[Chem. Pharm. Bull. 35(9)3894—3897(1987)]

5-Fluorouracil Derivatives. XII.[1] Synthesis and Antitumor Activity of α-Alkylthiomethyl-, α-Alkylsulfinylmethyl-, α-Alkylsulfonylmethyl-, and α-Acylthiomethyl-5-fluorouracils

SHOICHIRO OZAKI,*[,a] TOSHIO NAGASE,[a] HIROFUMI TAMAI,[a] HARUKI MORI,[b] AKIO HOSHI,[c] and MASAAKI IIGO[c]

Faculty of Engineering, Ehime University,[a] Bunkyo-cho, Matsuyama 790, Japan, Research Center. Mitsui Toatsu Chemicals,[b] Totsuka-ku, Yokohama 247, Japan, and National Cancer Center Research Institute,[c] Chuo-ku, Tokyo 104, Japan

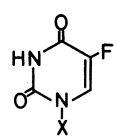
(Received February 26, 1987)

For the purpose of diminishing the toxicity of 5-fluorouracil (**1**) and obtaining biologically active derivatives of **1** suitable for oral administration, alkylthiomethyl, alkylsulfinylmethyl, alkylsulfonylmethyl, and acylthiomethyl groups were introduced at the 1- and 3-positions of **1**. The antitumor activity of these synthetic compounds was tested against L1210 leukemia in mice. 1-Alkylthiomethyl-5-fluorouracils showed weak antitumor activity at a high dose (300 mg/kg).

Keywords——5-fluorouracil; α-alkylthiomethyl-5-fluorouracil; α-alkylsulfinylmethyl-5-fluorouracil; α-alkylsufonylmethyl-5-fluorouracil; antitumor agent

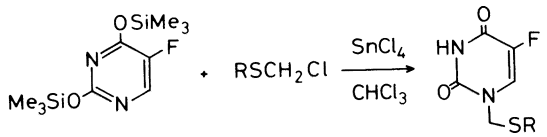
During various synthetic studies on masked 5-fluorouracil derivatives, we have realized that the bond strength between the nitrogen atom in the uracil ring and the substituent X is an important factor influencing the antitumor activity and the toxicity of the compounds. The previous results indicated that the weaker the bond strength, the stronger the antitumor activity and the toxicity of the masked compounds. In the case of *N*-carbonyl-5-fluorouracil derivatives,[2,3] for example, the N–C bond was relatively labile and its lability resulted in more potent antitumor activities as well as undesired side-effects. In the case of *N*-alkyl-5-fluorouracil derivatives,[4,5] the strong N–C bond conversely prevented these derivatives from being easily hydrolyzed *in vivo* and from showing any antitumor activity against L1210 leukemia. When oxygen was introduced at the α-position to the alkyl group, the N–C bond became labile under hydrolytic conditions and the resulting 1-acyloxyalkyl-,[6] 1-alkoxyalkyl-,[7,8] and 1-(tetrahydro-2-furyl)-5-fluorouracils[2] showed moderate antitumor activity. In view of these results, the authors thought that if sulfur instead of oxygen were introduced at the α-position to the alkyl substituent, this perturbation might modify the antitumor activity. In this report we wish to present details of the synthesis of α-alkylthiomethyl, α-alkylsulfinylmethyl-, α-alkylsulfonylmethyl-, and acylthiomethyl-5-fluorouracil derivatives[9] and their antitumor activities.

Introduction of sulfur-containing substituents into the 1- and/or 3-position(s) of 5-fluorouracil was effected in two different ways. In the first process, 2,4-bis(trimethylsiloxy)-5-fluoropyrimidine was chosen as the starting material and treated with alkylthiomethyl



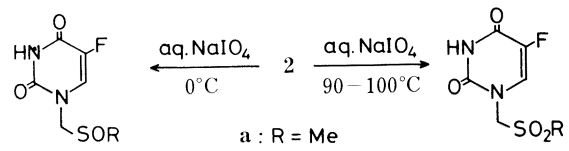
X: alkyl, $-\overset{O}{\overset{\|}{C}}R$, $-CH_2OR$, $-CH_2SO_nR$ ($n=0,1,2$)

Fig. 1



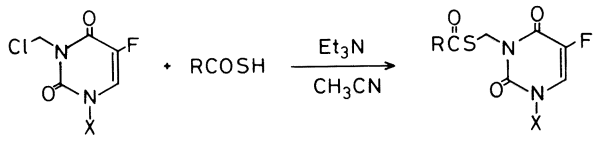
2a: R = Me
2b: R = Et

Chart 1



3 **a**: R = Me **b**: R = Et **4**

Chart 2



5: X = H
6: X = CH_2Cl

7: X = H
8: X = CH_2SCR (O)
a: R = Me
b: R = Ph

Chart 3

TABLE I. Antitumor Activity of 5-Fluorouracil Derivatives in the L1210 Leukemia

Compound No.	Substituent	Dose[a] (mg/kg/d)	ILS[b] (%)
2a	1-CH_2SCH_3	i.p. 100	−4
		p.o. 100	−6
		p.o. 300	11
2b	1-$CH_2SC_2H_5$	i.p. 30	−1
		i.p. 100	−4
		p.o. 100	−2
		p.o. 300	8
3a	1-CH_2SOCH_3	i.p. 100	−8
		p.o. 100	3
3b	1-$CH_2SOC_2H_5$	i.p. 30	−10
		i.p. 100	−5
		p.o. 100	−2
		p.o. 300	−8
4a	1-$CH_2SO_2CH_3$	*p.o.* 100	2
4b	1-$CH_2SO_2C_2H_5$	*p.o.* 100	0
7a	3-CH_2SCOCH_3	*p.o.* 100	4
7b	3-$CH_2SCOC_6H_5$	*p.o.* 100	0
8a	1,3-CH_2SCOCH_3	*p.o.* 100	−9

a) i.p. and *p.o.* mean intraperitoneal and *per os* administration, respectively. *b*) ILS means increase in life span; see Experimental.

chlorides in the presence of stannic chloride to afford 1-alkylthiomethyl-5-fluorouracils (**2**) after usual work-up (Chart 1). In this step, no 3-alkylthiomethyl derivatives were isolated. Both 1-alkylsulfinylmethyl- and 1-alkylsulfonylmethyl-5-fluorouracils (**3** and **4**) were easily prepared by the subsequent oxidation of **2** with sodium periodate. Compound **2** was converted to **3** by low-temperature oxidation (at 0 °C), and to **4** by high-temperature oxidation (at 90—100 °C). In both cases, the desired derivatives were obtained in good to excellent yields (Chart 2).

Acylthiomethyl-5-fluorouracils were prepared by direct esterification of thiocarboxylic acids with *N*-chloromethyl-5-fluorouracils **5** and **6**.[10] The reactions did not occur in the absence of triethylamine, but **5** and **6** were consumed within several hours even at low temperature in the presence of a base (Chart 3).

The antitumor activity of the nine-prepared compounds was tested against L1210 leukemia in mice,[11] and the results (increase in life span) are shown in Table I. 1-Alkylthiomethyl-5-fluorouracils **2** showed weak antitumor activity at a high dose (300 mg/kg) but compounds **3**, **4**, **7**, and **8** showed no antitumor activity. These data suggested that the perturbation caused by introducing the sulfur atom as the α-position to the alkyl group was not effective in weakening the N–C bond *in vivo*, compared with that caused by the presence of the oxygen atom, so that the sulfur-containing *N*-alkyl-5-fluorouracil derivatives unfortunately had little or no antitumor activity against L1210 leukemia.

Experimental

The melting points were recorded on a Büchi melting point apparatus and are uncorrected. The infrared (IR) spectra were obtained on a JASCO IR-A-1 spectrometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on JEOL-60HL, JEOL FX-100, and Hitachi R-24 spectrometers.

1-Methylthiomethyl-5-fluorouracil (2a)——Stannic chloride (5.21 g, 0.02 mol) was added dropwise to a mixture of 2,4-bis(trimethylsiloxy)-5-fluoropyrimidine (13.72 g, 0.05 mol), chloromethyl methyl sulfide (4.83 g, 0.05 mol), and chloroform (10 ml) at 60 °C. After stirring of the mixture for 1 h, ethanol (19 ml) was added and the whole was evaporated. The residue was taken up in dichloromethane (200 ml), and this solution was washed with water, dried, and evaporated. Ether was added to the residual oil to solidify the product. Filtration of this mixture gave **2a** (4.87 g, 52%). mp 147—148 °C. IR (KBr): 3180, 3030, 2840, 1775, 1735, 1690, 1670, 1488, 1432, 1381, 1350, 1295, 1250, 1234, 1127, 1003, 756, 711 cm^{-1}. ^{1}H-NMR (CD_3COCD_3) δ: 2.23 (3H, s, CH_3), 4.88 (2H, s, CH_2), 7.78 (1H, d, J=6 Hz, C_6-H), 10.50 (1H, br, N_3-H). *Anal.* Calcd for $C_6H_7FN_2O_2S$: C, 37.89; H, 3.71; F, 9.99; N, 14.73. Found: C, 37.69; H, 3.70; F, 9.73; N, 14.66.

1-Ethylthiomethyl-5-fluorouracil (2b)——Compound **2b** was prepared from 2,4-bis(trimethylsiloxy)-5-fluoropyrimidine (5.49 g, 0.02 mol) and ethylthiomethyl chloride (2.21 g, 0.02 mol) in the presence of stannic chloride (5.21 g, 0.02 mol) at 70 °C. **2b** (1.7 g, 42%). mp 148—149 °C. IR (KBr): 3190, 3070, 2860, 1730 (C=O), 1675, 1430, 1382, 1360, 1252, 1241, 1134, 928, 771, 704 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=8 Hz, CH_3), 2.64 (2H, q, J=8 Hz, CH_2), 4.86 (2H, s, CH_2S), 7.53 (1H, d, J=6 Hz, C_6-H), 9.92 (1H, br, N_3-H). *Anal.* Calcd for $C_7H_9FN_2O_2S$: C, 41.17; H, 4.44; F, 9.30; N, 13.72. Found: C, 40.87; H, 4.20; F, 9.10; N, 13.48.

1-Methylsulfinylmethyl-5-fluorouracil (3a)——Compound **2a** (2.85 g, 0.015 mol) was added to a cooled solution of $NaIO_4$ (3.37 g, 0.0158 mol) in water (40 ml) at 0 °C, and this mixture was stirred for 30 min, then filtered. The filtrate was evaporated to dryness. The residue was dissolved in acetone and the insoluble materials were filtered off. This filtrate was evaporated to dryness, acetone was again added, the insoluble materials were filtered off, and the filtrate was evaporated to dryness. The residue was washed with ethanol to afford **3a** (2.07 g, 67%) as colorless crystals. mp 200—201 °C. IR (KBr): 3190, 3030, 2915, 2858 1727, 1706, 1672, 1489, 1418, 1390, 1365, 1252, 1146 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.20 (3H, s, CH_3), 4.67 (1H, d, J=13 Hz, CH), 5.00 (1H, d, J=13 Hz, CH), 7.93 (1H, d, C_6-H, J=6 Hz), 11.97 (1H, br, N_3-H). *Anal.* Calcd for $C_6H_7FN_2O_3S$: C, 34.95; H, 3.42; F, 9.21; N, 13.59; S, 15.55. Found: C, 34.83; H, 3.07; F, 8.96; N, 13.29; S, 15.25.

1-Ethylsulfinylmethyl-5-fluorouracil (3b)——In the same manner as used for the preparation of **3a**, **3b** (3.71 g, 84%) was obtained from **2b** (4.08 g, 0.02 mol) and $NaIO_4$ (4.49 g, 0.022 mol). mp 204 °C. IR (KBr): 3180, 1720, 1704, 1670, 1486, 1384, 1067 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.25 (3H, t, J=8 Hz, CH_3), 2.80 (2H, dq, J=8, 13 Hz, CH_2), 4.69 (1H, d, J=13 Hz, CH), 7.98 (1H, d, J=7 Hz, C_6-H), 12.02 (1H, br, N_3-H). *Anal.* Calcd for $C_7H_9FN_2O_3S$: C, 38.18; H, 4.12; F, 8.63; N, 12.72; S, 14.56. Found: C, 38.46; H, 4.05; F, 8.45; N, 12.44; S, 14.38.

1-Methylsulfonylmethyl-5-fluorouracil (4a)——Compound **2a** was added to a solution of $NaIO_4$ (4.91 g, 0.022 mol) in water (50 ml) with stirring at 90—100 °C. Compound **2a** soon dissolved and after 30 min, new crystals

deposited from the solution. Stirring was continued for an additional 1.5 h, then the solution was cooled in an ice bath and the precipitate was filtered off, washed with water, and dried to obtain **4a** (2.09 g, 91%). mp 248 °C (dec.). IR (KBr): 3200, 3080, 1732, 1708, 1675, 1475, 1389 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 3.06 (3H, s, CH_3), 5.14 (2H, s, CH_2), 8.03 (1H, d, C_6-H), 12.04 (1H, br, N_3-H). *Anal.* Calcd for $C_6H_9FN_2O_4S$: C, 32.43; H, 3.18; F, 8.53; N, 12.61; S, 14.43. Found: C, 32.73; H, 2.99; F, 8.44; N, 12.32; S, 14.51.

1-Ethylsulfonylmethyl-5-fluorouracil (4b)——Compound **4b** (0.87 g, 89%) was obtained by oxidation of **3b** (0.85 g, 0.0042 mol) with $NaIO_4$ (1.96 g, 0.009 mol) in the same manner as described for **4a**. mp 240—241.5 °C. IR (KBr): 3200, 1752, 1715, 1677, 1475, 1231, 1131 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.24 (3H, t, J=8 Hz, CH_3), 3.12 (2H, q, J=8 Hz, CH_2), 5.12 (2H, s, NCH_2), 8.03 (1H, d, J=6 Hz, C_6-H), 12.06 (1H, br, N_3-H). *Anal.* Calcd for $C_7H_9FN_2O_4S$: C, 35.59; H, 3.84; F, 8.04; N, 11.86; S, 13.57. Found: C, 35.74; H, 3.84; F, 8.34; N, 11.56; S, 13.86.

3-Acetylthiomethyl-5-fluorouracil (7a)——Triethylamine (0.105 ml, 0.75 mmol) was added to a mixture of 3-chloromethyl-5-fluorouracil (**5**)[10] (134 mg, 0.75 mmol) and thioacetic acid (74.3 mg, 0.975 mmol) in acetonitrile (10 ml) over 5 min at room temperature. Exothermic reaction occurred to produce a precipitate of triethylamine hydrochloride. After a few hours, the precipitate was filtered off and the filtrate was evaporaed to dryness. The residue was subjected to silica gel column chromatography (hexane: ethyl acetate = 1 : 1) to give **7a** (128 mg, 78%). mp 136—137 °C. IR (KBr): 3340, 3080, 1670 (vs), 1472, 1415, 1244 (s), 1192, 1116, 990 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 2.34 (3H, s, CH_3), 5.38 (2H, s, CH_2), 7.87 (1H, t, J=6 Hz, C_6-H, became a doublet when D_2O was added), 11.25 (1H, br, N_1-H). *Anal.* Calcd for $C_6H_6FN_2O_3S$: C, 38.53; H, 3.23; N, 12.84. Found: C, 38.66; H, 3.04; N, 12.95.

3-Benzoylthiomethyl-5-fluorouracil (7b)——According to the same method as used for **7a**, **7b** (1.5 g, 54%) was obtained from 3-chloromethyl-5-fluorouracil (1.78 g, 0.01 mol) and thiobenzoic acid (1.79 g, 0.015 mol). mp 240—241 °C. IR (KBr): 3207, 3080, 1707, 1665 (vs), 1630, 1418, 1312, 1200 (s), 900 (s), 800, 760 cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.54 (2H, s, CH_2), 7.5—8.0 (6H, m, C_6H_5 and C_6-H), 11.32 (1H, s, NH).

1,3-Bis(acetylthiomethyl)-5-fluorouracil (8a)——Compound **8a** was prepared from 1,3-bis(chloromethyl)-5-fluorouracil and 2 eq of thioacetic acid in the presence of 2 eq of triethylamine as described above for **7a** and **7b**. **8a** (0.52 g, 70%). mp 70 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.13 (3H, s, CH_3 at 1-position), 2.37 (3H, s, CH_3 at 3-position), 5.45 (2H, s, CH_2 at 3-position), 5.68 (2H, s, CH_2 at 1-position), 7.66 (1H, d, J=6 Hz, C_6-H). *Anal.* Calcd for $C_{10}H_{11}FN_2O_4S_2$: C, 39.22; H, 3.62; F, 6.20; N, 9.14. Found: C, 38.95; H, 3.35; F, 5.90; N, 9.04.

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 L1210 leukemia. The compound to be tested was injected intraperitoneally or administered orally once daily for 5 d, starting 24 h after tumor implantation.

Evaluation of Antitumor Activity——The increase in life span was calculated by using the following formula:

$$\text{ILS (increase of life span) } (\%) = (T - C)/C \times 100$$

where T is the average number of days before death in the test group and C is the average number of days before death in the control group.[11]

Acknowledgment The authors wish to thank the staff of the Analytical Laboratory of the Mitsui Toatsu Research Center and Ehime University Advanced Instrumentation Center for the elemental analysis.

References and Notes

1) Part XI: S. Ozaki, Y. Watanabe, T. Nagase, Y. Ike, and H. Mori, *Chem. Pharm. Bull.*, **34**, 893 (1986).
2) S. Ozaki, Y. Ike, H. Mizuno, K. Ishikawa, and H. Mori, *Bull. Chem. Soc. Jpn.*, **50**, 2406 (1977).
3) T. Hoshiko, S. Ozaki, Y. Watanabe, T. Ogasawara, Y. Yamauchi, K. Fujiwara, A. Hoshi, and M. Iigo, *Chem. Pharm. Bull.*, **33**, 2832 (1985).
4) T. Hoshiko, Y. Watanabe, and S. Ozaki, *Heterocycles*, **20**, 2429 (1983).
5) S. Ozaki, Y. Watanabe, H. Fujisawa, and T. Hoshiko, *Heterocycles*, **22**, 527 (1984).
6) S. Ozaki, Y. Watanabe, T. Hoshiko, H. Mizuno, K. Ishikawa, and H. Mori, *Chem. Pharm. Bull.*, **32**, 733 (1984).
7) J. Yamashita, I. Yamawaki, S. Ueda, M. Yasumoto, N. Unemi, and S. Hashimoto, *Chem. Pharm. Bull.*, **30**, 4258 (1982).
8) S. Ozaki, Y. Watanabe, T. Hoshiko, T. Nagase, T. Ogasawara, M. Furukawa, A. Uemura, T. Ishikawa, H. Mori, A. Hoshi, M. Iigo, and R. Tokuzen, *Chem. Pharm. Bull.*, **34**, 150 (1986).
9) Some portions of synthetic procedures have been briefly described in patents; see Mitsui Toatsu Chemicals Inc., Japan. Patent Open 55-24124 (1980) [*Chem. Abstr.*, **93**, 186388*f* (1980)], and 55-98171 (1980) [*Chem. Abstr.*, **94**, 175146*v* (1981)].
10) 1,3-Bis(chloromethyl)-5-fluorouracil (**6**) was easily prepared by chlorination of 1,3-bis(hydroxymethyl)-5-fluorouracil with sulfonyl chloride in *ca.* 50% yield. Compound **5** was obtained by hydrolysis of **6** in aqueous acetone in 75—80% yield. The details of the chemistry of *N*-chloromethyl-5-fluorouracils will be described elsewhere.
11) A. Hoshi, M. Iigo, A. Nakamura, M. Inomata, and K. Kuretani, *Chem. Pharm. Bull.*, **26**, 161 (1978).