompose moderately in neutral media.[12] 1-Hexylcarbamoyl-5-fluorouracil (HCFU, Carmofur) has been used in Japan since 1981. HCFU showed remarkable effect on colorectal,[13,14] lung[13] breast[14,15] and gastric[14] cancers. In terms of the antitumor response rate[13] (20—43%), HCFU ranks in the top group of all antitumor agents. Nevertheless, HCFU has some side effects[16,17] such as hot sensation and pollakiuria syndrome. Thus, we are still trying to find a better compound.

This time, we synthesized[18] 1-(alkylthio)carbonyl-5-fluorouracils (**1**) and found that these compounds showed strong antitumor activity comparable to that of 1-carbamoyl-5-fluorouracils. Several 3- and 1,3-substituted 5-fluorouracils having a (alkylthio)carbonyl group were also synthesized. These compounds were obtained by three general methods.

Method 1: The reaction of S-alkyl chlorothioformate (**8**) with 5-fluorouracil (**7**) afforded **1** and **3**. This type of reaction was carried out in the presence of a base such as triethylamine or



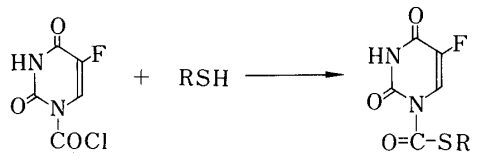
7 8 1 3

Chart 3

pyridine in a polar solvent such as *N*,*N*-dimethylformamide or pyridine. When equimolar amounts of **7** and **8** were reacted, the products consisted of mostly **1** with a very small amount of **3** (method 1(a)), and 3-substituted **2** was not detected by thin-layer chromatography (TLC), in contrast to the cases of the acyloxyalkylation and acylation.[8] As the molar ratio of **8** and **7** was increased, the yield of **3** was increased (method 1(b)).

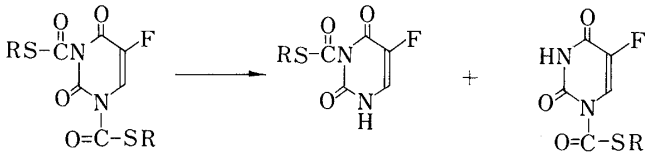
Method 2: The reaction of 1-chlorocarbonyl-5-fluorouracil (**9**) with a thiol (**10**) afforded **1**. This type of reaction was carried out in the presence of a base such as triethylamine in a polar solvent. The reaction is similar to the reaction[2] of **9** with an amine to afford 1-carbamoyl-5-fluorouracil.

Method 3: Compound **3** was subjected to aminolysis to afford 3-(alkylthio)carbonyl-5-fluorouracils (**2**) and 1-(alkylthio)carbonyl-5-fluorouracils (**1**). Basic reagents such as sodium hydroxide, ammonia, and amines were examined. Hydrolysis using aqueous sodium hydroxide gave 5-fluorouracil (**7**). Ammonia gave **2** and **1** unselectively. Ammonolysis of 1,3-



9 10 1

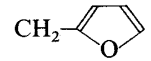
Chart 4



3 2 1

Chart 5

TABLE I. 1-(Alkylthio)carbonyl- and 3-(Alkylthio)carbonyl-5-fluorouracils

Compd. No.	R	Method	Yield (%)	mp (°C)	^{1}H-NMR ($CDCl_3$)	Formula	Analysis (%) Calcd (Found)		
							C	H	N
1a	*n*-C_6H_{13}	1 (a)	67	112	0.89 (3H, t), 1.10—1.90 (8H, br), 9.62—9.84 (1H, br)	$C_{11}H_{15}FN_2O_3S$	48.16 (48.07	5.51 5.47	10.21 10.21)
1b	*n*-C_8H_{17}	1 (a)	84	111—112	0.88 (3H, br t), 1.16—1.83 (12H, br), 2.97 (2H, t), 8.25 (1H, d), 9.00 (1H, br)	$C_{13}H_{19}FN_2O_3S$	51.64 (51.70	6.33 6.30	9.26 9.16)
1c	$CH_2CO_2C_2H_5$	1 (a)	52	164	1.28 (3H, t), 3.69 (2H, s), 4.18 (2H, q)	$C_9H_9FN_2O_5S$	39.13 (38.82	3.28 3.27	10.14 10.00)
1d	C_2H_5	1 (a)	40	175—176[a]	1.36 (3H, t), 3.01 (2H, q), 8.30 (1H, d), 8.86 (1H, br s)	$C_7H_7FN_2O_3S$	38.53 (38.42	3.23 3.25	12.84 12.84)
1e	*n*-C_4H_9	1 (a)	62	154—155	0.95 (3H, t), 1.23—1.84 (4H, m), 2.98 (2H, t), 8.26 (1H, d), 8.70 (1H, br)	$C_9H_{11}FN_2O_3S$	43.90 (43.89	4.50 4.53	11.38 11.36)
1f	*sec*-C_4H_9	1 (a)	63	140	1.02 (3H, t), 1.37 (3H, d), 1.69 (2H, m), 3.57 (1H, q), 8.24 (1H, d), 8.92 (1H, br)	$C_9H_{11}FN_2O_3S$	43.90 (43.90	4.50 4.86	11.38 11.34)
1g	*tert*-C_4H_9	1 (a)	43	199	1.54 (9H, s), 8.23 (1H, d), 8.31 (1H, br)	$C_9H_{11}FN_2O_3S$	43.90 (43.81	4.50 4.53	11.38 10.94)
1h	Cyclo-C_6H_{11}	2	34	197—198	1.13—2.15 (10H, br), 3.33—3.72 (1H, br), 8.22 (1H, br), 8.66 (1H, br)	$C_{11}H_{13}FN_2O_3S$	48.52 (48.28	4.81 4.79	10.29 10.29)
1i	C_6H_5	1 (a)	35	236	7.49 (5H, s), 8.16 (1H, d)	$C_{11}H_7FN_2O_3S$	49.62 (49.82	2.65 2.74	10.82 10.41)
1j	$CH_2C_6H_5$	1 (a)	44.3	168	4.19 (2H, s), 7.32 (5H, br s), 8.23 (1H, d)	$C_{12}H_9FN_2O_3S$	51.42 (51.75	3.24 3.27	10.00 9.94)
1k	CH_2– 	1 (a)	14	167	4.22 (2H, s), 6.30 (2H, m), 7.36 (1H, m), 8.24 (1H, d), 12.30 (1H, br)	$C_{10}H_7FN_2O_4S$	44.45 (43.13	2.61 2.60	10.37 10.38)
2a	*n*-C_6H_{13}	3 (a) 3 (b)	67 90	131—132	0.89 (3H, t), 1.10—1.90 (8H, br), 3.12 (2H, t), 9.62—9.84 (1H, br)	$C_{11}H_{15}FN_2O_3S$	48.16 (48.15	5.51 5.24	10.21 9.99)
2b	*n*-C_8H_7	3 (c)	95	120—121	0.87 (3H, t), 1.10—2.15 (12H, br), 3.10 (2H, t), 7.27 (1H, d), 9.39—9.99 (1H, br)	$C_{13}H_{19}FN_2O_3S$	51.64 (51.89	6.33 6.34	9.27 9.36)
2c	$CH_2CO_2C_2H_5$	3 (a)	60	176	1.33 (3H, t), 3.74 (2H, s), 4.28 (2H, q), 7.25 (1H, br), 9.34 (1H, br)	$C_9H_9FN_2O_5S$	39.13 (38.84	3.28 3.27	10.14 10.00)

a) mp of compound **1d** was reported to be 192 °C.[10]

TABLE II. Antitumor Activity of 5-FU Derivatives in the L-1210 Leukemia System (*p.o.*) in Mice

Compd. No.	R	Dose (mg/kg/d)	ILS (%)	Compd. No.	R	Dose (mg/kg/d)	ILS (%)
1a	*n*-C_6H_{13}	30	3	**1i**	C_6H_5	30	0
		100	48	**1j**	$CH_2C_6H_5$	30	11
		300	47			100	41
1b	*n*-C_8H_{17}	30	27			300	33
		100	44	**1k**	CH_2– 	30	13
		300	69			100	48
1c	$CH_2COOC_2H_5$	30	2	**2b**	*n*-C_8H_{17}	30	−3
		100	34			100	2
1d	C_2H_5	30	2			300	29
		100	43	**3b**	*n*-C_8H_{17}	100	0
1e	*n*-C_4H_9	30	8			300	6
		100	27		HCFU	30	21
		300	−7			100	53
1f	*sec*-C_4H_9	30	6			300	23
		100	33		Tegafur	30	0
1g	*tert*-C_4H_9	30	0			100	31
1h	Cyclo-C_6H_{11}	30	10			300	13
		100	19				

bis(hexylthio)carbonyl-(**3a**), 1,3-bis(butylthio)carbonyl-(**3e**), 1,3-bis(ethoxycarbonylmethylthio)carbonyl-5-fluorouracil (**3c**) in methanolic ammonia gave 67, 72 and 60% yield of **2** and 30, 25 and 26% yields of **1**, respectively, in 30 min at room temperature (method 3(a)). Aminolysis of 1,3-bis(hexylthio)carbonyl-5-fluorouracil (**3a**) by ethylamine in methanol at room temperature gave a 90% yield of 3-(hexylthio)-carbonyl-5-fluorouracil (**2a**) and 4% of 1-(hexylthio)carbonyl-5-fluorouracil (**1a**) (method 3(b)). Aminolysis of 1,3-bis(octylthio)-carbonyl-5-fluorouracil (**3b**) by isopropylamine in ether gave 3-(octylthio)carbonyl-5-fluorouracil (**2b**) selectively and almost quantitatively at 0 °C in 10 min (method 3(c)). Therefore it was found that isopropylamine is the best reagent to remove the 1-(alkylthio)carbonyl group to produce **2**.

The antitumor activity of thirteen compounds against L-1210 leukemia was examined, and the results are shown in Table II. 5-Fluorouracil derivatives having an (alkylthio)-carbonyl group at the N-1 position were markedly active against L-1210 leukemia. Their activity is comparable to that of 1-alkylcarbamoyl-5-fluorouracil. In particular, 1-(octylthio)-carbonyl-5-fluorouracil (**1b**) was more active and less toxic than HCFU, as shown by a comparison of the increase of life span (ILS) of **1b** with that of HCFU. Compound **1b** showed ILS values of 27% (*p.o.* 30 mg/kg), 44% (*p.o.* 100 mg/kg), and 69% (*p.o.* 300 mg/kg). HCFU showed ILS values of 21% (*p.o.* 30 mg/kg), 53% (*p.o.* 100 mg/kg), and 23% (*p.o.* 300 mg/kg). HCFU showed toxicity at 300 mg/kg, whereas **1b** showed no toxicity at the same dose. 1-(Butylthio)carbonyl-(**1e**) and 1-(cyclohexylthio)carbonyl-5-fluorouracil (**1h**) have weak antitumor activity, while, 1-(phenylthio)carbonyl-(**1i**), 3-(octylthio)carbonyl-(**2b**) and 1,3-bis(octylthio)carbonyl-5-fluorouracil (**3b**) have no antitumor activity. Thus, 1-(octylthio)-carbonyl-5-fluorouracil (**1b**) was selected as the best compound among the derivatives tested. tested.

Experimental

Melting points were determined on a Yamato melting point apparatus and are uncorrected. Infrared (IR) spectra were taken on a Hitachi EPI-G3 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded

on a JEOL JNM FX-100S with tetramethylsilane as an internal standard.

General Procedure of Method 1(a)——For the Synthesis of **1**: S-Alkyl chlorothioformate (**8**) (20 mmol) and triethylamine (19.8 mmol) were added to a solution of 5-fluorouracil (**7**) (9 mmol) in pyridine stirred for 18 h at room temperature and then heated at 40 °C for 10 h. After cooling triethylamine hydrochloride was filtered off. Pyridine was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 and the solution was washed with 1 N HCl (20 ml) and H_2O (20 ml) then dried over Na_2SO_4. The residue obtained by concentrating the solution was chromatographed on a column of silica gel to afford **1**. Yields and physical properties are listed in Table I. In the case of the preparation of **1c**, 10% of **3c** was also isolated; **3c** was identified by ^{1}H-NMR spectroscopy.

3c: Amorphous. ^{1}H-NMR ($CDCl_3$) δ: 1.28 (3H, t), 1.32 (3H, t), 3.69 (2H, s), 3.37 (2H, s), 4.02—4.41 (4H, m).

General Procedure of Method 1(b)——For the Synthesis of **3**: This method is the same as method 1(a) except for the use of 3 eq of **8**. **3a** was identified by ^{1}H-NMR spectroscopy.

3a: Yield 57%. mp 52 °C. ^{1}H-NMR ($CDCl_3$) δ: 0.88 (6H, t), 1.04—2.00 (16H, br), 3.00 (2H, t), 3.13 (2H, t), 8.24 (1H, d).

3b: Yield 79%. mp 60—61 °C. IR (Nujol): 1740, 1720, 1695, 1680, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 0.66—0.78 (6H, br), 0.78—2.03 (24H, br), 2.77—3.35 (4H, m), 8.24 (1H, d, J=6 Hz). *Anal.* Calcd for $C_{22}H_{35}FH_2O_4S_2$: C, 55.67; H, 7.43; N, 5.90. Found: C, 55.55; H, 7.28; N, 5.96.

1-(Cyclohexylthio)carbonyl-5-fluorouracil (1h)——Method 2: Phosgene gas (15 mmol) was bubbled into a pyridine (15 ml) solution of 5-fluorouracil (**7**) (0.65 g, 5 mmol) at 0 °C to prepare 1-chlorocarbonyl-5-fluorouracil (**9**). Cyclohexanethiol (0.85 g, 5 mmol) and triethylamine (1.4 ml) were dropped into the above suspension, and the reaction mixture was stirred overnight. After removal of pyridine under reduced pressure, the residue was dissolved in CH_2Cl_2, and the solution was washed with 1 N HCl then dried over Na_2SO_4. After removal of the solvent, the residue was recrystallized from EtOH to yield 0.28 g (35%) of **1h**. The physical properties are listed in Table I.

3-(Hexylthio)carbonyl-5-fluorouracil (2a)——Method 3(a): A solution of 1,3-bis[(hexylthio)carbonyl]-5-fluorouracil (**3a**) (0.279 g, 1.02 mmol) in MeOH (5 ml) was treated with 1 ml of 28% aqueous ammonia. After 30 min, the solvent and ammonia were evaporated and the residue was dissolved in CH_2Cl_2 (40 ml). The CH_2Cl_2 solution was washed with 1 N HCl, the solvent was evaporated off and the residue was chromatographed to afford 0.122 g (67%) of **2a**, and 0.54 g (30%) of **1a**.

Method 3(b): $EtNH_2$ (0.49 mmol) in MeOH was added to a mixture of **3a** (0.155 g, 0.38 mmol) in MeOH (4 ml) at room temperature. The resulting solution was stirred for 30 min. After removal of the solvent under reduced pressure, the residue was chromatographed on silica gel to afford 0.094 g (90%) of **2a** and 0.0042 g (4%) of **1a**. The physical properties are listed in Table I.

3-(Ethoxycarbonylmethylthio)carbonyl-5-fluorouracil (2c)——Method 3(a): 1,3-Bis(ethoxycarbonylmethylthio)carbonyl-5-fluorouracil (**3c**) (0.14 g, 0.507 mmol) was treated with methanolic ammonia as described above to afford 0.04 g (60%) of **2c**, and 0.02 g (26%) of **1c**. The physical properties are listed in Table I.

3-(Octylthio)carbonyl-5-fluorouracil (2b)——Method 3(c): Isopropylamine (0.35 g, 5.9 mmol) was added to a solution of 1,3-bis(octylthio)carbonyl-5-fluorouracil (**3b**) (2.81 g, 5.9 mmol) in ether (50 ml) at 0 °C, and the mixture was stirred for 10 min. The solvent was evaporated off and the residue was chromatographed on silica gel to afford 1.60 g (90%) of **2b**. The physical properties are listed in Table I.

Animals and Tumor System——Male BDF_1 mice weighing 20±2 g were used. Six mice in each group, either test or control, were implanted intraperitoneally with 1×10^5 cells of L-1210 leukemia. The compound to be tested was administered orally once daily for 5 d, starting 24 h after tumor implantation. Antitumor activity was evaluated in terms of the increase in life span over the controls (ILS=T/C%−100).[11]

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