mean ± SD) were randomly distributed into groups of 10 animals. They were subcutaneously injected daily for 3 days with 0.1 mL of olive oil solutions containing the test compound. The uteri were removed 24 h after the last injection, fixed with Bouin's solution, washed, dried, and weighed.

Acknowledgment. Thanks are due to the Deutsche Forschungsgemeinschaft and to the Verband der Chemischen Industrie, Fonds der Chemischen Industrie, who supported this work by grants. The technical assistance of Fritz Birk is gratefully acknowledged.

Registry No. 1, 79199-51-2; **1a**, 85720-33-8; **1b**, 85720-34-9; **1c**, 1835-04-7; **2**, 74536-61-1; **2a**, 74536-60-0; **2b**, 85720-35-0; **2c**, 586-22-1; **3**, 79140-57-1; **3a**, 79140-65-1; **3b**, 85720-36-1; **3c**, 4394-54-1; **4**, 74536-64-4; **5**, 55508-15-1; **6**, 85720-37-2; **7**, 10465-10-8; **8**, 85720-38-3; **8a**, 85720-39-4; **9**, 85720-40-7; **10**, 85720-41-8; **11**, 66877-40-5; **12**, 66877-41-6; **13**, 85720-42-9; **14**, 85720-43-0; **14a**, 85720-44-1; **15**, 85720-45-2; **15a**, 85720-46-3; **16**, 85720-47-4; **16a**, 85720-48-5; **16b**, 830-99-9; **17**, 85720-49-6; **17a**, 85720-50-9; **17b**, 85720-51-0; **18**, 85720-52-1; **18a**, 85720-53-2; **18b**, 85720-54-3; **18c**, 13329-61-8; **19**, 85720-55-4; **19a**, 85720-56-5; **19b**, 23600-60-4; **20**, 85720-57-6; **20a**, 85735-20-2; **20b**, 53773-75-4; **21**, 85720-58-7; **21a**, 85720-59-8; **21b**, 85720-60-1; hexestrol, 84-16-2; hexestrol dimethyl ether, 28231-25-6.

Supplementary Material Available: ^{1}H NMR data (Tables VI–VIII) of the 2,2′- and 3,3′-disubstituted hexestrol derivatives (**1–21** and **1a–21a**) and the 1-(2-substituted-4-methoxyphenyl)-1-propanols (**16b–21b**) (8 pages). Ordering information is given on any current masthead page.

Pyridazinones. 3. Synthesis, Antisecretory, and Antiulcer Activities of 2-Cyanoguanidine Derivatives

Toshihiro Yamada,* Hiroshi Shimamura, Yoshitsugu Tsukamoto, Azuma Yamaguchi, and Masahiko Ohki

Research Laboratories, Morishita Pharmaceutical Co., Ltd., 1658, Ohshinohara, Yasu-cho, Yasu-gun, Shiga, 520-23, Japan.
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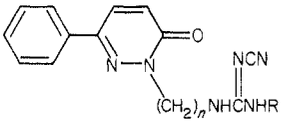
3(2*H*)-Pyridazinone derivatives having a 2-cyanoguanidine moiety, as well as a sulfur or an oxygen atom in the alkylene side chain, were synthesized and evaluated for gastric antisecretory and antiulcer activities. The key intermediates, free amines having a thioether linkage, were synthesized by the reaction of 2-(ω-chloroalkyl) derivatives with cysteamine, while other intermediates having an ether linkage were synthesized from 2-(ω-chloroalkyl)oxymethyl derivatives. These free amines were converted via the 3-cyano-2-methyl-1-isothiourea derivatives into the desired 2-cyano-3-substituted-1-guanidine derivatives. All compounds synthesized were evaluated for gastric antisecretory activity in the pylorus-ligated rat by the method of Shay, and selected compounds were evaluated in the stress-induced ulcer test in rat. Structure–activity relationships are discussed. The molecular features for the best activities are a phenyl group in the C-6 position of the 3(2*H*)-pyridazinone ring, a four-atom chain length between the 3(2*H*)-pyridazinone ring and the 2-cyanoguanidine moiety, and a thioether rather than an ether linkage. Among them, compound **14**, 2-[[[2-(2-cyano-3-methyl-1-guanidino)ethyl]thio]methyl]-6-phenyl-3(2*H*)-pyridazinone, had the most potent antisecretory and antiulcer activities. These compounds are neither histamine H_2 receptor inhibitors nor anticholinergic agents.

A variety of derivatives that incorporate an aminoalkylthio unit [$NH(CH_2)_nS$] have been synthesized, and their pharmacological activities, such as cardiovascular,[1] cholinesterase inhibitor,[2] active transport,[3] antibacterial,[4] and antidepressant,[5] have been reported. Since the discovery of metiamide by Black et al.[6] as a histamine H_2 receptor antagonist, several histamine H_2 receptor antagonists, e.g., cimetidine,[7] ranitidine (AH 19065),[8] tiotidine (ICI 125211),[9] oxmetidine (SKF 92994),[10] and etintidine (BL-5641),[11] have been reported. All these compounds bear an aminoethylthio unit as a common structural feature; hence, the unit may be thought to be general for the antagonistic activity.

For the development of new types of antiulcer agents without anticholinergic activity, a series of novel 3(2*H*)-pyridazinone derivatives were synthesized, and the structural requirements for activity were defined by molecular modification. We recently reported the synthesis of a series of 3(2*H*)-pyridazinone derivatives (1) having a



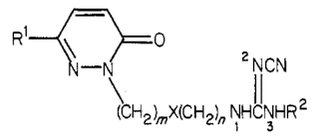
1
MUN-114, n = 4; R = CH_3
MUN-118, n = 5; R = C_2H_5

thiourea or a 2-cyanoguanidine moiety, which were shown

(1) Wise, L. D.; Morrison, G. C.; Egan, K.; Commarato, M. A.; Kopia, G.; Lattime, E. C. *J. Med. Chem.* **1974**, *17*, 1232.
(2) Kutty, K. M.; Jacob, J. C.; Hutton, P. J.; Peterson, S. C. *Clin. Biochem.* **1975**, *8*, 379.
(3) Schuldrin, S.; Kerwar, G.; Kaback, H.; Weil, R. *J. Biol. Chem.* **1975**, *250*, 1361.
(4) Neu, H. C.; Labthavikul, P. *Int. Conf. Antimicrob. Agents Chemother., 20th* **1980**, 260.
(5) Ong, H. H.; Profitt, J. A.; Anderson, V. B.; Spaulding, T. C.; Wilker, J. C.; Geyer III, H. M.; Kruse, H. *J. Med. Chem.* **1980**, *23*, 494.
(6) Black, J. W.; Duncan, W. A. M.; Emmett, J. C.; Ganellin, C. R.; Hesselbo, T.; Parsons, M. E.; and Wyllie, J. H. *Agents Actions* **1973**, *3*, 133.
(7) Brimblecombe, R. W.; Duncan, W. A. M.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Parsons, M. E. *J. Int. Med. Res.* **1975**, *3*, 86.
(8) Bradshaw, J.; Brittain, R. T.; Clitherow, J. W.; Daly, M. J.; Jack, D.; Price, B. J.; Stables, R. *Br. J. Pharmacol.* **1979**, *66*, 464p.
(9) Yellin, T. O.; Buck, S. H.; Gilman, D. J.; Jones, D. F.; Waldleworth, J. M. *Life Sci.* **1979**, *25*, 2001.
(10) Blakemore, R. C.; Brown, T. H.; Durant, G. J.; Emmett, J. C.; Ganellin, C. R.; Parsons, M. E.; Rasmussen, A. C. *Br. J. Pharmacol.* **1980**, *70*, 105p.
(11) Cavanagh, R. L.; Usakewicz, J. J.; Buyniski, J. P. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1980**, *39*, 768.

0022-2623/83/1826-1144$01.50/0

Table I. 2-[[[(2-Cyano-3-substituted-1-guanidino)alkyl]thio- or -oxy]alkyl]-3(2*H*)-pyridazinones



no.	R^1	R^2	X	*m*	*n*	yield,[a] %	mp, °C	crystn solvent[b]	formula[c]
10	CH_3	CH_3	S	1	2	89	153-155	EtOH-IPE	$C_{11}H_{16}N_6OS$
11	CH_3	C_2H_5	S	1	2	61	110-112	EtOH-IPE	$C_{12}H_{18}N_6OS$
12	C_4H_8NO[d]	CH_3	S	1	2	95	188-189	EtOH-IPE	$C_{14}H_{21}N_7O_2S$
13	$(CH_3)_2N$	CH_3	S	1	2	63	172-173	EtOH-IPE	$C_{12}H_{19}N_7OS$
14	C_6H_5	CH_3	S	1	2	84	157-158	EtOH	$C_{16}H_{18}N_6OS$
15	C_6H_4(4-Cl)	CH_3	S	1	2	68	218-219	EtOH	$C_{16}H_{17}ClN_6OS$
16	C_6H_4(4-CH_3)	CH_3	S	1	2	78	172-174	EtOH	$C_{17}H_{20}N_6OS$
17	C_6H_5	C_2H_5	S	1	2	80	170-172	EtOH	$C_{17}H_{20}N_6OS$
18	C_6H_5	C_3H_7	S	1	2	56	143-146	EtOH	$C_{18}H_{22}N_6OS$
19	C_6H_5	CH_3	S	2	2	81	139-140	EtOH	$C_{17}H_{20}N_6OS$
20	C_6H_5	C_2H_5	S	2	2	88	143-144	EtOH	$C_{18}H_{22}N_6OS$
21	C_6H_5	C_3H_7	S	2	2	54	104-107	EtOH	$C_{19}H_{24}N_6OS$
22	C_6H_5	CH_3	S	3	2	93	117-120	EtOH	$C_{18}H_{22}N_6OS$
23	C_6H_5	C_2H_5	S	3	2	61	87-90	EtOH	$C_{19}H_{24}N_6OS$
24	C_6H_5	CH_3	O	1	2	96	144-146	EtOH-IPE	$C_{16}H_{18}N_6O_2$
25	C_6H_5	CH_3	O	1	3	95	121-122	EtOH-IPE	$C_{17}H_{20}N_6O_2$
26	C_6H_5	CH_3	O	1	4	33	119-120	CH_3CN	$C_{18}H_{22}N_6O_2$

[a] The yield quoted was for isolated purified product. [b] IPE = isopropyl ether. [c] All compounds analyzed within ±0.4% theory for C, H, and N. [d] Morpholino.

to possess marked gastric antisecretory and/or antiulcer activities in rat.[12] We have also reported that 2-[4-(2-cyano-3-methyl-1-guanidino)butyl]-6-phenyl-3(2*H*)-pyridazinone (MUN-114) and 2-[5-(2-cyano-3-ethyl-1-guanidino)pentyl]-6-phenyl-3(2*H*)-pyridazinone (MUN-118) showed potential as new antiulcer agents without histamine H_2 receptor antagonistic action.

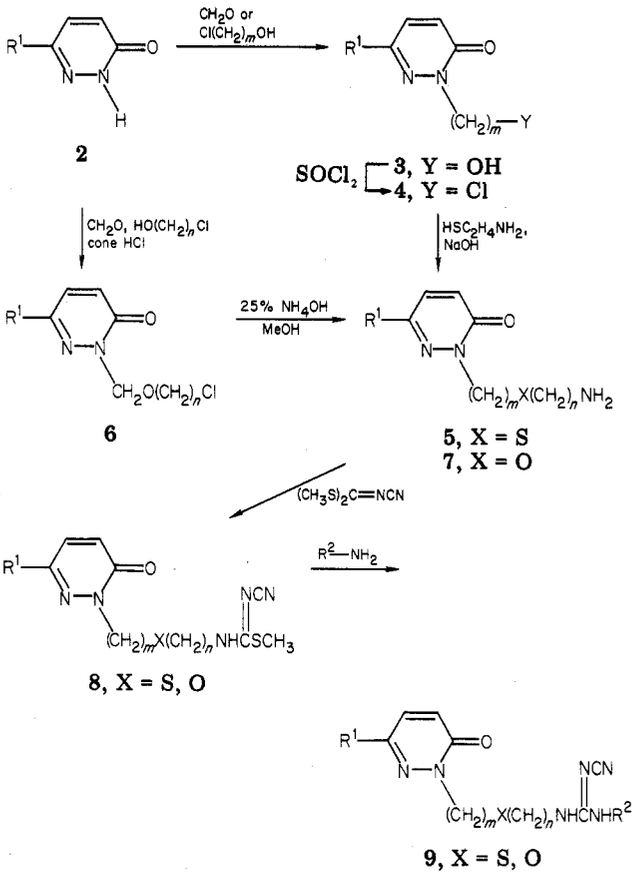
Based on these considerations, it was decided to prepare a series of 3(2*H*)-pyridazinone derivatives having a 2-cyanoguanidine moiety that incorporate a sulfur or an oxygen atom in an alkylene side chain between the 3-(2*H*)-pyridazinone ring and the 2-cyanoguanidine moiety.

Chemistry. The desired 2-cyanoguanidine derivatives having a thioether or an ether linkage (Table I) were prepared by the general reaction sequence depicted in Scheme I. 2-(ω-Hydroxyalkyl) derivatives (**3**) were generally synthesized by the reaction of ω-hydroxyalkyl chlorides with 3(2*H*)-pyridazinones (**2**) in EtOH in the presence of NaOH. N-Hydroxymethylation of **2** to obtain **3** having a methylene unit (*m* = 1) was most conveniently carried out with 37% formalin in H_2O or EtOH.[13] Chlorination of **3** by refluxing with $SOCl_2$ in $CHCl_3$ gave **4** in good yield. The key intermediates, free amines **5**, were prepared by reaction of **4** with cysteamine in the presence of NaOH.

For the preparation of free amines having an ether linkage, the 2-(ω-chloroalkyl)oxymethyl derivatives **6** were obtained by reaction of ω-hydroxyalkyl chloride and paraformaldehyde in the presence of concentrated HCl solution. The chlorides **6** were converted to the free amines **7** by reaction with ammonium hydroxide.

The desired 2-cyanoguanidine derivatives **9** were prepared from the key intermediates **5** and **7**. The free amines **5** and **7** were converted with dimethyl cyanodithioimidocarbonate[14] to 3-cyano-2-methyl-1-isothioureas **8** (Table III), which gave the desired 2-cyano-3-substituted-1-guanidines **9** by reaction with suitable amines in acceptable yield (Table I).

Scheme I[a]



[a] R^1 = CH_3, c-$O(CH_2CH_2)_2N$, $N(CH_3)_2$, C_6H_5, C_6H_4(4-Cl), C_6H_4(4-CH_3); *m* = 1-3; *n* = 2-4; R^2 = CH_3, C_2H_5, C_3H_7.

Pharmacological Results and Discussion

All compounds synthesized in the present studies were tested for gastric antisecretory activity at a dose of 100 mg/kg in the pylorus-ligated rat by the method of Shay.[15]

(12) Yamada, T.; Nobuhara, Y.; Shimamura, H.; Tsukamoto, Y.; Yoshihara, K.; Yamaguchi, A.; Ohki, M. *J. Med. Chem.* **1983**, *26*, 373.
(13) Gregory, H.; Hills, J.; Wiggins, L. *J. Chem. Soc.* **1949**, 1248.
(14) Timmons, R. J; Wittenbrook, L. S. *J. Org. Chem.* **1967**, *32*, 1566.

Table II. Antisecretory and Antiulcer Activities of 3(2*H*)-Pyridazinones

no.	antisecretory act.[a] % inhibn at 100 mg/kg	antisecretory act.[a] ED_{50}, mg/kg	antiulcer act.[b] ED_{50}, mg/kg
10	30.0	>100	>100
11	26.7	>100	
12	−12.5 (NS)[c]		NS
13	6.9 (NS)		
14	83.3	30.5 (25.4-36.5)	28.5 (18.2-44.7)
15	70.3	39.1 (33.1-46.1)	
16	62.5	53.8 (46.4-62.4)	
17	50.1	100[d]	63.1 (43.1-92.3)
18	37.3	>100	97.6 (70.7-134.7)
19	58.3	80.0 (65.0-98.4)	77.6 (56.2-107.1)
20	50.8	100[d]	
21	33.5	>100	
22	18.2 (NS)		>100
23	22.9 (NS)		
24	60.6	82.3 (63.9-106.0)	>100
25	49.8	100[d]	NS
26	32.8	>100	NS
cimetidine	80.6	60.3 (50.3-72.4)	43.2 (25.4-73.4)
MUN-114	79.0	32.4 (27.0-38.9)	38.5 (25.7-57.8)

[a] Statistically significant activity ($p < 0.05$) was determined in the 4-h pylorus-ligated rat by using the technique of Shay. [b] Stress ulcer (see Experimental Section). [c] NS = not statistically significant. [d] Graphically calculated.

For the compounds that showed about 50% or more inhibition in this first screening, dose range studies were performed, and ED_{50} values were determined. In addition, selected compounds that showed substantial antisecretory activity were tested for inhibition of the generation of experimental ulcer-induced by stress.[16] For comparison, the active references cimetidine and MUN-114 were included in the biological determinations. The structures and physicochemical data of the compounds synthesized are shown in Table I, and their pharmacological activities are shown in Table II.

A series of 3(2*H*)-pyridazinone derivatives with a thioether or an ether linkage in the alkylene side chain between the 3(2*H*)-pyridazinone ring and the 2-cyanoguanidine moiety had considerable antisecretory and/or antiulcer activities, and the compounds that showed about 50% or more inhibition in the first screening had no histamine H_2 receptor antagonistic activity at 1×10^{-5} M. A close inspection of the results reveals some interesting facts with respect to structure–activity relationships.

The relative antisecretory activities of C-6 substituted derivatives of the 3(2*H*)-pyridazinone ring were examined. The 6-phenyl-3(2*H*)-pyridazinone derivatives (**14–18**) were more active than the 6-methyl (**10**), 6-morpholino (**12**), or 6-dimethylamino derivatives (**13**). The effect of substituents on the C-6 phenyl ring (**15** and **16**) on biological activity was examined, but the biological advantage could not be elucidated.

The effects of alkyl substitution on the N-3 position of the 2-cyanoguanidine moiety for activity was surveyed in a series of thioether linkage derivatives. The introduction of a longer alkyl group in the N-3 position evidently decreased the activity according to their lipophilicity (**14** > **17** > **18** and **19** > **20** > **21**). The methyl group was found to be very preferable for the activity.

The effects of the length of the side chain linking the 2-cyanoguanidine moiety with the 3(2*H*)-pyridazinone ring were examined in the group of 2-cyano-3-methyl-1-guanidines (**14**, **19**, and **22**), 2-cyano-3-ethyl-1-guanidines (**17**, **20**, and **23**), and 2-cyano-3-propyl-1-guanidines (**18** and **21**). With respect to the number of atoms in the side chain, the relative potencies of these compounds can generally be summarized in the following order (number of atoms =) 4 > 5 > 6. Compound **14** showed the highest potency among the 2-cyanoguanidine derivatives.

Compounds **24–26** having an ether linkage in the alkylene side chain generally showed a decrease in potency, compared with the series of thioether linkage derivatives (**14** and **19**). Herke and Schunack[17] reported the preparation of a compound wherein the thioether linkage in cimetidine was replaced by an ether linkage as potential H_2 antihistamines, and the analogue did not show any inhibition of gastric acid secretion. Hence, they concluded that the replacement results in a marked loss of activity.

In addition, the effects on activity of the side chain between the oxygen and the N-1 position of the 2-cyanoguanidine moiety in the ether linkage group (**24–26**) was influenced by its length (*n*): 2 > 3 > 4.

The 3(2*H*)-pyridazinone derivatives with four atoms in the side chain between the ring an the 2-cyanoguanidine moiety generally exhibited higher inhibitory activity. This probably means that these compounds require a specific distance between the carbonyl group of the 3(2*H*)-pyridazinone ring and the N-1 or N-3 position of the 2-cyanoguanidine moiety. These distances were estimated to be 7.6 or 10.2 Å by using Dreiding stereomodels, respectively.

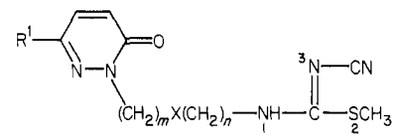
Ten compounds were selected to define the structural requirements for the antiulcer activity on the basis of their gastric antisecretory activity described above. These compounds were subjected to the stress-induced gastric lesion in the rat by the method of Takagi et al.,[16] which is considered to be important in the pathogenesis of the stress-induced gastric ulcer causing the disturbances of the gastric mucosal microcirculation. When substantial activity (≥50%) was observed at a dose of 100 mg/kg, full dose range studies were performed on the active compounds, and ED_{50} values were determined (Table II).

Among the compounds having four atoms in the side chain, the compounds containing a phenyl group in the C-6 position of the 3(2*H*)-pyridazinone ring (**14**, **17**, and **18**) possessed significantly potent antiulcer activity, while the methyl derivative (**10**) decreased the activity and the morpholino (**12**) further diminished the activity. The phenyl group in the C-6 position of the 3(2*H*)-pyridazinone ring is preferred for enhanced stress-induced antiulcer activity.

The relationship between the length of the side chain and the potency of compounds **14**, **19**, and **22** were examined. As shown in Table II, four-atoms length is favorable for the activity. Compounds **24–26** with an ether linkage in the alkylene side chain generally showed a decrease in

(15) Shay, H. G.; Sun, D. C. H.; Gruenstein, M. *Gastroenterology* **1954**, *26*, 906.
(16) Takagi, K.; Okabe, S. *Jpn. J. Pharmacol.* **1968**, *18*, 9.
(17) Herke, J.; Schunack, W. *Eur. J. Med. Chem.* **1979**, *14*, 203.

Table III. 2-[[[(3-Cyano-2-methyl-1-isothioureido)alkyl]thio- or -oxy]alkyl]-3(2*H*)-pyridazinones (8)



no.	R^1	X	*m*	*n*	yield,[a] %	mp, °C	crystn solvent[b]	formula[c]
8a	CH_3	S	1	2	49	146-149	EtOH-IPE	$C_{11}H_{15}N_5OS_2$
8b	C_4H_8NO[d]	S	1	2	74	98-99	EtOH	$C_{14}H_{20}N_6O_2S_2$
8c	$(CH_3)_2N$	S	1	2	38	114-116	EtOH	$C_{12}H_{18}N_6OS_2$
8d	C_6H_5	S	1	2	73	136-138	EtOH-IPE	$C_{16}H_{17}N_5OS_2$
8e	C_6H_4(4-Cl)	S	1	2	33	159-162	EtOH	$C_{16}H_{16}ClN_5OS_2$
8f	C_6H_4(4-CH_3)	S	1	2	49	59-61	EtOH	$C_{17}H_{19}N_5OS_2$
8g	C_6H_5	S	2	2	93	99-102	EtOH	$C_{17}H_{19}N_5OS_2$
8h	C_6H_5	S	3	2	94	131-134	EtOH	$C_{18}H_{21}N_5OS_2$
8i	C_6H_5	O	1	2	59	69-70	EtOH-IPE	$C_{16}H_{17}N_5O_2S$
8j	C_6H_5	O	1	3	47	110-113	EtOH-IPE	$C_{17}H_{19}N_5O_2S$
8k	C_6H_5	O	1	4	34	97-100	EtOH-IPE	$C_{18}H_{21}N_5O_2S$

[a] The yield quoted was for isolated purified product. [b] IPE = isopropyl ether. [c] All compounds analyzed within ±0.4% theory for C, H, and N. [d] Morpholino.

activity, but compounds **25** and **26** were inactive. These compounds were shown not to have anticholinergic activity at 1×10^{-5} M.

In summary, the antisecretory and/or antiulcer activity of 3(2*H*)-pyridazinone derivatives was influenced by the kind of substituent in the C-6 position of the 3(2*H*)-pyridazinone ring, by the substitution in the 2-cyanoguanidine moiety, and by the length of the side chain containing either a sulfur or an oxygen. The activity was due to neither histamine H_2 receptor antagonistic action nor anticholinergic action. Among these compounds, 2-[[[2-(2-cyano-3-methyl-1-guanidino)ethyl]thio]methyl]-6-phenyl-3(2*H*)-pyridazinone (**14**) was found to be the most potent with respect to the antisecretory and antiulcer activities in the rat model.

Experimental Section

Chemistry. All melting points were obtained with a Yanagimoto micro melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi Type 215 spectrophotometer, and proton nuclear magnetic resonance spectra (^{1}H NMR) were recorded on a JEOL JNM-PS-100 spectrometer and reported as parts per million (ppm, δ) relative to tetramethylsilane. Analyses were performed with a Hitachi 026 CHN analyzer; analyses indicated by elemental symbols were within 0.4% of the theoretical values.

Starting Materials (2). 6-Methyl-3(2*H*)-pyridazinone,[18] 6-phenyl-3(2*H*)-pyridazinone,[19] and related derivatives were prepared by the cited procedure.[20]

2-(Hydroxymethyl)-6-phenyl-3(2*H*)-pyridazinone (3, R^1 = C_6H_5; Y = OH; *m* = 1). A mixture of 8.6 g (0.05 mol) of 6-phenyl-3(2*H*)-pyridazinone (**2**) and 30 mL of 37% formalin was heated at reflux for 1 h. The solid that separated on cooling was filtered and washed with water. The solid was recrystallized from H_2O–EtOH to provide 9.0 g (88%) of **3** (R^1 = C_6H_5; Y = OH; *m* = 1): mp 158–160 °C; IR (Nujol) 3240 (OH), 1650 (C=O) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 5.48 (d, 2 H, *J* = 7 Hz, CH_2OH), 6.73 (t, 1 H, *J* = 7 Hz, OH), 7.10 (d, 1 H, *J* = 10 Hz, C_4 H), 7.5 (m, 3 H, Ar H), 7.9 (m, 2 H, Ar H), 8.10 (d, 1 H, *J* = 10 Hz, C_5 H).

2-(2-Hydroxyethyl)-6-phenyl-3(2*H*)-pyridazinone (3, R^1 = C_6H_5; Y = OH; *m* = 2). A mixture of 8.6 g (0.05 mol) of 6-phenyl-3(2*H*)-pyridazinone (**2**) and 2.8 g (0.05 mol) of KOH in 200 mL of EtOH was warmed at 50 °C for 1 h. The mixture was evaporated to dryness in vacuo. The residue was suspended with 200 mL of DMF–benzene (1:1), and then 7.5 g (0.06 mol) of ethylene chlorohydrin was added dropwise over a period of 0.5 h to the suspension in an ice–water bath. The suspension was heated at 120 °C for 24 h, and the mixture gradually became homogeneous as rate of reaction increased. The mixture was then evaporated to dryness in vacuo. The residue was extracted with $CHCl_3$ (100 mL × 2), dried over anhydrous Na_2SO_4, and filtered. The filtrate was evaporated under reduced pressure to give a crude solid. The solid was recrystallized from EtOH–isopropyl ether to give 8.0 g (74%) of **3** (R^1 = C_6H_5; Y = OH; *m* = 2): mp 98–99 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.32 (t, 2 H, *J* = 7 Hz, $CONCH_2$), 4.96 (t, 1 H, *J* = 7 Hz, CH_2OH).

2-(2-Chloroethyl)-6-phenyl-3(2*H*)-pyridazinone (4, R^1 = C_6H_5; Y = Cl; *m* = 2). To a solution of 19.5 g (0.09 mol) of 2-(2-hydroxyethyl)-6-phenyl-3(2*H*)-pyridazinone (**3**) in 100 mL of $CHCl_3$, maintained at < 5 °C, was gradually added 11.9 g (0.1 mol) of $SOCl_2$. The mixture was heated at reflux (80 °C) on oil bath for 1 h. After cooling, the reaction mixture was evaporated, and the residue was dissolved with 200 mL of $CHCl_3$. The organic phase was washed with water (100 mL × 3) and dried over Na_2SO_4. The dried solution was evaporated to give crude solid, which was recrystallized from EtOH–isopropyl ether to give 18.9 g (89%): mp 57–59 °C; ^{1}H NMR (Me_2SO-d_6) δ 4.12 (t, 2 H, *J* = 7 Hz, $CONCH_2$), 4.56 (t, 2 *H*, *J* = 7 Hz, CH_2Cl).

2-[[(2-Aminoethyl)thio]methyl]-6-phenyl-3(2*H*)-pyridazinone (5, R^1 = C_6H_5; *m* = 1; *n* =2). To a solution of 4.6 g (0.2 mol) of Na in 300 mL of absolute EtOH was added 11.4 g (0.1 mol) of cysteamine hydrochloride in portions. The resultant reaction mixture was allowed to warm to ambient temperature over 1 h. After the mixture was cooled, 20 g (0.09 mol) of 2-(chloromethyl)-6-phenyl-3(2*H*)-pyridazinone (**4**) was added, and the mixture was heated at reflux for 5 h. The reaction mixture was then filtered and concentrated in vacuo to give a dark orange oil. The oily product was dissolved in $CHCl_3$ (300 mL) and water (100 mL), and the pH of the water layer was adjusted to 10 with aqueous 10% NaOH solution. The organic layer was washed with three 200-mL portions of saturated $NaHCO_3$ and water, and evaporated to give 14 g (60%) of **5** (R^1 = C_6H_5; *m* = 1; *n* = 2) as a slight yellow oil. This free amine was used without further purification: IR (film) 3350 (NH), 1670 (C=O) cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 2.52 (s, 2 H, CH_2NH_2), 2.84 (s, 4 H, $SCH_2CH_2NH_2$), 5.26 (s, 2 H, $CONCH_2S$), 7.12 (d, 1 H, *J* = 10 Hz, C_4 H), 7.5 (m, 3 H, Ar H), 7.9 (m, 2 H, Ar H), 8.08 (d, 1 H, *J* = 10 Hz, C_5 H).

2-[[(2-Aminoethyl)thio]methyl]-6-methyl-3(2*H*)-pyridazinone (5, R^1 = CH_3; *m* = 1; *n* = 2). The title compound was prepared from 2-(chloromethyl)-6-methyl-3(2*H*)-pyridazinone (**4**) and cysteamine hydrochloride as described for the preparation of **5** (R^1 = C_6H_5; *m* = 1; *n* = 2): ^{1}H NMR (Me_2SO-d_6) δ 1.98 (s, 2 H, CH_2NH_2), 2.80 (s, 4 H, $SCH_2CH_2NH_2$), 5.18 (s, 2 H, $CONCH_2S$).

2-[2-[(2-Aminoethyl)thio]ethyl]-6-phenyl-3(2*H*)-pyridazinone (5, R^1 = C_6H_5; *m* = 2; *n* = 2). The title compound was prepared from 2-(2-chloroethyl)-6-phenyl-3(2*H*)-pyridazinone (**4**) and cysteamine hydrochloride, as described for the preparation

(18) Kumagai, M. *Nippon Kagaku Zasshi* **1960**, *81*, 489.
(19) Steck, E. A.; Brundage, R. P.; Fletcher, L. T. *J. Am. Chem. Soc.* **1953**, *73*, 1117.
(20) Castle, R. N. *Chem. Heterocycl. Compd.* **1973**, *28*.

of **5** ($R^1 = C_6H_5$; $m = 1$; $n = 2$): ^1H NMR (Me_2SO-d_6) δ 2.05 (br, 2 H, CH_2NH_2), 2.70 (s, 4 H, $SCH_2CH_2NH_2$), 2.95 (t, 2 H, $J = 7$ Hz, $CONCH_2CH_2S$), 4.44 (t, 2 H, $J = 7$ Hz, $CONCH_2CH_2S$).

2-[3-[(2-Aminoethyl)thio]propyl]-6-phenyl-3(2*H*)-pyridazinone (5, $R^1 = C_6H_5$; $m = 3$; $n = 2$). The title compound was prepared from 2-(3-chloropropyl)-6-phenyl-3(2*H*)-pyridazinone (**4**) and cysteamine hydrochloride, as described for the preparation of **5** ($R^1 = C_6H_5$; $m = 1$; $n = 2$): ^1H NMR (Me_2SO-d_6) δ 1.97 (br, 2 H, CH_2NH_2) 2.09 (quintet, 2 H, $J = 7$ Hz, $CONCH_2CH_2CH_2S$), 2.7 (m, 6 H, $CONCH_2CH_2CH_2SCH_2CH_2NH_2$), 4.28 (t, 2 H, $J = 7$ Hz, $CONCH_2$).

2-[[(2-Chloroethyl)oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (6, $R^1 = C_6H_5$; $n = 2$). A mixture of 17.2 g (0.1 mol) of 6-phenyl-3(2*H*)-pyridazinone (**2**), 3.3 g (0.12 mol) of paraformaldehyde, and 10 mL of concentrated HCl solution in 100 mL of ethylene chlorohydrin was heated at 120 °C for 24 h. After cooling, the reaction mixture was concentrated to one-third of its original volume and filtered, and then the filtrate was evaporated to dryness in vacuo. The residue was dissolved with 100 mL of $CHCl_3$, and the $CHCl_3$ solution was washed with saturated $NaHCO_3$, 2% HCl solution, and then water and dried over Na_2SO_4. The filtrate was evaporated under reduced pressure, and the residue was dissolved in 100 mL of $CHCl_3$ and applied to a 350-g silica gel (Wako's gel C-200) column in $CHCl_3$. The fraction that eluted with $CHCl_3$ was evaporated in vacuo to give 15.7 g (59.4%) of **6** ($n = 2$) as a slight yellow oil: IR (film) 1660 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 3.70 (m, 4 H, OCH_2CH_2Cl), 5.54 (s, 2 H, $CONCH_2O$), 7.13 (d, 1 H, $J = 10$ Hz, C_4H), 7.54 (m, 3 H, Ar H), 7.79 (m, 2 H, Ar H), 8.06 (d, 1 H, $J = 10$ Hz, C_5 H).

2-[[(2-Aminoethyl)oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (7, $R^1 = C_6H_5$; $n = 2$). A mixture of 13.7 g (0.05 mol) of 2-[[(2-chloroethyl)oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (**6**, $R^1 = C_6H_5$; $n = 2$) and 130 mL of 25% ammonium hydroxide in 150 mL of MeOH was heated at 150 °C in an autoclave for 24 h. After cooling, the reaction mixture was evaporated to dryness in vacuo, and the residue was dissolved with 100 mL of water and 100 mL of benzene. After the addition of 200 mL of $CHCl_3$ to the water layer, the pH of the water phase was adjusted to 10 with aqueous 30% NaOH solution. The $CHCl_3$ phase was washed with saturated NaCl solution and dried over Na_2SO_4. The dried solution was evaporated to dryness in vacuo to give 6.6 g (55%) as a slight yellow oil: ^1H NMR (Me_2SO-d_6) δ 2.04 (m, 2 H, NH_2), 2.78 (t, 2 H, $J = 7$ Hz, CH_2NH_2), 3.71 (t, 2 H, $J = 7$ Hz, $OCH_2CH_2NH_2$), 5.54 (s, 2 H, $CONCH_2$).

2-[[(3-Aminopropyl)oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (7, $R^1 = C_6H_5$; $m = 1$; $n = 3$). The title compound was prepared from 2-[[(3-chloropropyl)oxy]methyl]-6-phenyl-3-(2*H*)-pyridazinone (**6**, $R^1 = C_6H_5$; $n = 3$) and 25% ammonium hydroxide, as described for the preparation of **7** ($R^1 = C_6H_5$; $m = 1$; $n = 2$): ^1H NMR (Me_2SO-d_6) δ 1.62 (quintet, 2 H, $J = 7$ Hz, $OCH_2CH_2CH_2NH_2$), 2.60 (br, 2 H, NH_2), 2.68 (t, 2 H, $J = 7$ Hz, CH_2NH_2), 3.72 (t, 2 H, $J = 7$ Hz, OCH_2CH_2).

2-[[(4-Aminobutyl)oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (7, $R^1 = C_6H_5$; $m = 1$; $n = 4$). The title compound was prepared from 2-[[(4-chlorobutyl)oxy]methyl]-6-phenyl-3-(2*H*)-pyridazinone (**6**, $R^1 = C_6H_5$; $n = 4$) and 25% ammonium hydroxide, as described for the preparation of **7** ($R^1 = C_6H_5$; $m = 1$; $n = 2$): ^1H NMR (Me_2SO-d_6) δ 1.50 (m, 4 H, $OCH_2CH_2CH_2CH_2NH_2$), 2.46 (br, 2 H, NH_2), 2.60 (t, 2 H, $J = 7$ Hz, CH_2NH_2), 3.66 (t, 2 H, $J = 7$ Hz, OCH_2CH_2), 5.47 (s, 2 H, $CONCH_2O$).

2-[[[2-(3-Cyano-2-methyl-1-isothioureido)ethyl]thio]methyl]-6-phenyl-3(2*H*)-pyridazinone (8d). To a solution of 9.7 g (0.037 mol) of 2-[[(2-aminoethyl)thio]methyl]-6-phenyl-3-(2*H*)-pyridazinone (**5**, $R^1 = C_6H_5$; $m = 1$; $n = 2$) in 40 mL of EtOH was slowly added dropwise a solution of 5.4 g (0.037 mol) of dimethyl cyanodithioimidocarbonate in 20 mL of EtOH at room temperature for 15 min. The mixture was set aside overnight. The reaction mixture practically becomes solid, and the stirrer may have to be stopped. The precipitate was filtered, and the residue was washed with 20 mL of cooled EtOH. Recrystallization of this product from EtOH–isopropyl ether afforded 9.7 g (73%) of **8d** as a slight yellow powder: IR (Nujol) 3310 (NH), 2170 (C≡N), 1660 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.62 (s, 3 H, SCH_3), 3.03 (t, 2 H, $J = 7$ Hz, SCH_2CH_2NH), 3.68 (t, 2 H, $J = 7$ Hz, SCH_2CH_2NH), 5.36 (s, 2 H, $CONCH_2S$), 7.20 (d, 1 H, $J = 10$ Hz, C_4 H), 7.6 (m, 3 H, Ar H), 8.0 (m, 2 H, Ar H), 8.22 (d, 1 H, $J = 10$ Hz, C_5 H), 8.6 (br, 1 H, NH).

2-[[[2-(3-Cyano-2-methyl-1-isothioureido)ethyl]oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (8i). The title compound was prepared from 2-[[(2-aminoethyl)oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (**7**, $R^1 = C_6H_5$; $m = 1$; $n = 2$) and dimethyl cyanodithioimidocarbonate, as described for the preparation of **8d** ($R^1 = C_6H_5$; X = S; $m = 1$; $n = 2$): IR (Nujol) 3270 (NH), 2160 (C≡N), 1665 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.56 (s, 3 H, SCH_3), 3.54 (t, 2 H, $J = 7$ Hz, OCH_2CH_2NH), 3.88 (t, 2 H, $J = 7$ Hz, OCH_2CH_2NH), 5.52 (s, 2 H, $CONCH_2$).

2-[[[2-(2-Cyano-2-methyl-1-guanidino)ethyl]thio]methyl]-6-phenyl-3(2*H*)-pyridazinone (14). A mixture of 5.0 g (0.014 mol) of 2-[[[2-(3-cyano-2-methyl-1-isothioureido)-ethyl]thio]methyl]-6-phenyl-3(2*H*)-pyridazinone (**8d**) and 40 mL of 30% $MeNH_2$–MeOH solution was heated at 60 °C for 7 h. The reaction mixture was evaporated under reduced pressure, and the residue was dissolved in 100 mL of $CHCl_3$. The organic layer was washed with three 100-mL portions of water and dried with Na_2SO_4. The dried solution was evaporated to give crude solid, which was chromatographed (silica gel, eluted with $CHCl_3$ only). The desired fraction was evaporated to dryness in vacuo, and the residue was recrystallized from EtOH and isopropyl ether to give 4.1 g (84%) of **14** as a colorless crystal: IR (Nujol) 3300 (NH), 2180 (C≡N), 1650 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.68 (d, 3 H, $J = 5$ Hz, $NHCH_3$), 2.90 (t, 2 H, $J = 7$ Hz, SCH_2CH_2NH), 3.46 (t, 2 H, $J = 7$ Hz, SCH_2CH_2NH), 5.27 (s, 2 H, $CONCH_2S$), 7.1 (br, 2 H, 2 NH), 7.10 (d, 1 H, $J = 10$ Hz, C_4 H), 7.5 (m, 3 H, Ar H), 7.9 (m, 2 H, Ar H), 8.10 (d, 1 H, $J = 10$ Hz, C_5 H).

2-[[[2-(2-Cyano-3-methyl-1-guanidino)ethyl]oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (24). The title compound was prepared from 2-[[[2-(3-cyano-2-methyl-1-isothioureido)ethyl]oxy]methyl]-6-phenyl-3(2*H*)-pyridazinone (**8i**) and 30% $MeNH_2$–MeOH solution, as described for the preparation of **14**: IR (Nujol) 3280, 3210 (NH), 2160 (C≡N), 1650 (C=O) cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.26 (d, 3 H, $J = 5$ Hz, $NHCH_3$), 3.31 (t, 2 H, $J = 7$ Hz, CH_2NH), 3.74 (t, 2 H, $J = 7$ Hz, OCH_2CH_2NH), 5.46 (s, 2 H, $CONCH_2O$), 7.00 (br, 2 H, 2 NH).

Pharmacology. Gastric Antisecretory Activity. Gastric antisecretory activity was evaluated by the technique of Shay.[15] Male Wistar rats, weighing 150–200 g, were fasted for 24 h prior to the test in cages with wire-mesh floor to prevent coprophagy, but they were allowed water ad libitum. After fasting, the rats were divided into groups of six animals each. One group served as the control. A small midline incision was performed, and the pylorus was ligated under ether anesthesia. The test compounds (dissolved or suspended in 1% carboxymethylcellulose solution) or the vehicle was administered intraduodenally to each group. Four hours after the abdomen was closed, the stomach was extirpated under ether anesthesia, and the volume of accumulated gastric juice therein was measured. The gastric juice was titrated against 0.1 N NaOH solution to determine the concentration of free acid (at pH 3.0), and hourly outputs of free acid were calculated for each rat. In the first experiment, the test compounds were administered at a dose level of 100 mg/kg, and the results were represented as percentage inhibition against control. In the next step, the selected test compounds from the first experiment were administered at several dose levels, and the ED_{50} value was calculated.[21]

Antiulcer Activity Induced by Stress. Ten male Wistar rats, weighing 200–220 g, per group were used. After oral administration of test compounds, animals were immobilized in the stress cage and immersed in a water bath according to the method described by Takagi et al.[16] Seven hours later, the stomach was extirpated, and the length of lesions in the glandular portion was measured. The ulcer index (millimeters) was obtained by the summation of the length of the lesions, and the ED_{50} value for antiulcer activity was calculated.[21]

Anticholinergic Activity. The anticholinergic activity was determined by the guinea pig isolated ileum preparation suspended in Tyrode's solution aerated with 95% O_2/5% CO_2 at 30

(21) Lichfield, J. T.,; Wilcoxon, F. *J. Pharmacol. Exp. Ther.* **1949**, *96*, 99.

°C. Cumulative dose–response curves for acetylcholine-induced contraction were determined in the absence or in the presence of test compounds (1×10^{-7} to 1×10^{-5} M) or atropine (3×10^{-7} to 1×10^{-4} M).

Histamine H_2 Receptor Antagonistic Activity. The histamine H_2 receptor antagonistic activity was determined by using the guinea pig isolated right atrium preparation suspended in Krebs solution aerated with 95% O_2/5% CO_2 at 32 °C. Cumulative dose–response curves for histamine-induced positive chronotropic action were determined in the absence or in the presence of test compounds (1×10^{-7} to 1×10^{-5} M) or cimetidine (3×10^{-6} to 3×10^{-5} M).

Registry No. **2** ($R^1 = C_6H_5$), 2166-31-6; **2** ($R^1 = CH_3$), 13327-27-0; **2** (R^1 = morpholino), 27464-00-2; **2** [$R^1 = N(CH_3)_2$], 35716-89-3; **2** [$R^1 = C_6H_4$(4-Cl)], 2166-13-4; **2** [$R^1 = C_6H_4$(4-CH_3)], 2166-32-7; **3** ($R^1 = C_6H_5$; $m = 1$), 32949-37-4; **3** ($R^1 = C_6H_5$; $m = 2$), 23916-77-0; **4** ($R^1 = C_6H_5$; $m = 2$), 74316-74-8; **4** ($R^1 = C_6H_5$, $m = 3$), 23916-79-2; **4** ($R^1 = CH_3$, $m = 1$), 34477-79-7; **4** ($R^1 = C_6H_5$, $m = 1$), 34477-77-5; **5** ($R^1 = CH_3$, $m = 1$, $n = 2$), 85748-96-5; **5** ($R^1 = C_6H_5$, $m = n = 2$), 85748-97-6; **5** ($R^1 = C_6H_5$, $m = 3$, $n = 2$), 85748-98-7; **5** ($R^1 = C_6H_5$, $m = 1$, $n = 2$), 79460-68-7; **6** ($R^1 = C_6H_5$, $n = 2$), 85748-99-8; **6** ($R^1 = C_6H_5$, $n = 3$), 85749-00-4; **6** ($R^1 = C_6H_5$, $n = 4$), 85749-01-5; **7** ($R^1 = C_6H_5$, $m = 1$, $n = 2$), 79460-67-6; **7** ($R^1 = C_6H_5$; $m = 1$; $n = 3$), 85749-02-6; **8a**, 85749-03-7; **8b**, 82023-15-2; **8c**, 82023-14-1; **8d**, 79460-53-0; **8e**, 79460-54-1; **8f**, 79460-55-2; **8g**, 85749-04-8; **8h**, 85749-05-9; **8i**, 79460-49-4; **8j**, 85658-25-9; **8k**, 85658-26-0; **10**, 85748-88-5; **11**, 85748-89-6; **12**, 82022-95-5; **13**, 82033-49-6; **14**, 79460-56-3; **15**, 79460-58-5; **16**, 79460-60-9; **17**, 79460-57-4; **18**, 85748-90-9; **19**, 85748-91-0; **20**, 85748-92-1; **21**, 85748-93-2; **22**, 85748-94-3; **23**, 85748-95-4; **24**, 79460-50-7; **25**, 85658-27-1; **26**, 85658-29-3; $(CH_3S)_2C{=}NCN$, 10191-60-3; ethylene chlorohydrin, 107-07-3; cysteamine hydrochloride, 156-57-0.

Mechanism of Action of 2′,5-Difluoro-1-arabinosyluracil

Jeffrey A. Coderre,† Daniel V. Santi,*,† Akira Matsuda,‡ Kyoichi A. Watanabe,‡ and Jack J. Fox‡

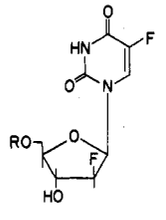
Department of Pharmaceutical Chemistry and Department of Biochemistry and Biophysics, University of California, San Francisco, California 94143, and Laboratory of Organic Chemistry, Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center, Rye, New York 10580. *Received December 29, 1982*

Results are described which demonstrate that the cytotoxic action of 2′,5-difluoro-1-arabinosyluracil (FFara-Ura) involves conversion to the corresponding 5′-phosphate, FFara-UMP, and subsequent inhibition of thymidylate synthetase. The evidence for this is as follows: (a) cells lacking thymidine kinase are 120-fold more resistant to FFara-Ura; (b) FFara-Ura markedly inhibits the incorporation of 2′-deoxyuridine (dUrd) into DNA with little or no effect on 2′-deoxythymidine (dThd) incorporation; (c) FFara-Ura causes changes in deoxynucleoside triphosphate pool sizes, which are characteristic of specific inhibition of dTMP synthetase. Binding and spectroscopic studies demonstrate that FFara-UMP inactivates dTMP synthetase from *Lactobacillus casei* in a manner analogous to that described for FdUMP. Furthermore, FFara-Ura is not a substrate for the pyrimidine phosphorylases; the significance of this finding with regard to the possible chemotherapeutic utility of FFara-Ura is discussed.

FdUrd[1] is a potent cytotoxic agent toward most tissue culture cells because of its direct conversion to FdUMP, which is a potent and specific mechanism-based inhibitor of dTMP synthetase.[2,3] With in vivo systems the anticipated direct effect of FdUrd is altered by its conversion to FUra, which, in addition to inhibition of dTMP synthetase, undergoes extensive metabolism and is incorporated into RNA.[4,5] As a result, it is not yet known whether specific inhibition of dTMP synthetase would be of chemotherapeutic benefit in the treatment of neoplastic diseases.

One approach that has been used in an attempt to overcome this problem has been the construction of analogues that (a) are not substrates for the pyrimidine nucleoside phosphorylases and, hence, are metabolically stable, (b) are converted to the corresponding 5′-nucleotides by a nucleoside kinase, and (c) provide specific and potent inhibitors of dTMP synthetase. Examples of compounds that fulfill, or partially fulfill, these requirements include the carbocyclic analogue of FdUrd,[6] 5-fluoro-2′-deoxycytidine,[7] and 1-β-D-arabinofuranosyl-5-fluorouracil.[8]

Recently, 2′,5-difluoro-1-arabinosyluracil (FFara-Ura) has been synthesized and shown to be moderately cytotoxic toward tissue culture cells.[9] In this report, we describe experiments which demonstrate that FFara-Ura is not a substrate for mammalian pyrimidine phosphorylases and that the cytotoxic action of FFara-Ura involves conversion to the corresponding nucleotide, FFara-UMP, and subsequent inhibition of dTMP synthetase. Experiments are described which demonstrate that FFara-Ura forms a covalent complex with dTMP synthetase in a manner analogous to FdUMP.



R = H, FFara-Ura
R = PO_3H_2, FFara-UMP

(1) The following abbreviations are used. FUra, 5-fluorouracil; FdUrd, 5-fluoro-2′-deoxyuridine; FdUMP, 5-fluoro-2′-deoxyuridylate; FFara-Ura, 1-(2-deoxy-2-fluoro-β-D-arabinofuranosyl)-5-fluorouracil or 2′,5-difluoro-1-arabinosyluracil; FFara-UMP, 2′,5-difluoro-1-arabinosyluridylate; H_2folate, 7,8-dihydrofolate; CH_2-H_4folate, 5,10-methylene-5,6,7,8-tetrahydrofolate; NMM, *N*-methylmorpholine; RV, retention volume. All other abbreviations used are those recommended by IUPAC.

(2) Pogolotti, Jr., A. L.; Santi, D. V. In "Bioorganic Chemistry"; Van Tamelen, E. E., Ed.; Academic Press: New York, 1977; Vol 1, p 277.
(3) Danenberg, P. V. *Biochim. Biophys. Acta* **1977**, *473*, 73.
(4) Heidelberger, C. *Handb. Exp. Pharmakol.* **1974**, *38*, 193.
(5) Myers, C. E. *Pharmacol. Rev.* **1981**, *33*, 1.
(6) Shealy, Y. F.; Frye, J. L.; Du Bois, N. F.; Shaddix, S. C.; Brockman, R. W. *J. Med. Chem.* **1981**, *24*, 1083.
(7) Newman, E. M.; Santi, D. V. *Proc. Natl. Acad. Sci. U.S.A.* **1982**, *79*, 6419.
(8) Nakayama, C.; Wataya, Y.; Santi, D. V.; Saneyoshi, M.; Udea, T. *J. Med. Chem.* **1981**, *24*, 1161.
(9) Watanabe, K. A.; Reichman, U.; Hirota, K.; Lopez, C.; Fox, J. J. *J. Med. Chem.* **1979**, *22*, 21.

† University of California.
‡ Memorial Sloan-Kettering Cancer Center.

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