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Synthesis and evaluation of 6-methylene-bridged uracil derivatives. Part 1: Discovery of novel orally active inhibitors of human thymidine phosphorylase

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Abstract—A series of novel 6-methylene-bridged uracil derivatives have been prepared as inhibitors of human thymidine phosphorylase (TP). To enhance the in vivo antitumor activity of fluorinated pyrimidine 2′-deoxyribonucleosides such as 2′-deoxy-5-(trifluoromethyl)uridine (F_3dThd), a potent TP inhibitor preventing their degradation to an inactive compound, has become a target of medicinal chemistry. We present here the synthesis and evaluation of novel human TP inhibitors. Introduction of an *N*-substituted aminomethyl side chain at the 6-position of 5-chlorouracil has improved water solubility and enhanced inhibitory activity compared with the known TP inhibitor, 6-amino-5-chlorouracil. Compound **42** was reasonably well absorbed in mice after oral administration. When combined with F_3dThd, compound **42** exerted its TP inhibitory potency by increasing the maximum plasma concentrations of the former as evidenced in experiments with monkeys. Positive changes in pharmacokinetic profile were accompanied by the enhanced in vivo antitumor activity of this combination when compared to F_3dThd alone, in mice bearing human tumor xenografts. Both biochemical and pharmacological effects appeared to fit the concept as anticipated.

1. Introduction

Fluorinated pyrimidine 2′-deoxyribonucleosides possessing chemotherapeutic activity, such as 2′-deoxy-5-(trifluoromethyl)uridine (F_3dThd) and 2′-deoxy-5-fluorouridine (FdUrd), are known to have strong in vitro and in vivo antitumor activities.[1,2] However, F_3dThd is easily metabolized to an inactive compound, 5-(trifluoromethyl)uracil while FdUrd is metabolized to still active 5-fluorouracil by pyrimidine nucleoside phosphorylases.[3,4] In particular, F_3dThd is rapidly converted to an inactive pyrimidine base in several animal species including mice, monkeys, and human.[5,6] Therefore, none of the above compounds have so far been able to provide satisfactory clinical antitumor effects.[7,8] To prevent such inactivation, several efforts have been made to develop an inhibitor of pyrimidine nucleoside phosphorylases, represented by uridine phosphorylase (UP) and thymidine phosphorylase (TP). The former, UP, predominantly expressed in rodents such as mice and rats, and the latter, TP, is a principal enzyme in human.[9,10] Therefore, the antitumor effects of fluorinated pyrimidine 2′-deoxyribonucleosides in human can be improved rather by an inhibitor of TP. Furthermore, human TP has been identified with platelet derived endothelial cell growth factor (PD-ECGF), known as an endogenous angiogenic factor.[11] Accordingly, an inhibitor of human TP may also inhibit the process of angiogenesis, which is closely associated with the malignant process of solid tumors and should bring supplementary effects in the treatment of cancer. Reported early examples of human or horse TP inhibitors are 6-aminouracil derivatives such as 6-aminothymine, 6-amino-5-bromouracil, and 6-amino-5-chlorouracil (6A5CU, **3**).[12–15] In our study, 6A5CU showed a moderate inhibitory effect against human TP and rat UP.[16] 6A5CU showed inhibitory activity against human TP-induced angiogenesis in mouse model.[15] Hence, we had selected 6A5CU as a lead compound, considered as the best inhibitor at that time. Baker's group synthesized several 6-anilino and 6-(1-naphthylmethylamino) derivatives of uracil as potent inhibitors of *E. coli* TP.[17,18]

Keywords: Thymidine phosphorylase inhibitor; TPI; 2′-Deoxy-5-(trifluoromethyl)uridine (F_3dThd); 6-Methylene-bridged uracil derivative.
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These compounds were recognized as 6-aminouracil derivatives, however, appeared to be insufficient in the inhibition of mammalian TP.[19] Woodman et al. have reported that hydrophobic bonding region adjacent to the active site of TP differed to a great extent among animal species.[20] Therefore, it was assumed that the design of a novel potent inhibitor should be based on the screening data obtained with human but not *E. coli* TP. Several research groups explored a variety of novel 6-aminouracil derivatives substituted at the 1-position or the 6-amino function as potent human TP inhibitors.[21–23] In our previous paper,[16] we reported on 5-chloro-6-(2-iminopyrrolidine-1-yl)methyluracil hydrochloride (TPI, **1**) as a novel specific inhibitor of human TP potentiating the antitumor effect of F_3dThd (Fig. 1).

In this paper, we describe the synthesis and biological evaluation of a series of novel 6-methylene-bridged uracil derivatives and disclose the full details of the discovery process of the lead compounds of TPI. Several derivatives have been synthesized to enhance the TP inhibitory activity and water solubility, since these properties should allow dosage reduction and improve clinical efficacy. In designing an inhibitor, we have focused on the hydrophobic bonding region that surround the active site of human TP and interact with the 6-position of thymine. The selection of a final candidate is based on the in vitro inhibition of TP, followed by a reasonable pharmacokinetic (PK) profile and an ability of increasing plasma concentration of F_3dThd under in vivo conditions, using mice. This study reveals that compound **42** elevates the plasma level of F_3dThd in mice, but the most pronounced effects are shown in monkeys. The in vivo antitumor activity of F_3dThd combined with **42** is evaluated in mice. The implication of the improved PK profile of F_3dThd after combined treatment with **42** in the therapeutic outcome of the former is clearly demonstrated.

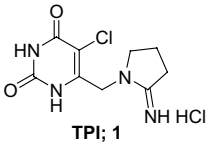
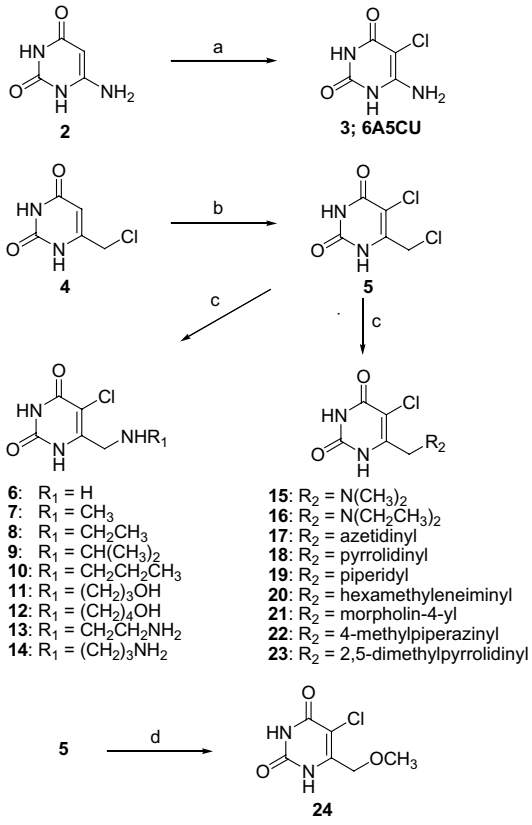


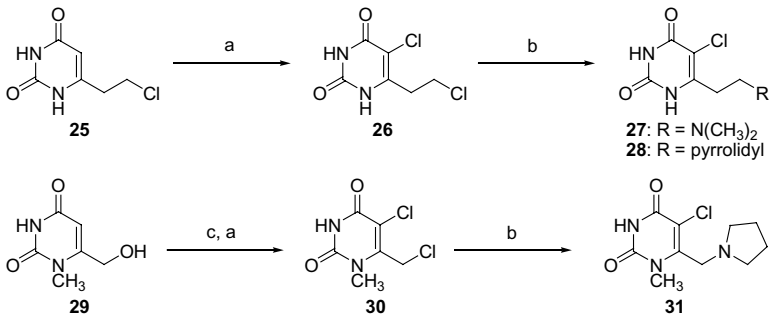
Figure 1.

2. Chemistry

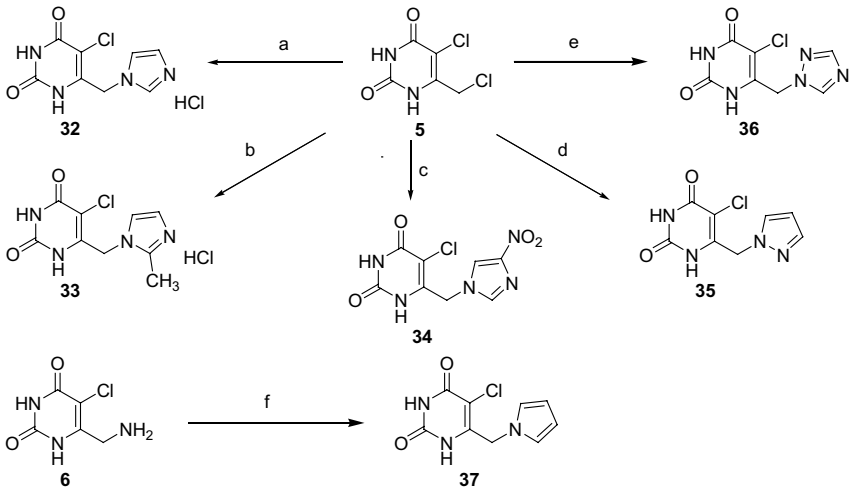
Scheme 1 illustrates the synthesis of 6-substituted 5-chlorouracil derivatives. 6A5CU was prepared from 6-aminouracil (**2**) in a previously described manner.[24] 5-Chloro-6-(chloromethyl)uracil (**5**) was prepared by chlorination of 6-(chloromethyl)uracil (**4**) with sulfuryl chloride in acetic acid. 6-Aminomethyl-5-chlorouracil (**6**) and its *N*-substituted derivatives **7**–**23** were obtained by reacting **5** with a large excess of aqueous ammonia or appropriate alkylamines in water. The methoxy analogue **24** was prepared by treating **5** with NaOMe in DMF. The synthesis of compounds **27**, **28**, and **31** is illustrated in Scheme 2. 2-Amino-substituted ethyl compounds **27** and **28** were prepared from 5-chloro-6-



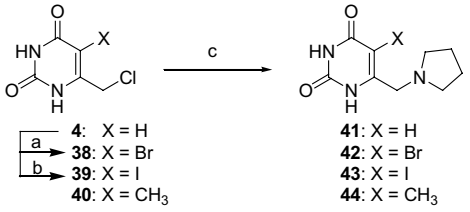
Scheme 1. Reagents and conditions: (a) SO_2Cl_2, DMF; (b) SO_2Cl_2, AcOH; (c) corresponding amine, H_2O; (d) NaOMe, DMF.



Scheme 2. Reagents and conditions: (a) SO_2Cl_2, AcOH; (b) dimethylamine or pyrrolidine, H_2O; (c) $SOCl_2$.



Scheme 3. Reagents and conditions: (a) *N*-acetylimidazole, MeOH; (b) 2-methylimidazole, KOH, H_2O; (c) 4-nitroimidazole, KOH, H_2O; (d) pyrazole, KOH, H_2O; (e) 1*H*-1,2,4-triazole, KOH, H_2O; (f) 2,5-dimethoxytetrahydrofuran, AcOH.



Scheme 4. Reagents and conditions: (a) NBS, DMF; (b) *N*-iodosuccinimide, DMF; (c) pyrrolidine, H_2O.

(2-chloroethyl)uracil (**26**), which was derived from 6-(2-chloroethyl)uracil (**25**)[25] by chlorination with sulfuryl chloride. Compound **31** was prepared from the known compound **29**[26] using a three-step sequence: (a) conversion of the hydroxyl group into a chloride with thionyl chloride, (b) chlorination at the 5-position with sulfuryl chloride, and (c) substitution with pyrrolidine at the allyl position.

Scheme 3 illustrates the synthesis of compounds possessing a five-membered ring at the side chain. Compound **32** was prepared by heating **5** with acetyl imidazole[27,28] in MeOH. Compounds **33**–**36** were prepared by treating **5** with corresponding heterocycles in the presence of KOH in water. Compound **37** was prepared by treating **6** with 2,5-dimethoxytetrahydrofuran[29] in acetic acid. Scheme 4 illustrates the synthesis of 5-substituted derivatives of compound **18**. 5-Bromo-6-(chloromethyl)uracil (**38**) and 6-chloromethyl-5-iodouracil (**39**) were prepared by halogenation of **4** with small excess of *N*-bromosuccinimide (NBS) or *N*-iodosuccinimide in DMF. 6-(Chloromethyl)thymine (**40**) was prepared according to a previously reported procedure.[30] They were converted to corresponding pyrrolidine analogues **41**–**44** in the same manner as described for **18**.

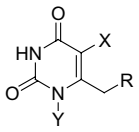
3. Results and discussion

To develop the potent human TP inhibitor, its maximum plasma concentration (C_{max}) value should be far beyond its IC_{50} value. Despite the presence of some pharmacological activity of 6A5CU, its water solubility and potency of inhibiting human TP activity were not sufficiently suitable to meet the criteria of good pharmacologically active agents. The water solubility of compound **6** was almost the same as that of 6A5CU (0.13 vs 0.12 mg/mL). However, the hydrochloride salt of **6** showed excellent solubility in water (>10 mg/mL). In contrast, 6A5CU did not form a hydrochloride salt, therefore excellent water solubility could not be achieved. The sufficient water solubility is important for the gastrointestinal absorption and is absolutely required for the intravenous drug formulation. Further derivation of **6** was expected to result in an improvement of the PK profile.

Since 1-methyluracil, 1-methylthymine, and 1-ethylthymine did not inhibit TP, Niedzwicki et al. predicted that substitution at the 1-position of uracil with an alkyl chain would not lead to create a potent TP inhibitor.[31] It indicated a low suitability of the analogues substituted at the 1-position. Hence, we focused on the substitution at the 6-position of uracil rather than the 1-position.

Table 1 shows the inhibitory effect of 6-methylene-bridged uracil derivatives on human TP. Firstly, the in vitro potency of **6** against human TP was almost the same as that of 6A5CU (IC_{50}, 15 μM). The potency of *N*-methyl derivative **7**, *N*-ethyl derivative **8**, and *N*-isopropyl derivative **9** were close to that of **6**. The inhibitory effect was decreased in the case of *N*-propyl derivative **10**. The compounds **11**–**14** having a hydroxy or an amino group at the end of alkyl side chains appeared to be less effective in the case of introducing longer methylene bonds. *N*,*N*-Dialkyl or *N*-substituted-heterocyclic derivatives of **6** were also evaluated, and dimethylamino derivative **15** showed the same potency as **6**, while diethylamino derivative **16** appeared to be less potent. These observations led to the conclusion that the hydrophobic region contacting the 6-position of 5-chlorouracil at the active site of human TP would not exist, contrasting the results derived from *E. coli* TP,

Table 1. Inhibitory effect of compounds **6**–**24**, **27**, **28**, **32**–**37**, and **41**–**44** on human TP



Compd	R	X	Y	IC_{50}[a] (μM)
6A5CU				15
6	NH_2	Cl	H	23
7	$NHCH_3$	Cl	H	12
8	$NHCH_2CH_3$	Cl	H	20
9	$NHCH(CH_3)_2$	Cl	H	32
10	$NHCH_2CH_2CH_3$	Cl	H	>100
11	$NH(CH_2)_3OH$	Cl	H	47
12	$NH(CH_2)_4OH$	Cl	H	>100
13	$NH(CH_2)_2NH_2$	Cl	H	81
14	$NH(CH_2)_3NH_2$	Cl	H	>100
15	$N(CH_3)_2$	Cl	H	21
16	$N(CH_2CH_3)_2$	Cl	H	>100
17	*N*-Azetidinyl	Cl	H	2.6
18	*N*-Pyrrolidinyl	Cl	H	2.2
19	*N*-Piperidyl	Cl	H	120
20	*N*-Hexamethyleneiminyl	Cl	H	>100
21	*N*-Morpholinyl	Cl	H	>100
22	*N*-(4-Methylpiperazinyl)	Cl	H	>100
23	*N*-(2,5-Dimethylpyrrolidinyl)	Cl	H	>100
24	OCH_3	Cl	H	>100
27	$CH_2N(CH_3)_2$	Cl	H	25
28	(*N*-Pyrrolidinyl)methyl	Cl	H	82
31	*N*-Pyrrolidinyl	Cl	CH_3	>100
32	*N*-Imidazolyl	Cl	H	1.0
33	*N*-(2-Methylimidazolyl)	Cl	H	28
34	*N*-(4-Nitroimidazolyl)	Cl	H	>100
35	*N*-Pyrazolyl	Cl	H	>100
36	*N*-(1,2,4-Triazolyl)	Cl	H	>100
37	*N*-Pyrrolyl	Cl	H	>100
41	*N*-Pyrrolidinyl	H	H	51
42	*N*-Pyrrolidinyl	Br	H	0.51
43	*N*-Pyrrolidinyl	I	H	1.3
44	*N*-Pyrrolidinyl	CH_3	H	3.8

[a] Inhibitory effect is reported as an IC_{50} value, indicating the concentration of the compound required to inhibit enzyme activity by 50%. A 0.6 mM concentration of [6-^{3}H]dThd is used as the substrate for the TP reactions. This assay tests at least three concentrations of each compound.

which has a wide space to fit 1-naphthylmethylamino substitution at the 6-position of 5-chlorouracil.[18] Therefore, the reduction of bulkiness of substitution at the 6-position of 5-chlorouracil was expected to improve the inhibitory activity, which led to the synthesis of small-sized cyclic amines. Azetidinyl and pyrrolidinyl derivatives **17** and **18**, respectively, appeared to better match the active site of human TP and showed almost 10-fold stronger activity than **6**. Further, introduction of larger heterocyclic amines did not provide good results (compounds **19**–**22**). Furthermore, dimethylated derivative **23** dramatically diminished the inhibitory activity when compared with **18**, because of tight limitation of the active site of human TP. Secondly, changing substitution at the 6-position of 5-chlorouracil from CH_2NHCH_3 to CH_2OCH_3 greatly diminished the activity. Hence, the nitrogen atom was essential to fit the active site of human TP. Thirdly, 6-ethylene-bridged 5-chlorouracil derivatives **27** and **28** were evaluated. Compound **28** containing pyrrolidine was found to be less active than corresponding methylene-bridged derivative **18**. In contrast, compound **27** showed the close inhibitory activity of compound **15** due to the reduction of bulkiness. Fourthly, 1-methylated derivative **31** was confirmed to possess low TP inhibitory activity (IC_{50} >100 μM) when compared with **18**, and this result was in agreement with previous prediction. This result suggests two possibilities. Firstly, the conformational freedom of the 6-pyrrolidylmethyl chain would be restricted by the *N*-methyl group, therefore the preferred conformation would not be present at the target site. Secondly, hydrogen donation from the 1-position of **18** would play an important role for enzyme–ligand interaction. In addition, compound **18** was more water soluble (7.6 mg/mL) than 6A5CU. Based on these results, we focused our study on the derivatives of **18**.

Among several five-membered aromatic heterocyclic derivatives **32**–**37**, only imidazole derivative **32** showed good potency. In contrast to the results of **33** and **34**, a small substitution at the imidazole dramatically influenced the ability to fit the active site of human TP.

In the case of F_3dThd and FdUrd, which are thought to possess a time-dependent mode of action, it is assumed that consecutive administration of these drugs would be more effective than a single dose or intermittent administration. Therefore, prolonged retention of those drugs in plasma would be of great importance and would be achieved by co-administration with human TP inhibitors. To directly demonstrate the potency of these compounds in vivo, small scale PK experiments were performed in mice. The test compounds were administered orally in different molar ratios together with F_3dThd. Table 2 shows the C_{max} and the area under the curve (AUC) values of each compound. Considering the IC_{50} value of 6A5CU against human TP, its C_{max} and $AUC_{0\text{-}6}$ at high dose such as 300 mg/kg in BALB/c mice were not high enough to exert pharmacological effect because of the limited absorption caused by its poor water solubility. In contrast, compound **32** and the hydrochloride salt of **18** had excellent water solubility (>50 mg/mL). Hence, compounds **18** and **32** were expected to have excellent PK profile. The C_{max} of compound **18** was much higher than that of 6A5CU, even when administered at lower doses. The C_{max} of compound **32** was also higher than that of 6A5CU, but lower than that of **18**. Compound **18** expected to have better pharmacological activity than compound **32** in vivo.

Finally, derivatives substituted at the 5-position of **18** were evaluated. Uracil derivative **41** was less active, but the potency of iodo and methyl derivatives (**43** and **44**) were close to that of **18**. In contrast, 5-bromo derivative **42** was almost 4-fold more potent than 18, and its water solubility was 4.6 mg/mL. In addition, the hydrochloride salt of **42** had excellent water solubility (>50 mg/mL). Hence, compound **42** was selected for further in vivo biological evaluation. The C_{max} of compound **42** was

Table 2. Oral absorption of compounds **18**, **32**, **42**, and 6A5CU in mice after combined administration with F_3dThd at different molar ratios[a]

Compd	Dose (mmol/kg)	Molar ratio[b]	C_{max}[c] (μM)	T_{max}[d] (h)	AUC_{0-8}[e] (μM h)
18	0.169	1	100	0.5	120
	0.507	3	270	0.5	440
32	0.169	1	17	0.5	14
	0.507	3	69	0.5	66
42	0.169	1	80	0.25	130
	0.845	5	340	0.5	690
6A5CU	0.169		12	0.5	24[f]
	1.86		19	0.5	79[f]

[a] Compounds **18**, **32**, or **42** with F_3dThd (0.169 mmol/kg) dissolved in 0.5% hydroxypropylmethylcellulose (HPMC) were orally administered to ICR mice (male, 6 weeks old, $n = 3$). 6A5CU suspended in 0.5% HPMC was orally administered to BALB/c mice (male, 9 weeks old, $n = 3$).
[b] Molar ratio of compounds based on F_3dThd.
[c] Maximum plasma concentration after oral dosing.
[d] Time to C_{max}.
[e] Area under the concentration–time curve for 0–8 h after oral dosing.
[f] AUC for 0–6 h after oral dosing.

close to that of compound **18**. We have previously reported that phosphorolysis of F_3dThd in monkey liver and intestine was catalyzed mainly by TP reflecting a typical pattern for human. On the other hand, UP was a primary enzyme degrading F_3dThd in mice, therefore the effect of the inhibition of TP may not be easily detected in mice.[16] However, it seemed reasonable to confirm positive PK changes in monkeys. Compound **42** (9.25 mg/kg) was administered orally with an equimolar amount of F_3dThd (10 mg/kg) in monkeys ($n = 3$) to evaluate the potency of elevating the plasma concentration of F_3dThd. Plasma F_3dThd levels increased very markedly, with a T_{max} of 2 h, a C_{max} of 14.1 μg/mL, and an AUC value of 39.6 μg h/mL. The respective parameters for F_3dThd administration alone were a T_{max} of 1 h, a C_{max} of 0.23 μg/mL, and an AUC value of 0.28 μg h/mL.

In addition, an antitumor experiment was performed to prove the concept of enhancing the in vivo potency of F_3dThd by co-administration with compound **42**. Table 3 shows the results of antitumor activity of orally administered F_3dThd alone and in combination with equimolar **42** against the AZ-521 human stomach cancer model in nude mice. F_3dThd alone inhibited tumor growth by 45.7%, and its effect was significantly enhanced up to 73.5% by co-administration with compound **42** (F_3dThd alone; $p = 0.033$, F_3dThd with **42**; $p = 1.5 \times 10^{-7}$, F_3dThd alone vs F_3dThd with **42**; $p = 0.10$ by Dunnett's *t*-test). The PK experiment provided evidence that the improved antitumor effect in vivo was accompanied by elevated plasma and human tumor tissue F_3dThd levels due to the inhibition of degradation by TP and by UP possibly.[32] These results suggest that compound **42** has sufficient in vivo activity to enhance F_3dThd plasma concentration, and therefore to improve its antitumor activity.

4. Conclusion

We evaluated novel 6-methylene-bridged uracil derivatives as potent human TP inhibitors. Starting from the lead compound 6A5CU, prominent improvements, both in in vitro activity (IC_{50}, 30-fold more potent) and oral absorption (C_{max}, >6-fold more) in mice, have been accomplished through introduction of a methylene-bridge and pyrrolidine ring at the 6-position and optimization of the substituent at the 5-position. Our study on 6-methylene-bridged uracil derivatives has revealed the tight limitation at the active site of human TP. The substitution at the 6-position of 5-chlorouracil with methylene-bridge was matched pyrrolidine and imidazole. This finding would significantly contribute to the development of more potent inhibitors of human TP.

Careful chemical design results in synthesis of inhibitors with good water solubility and improved oral absorption as shown in the case of **42**. Compound **42** has enhanced plasma concentration of F_3dThd and

Table 3. Antitumor effect and plasma level of F_3dThd on human gastric cancer (AZ-521) bearing nude mice with or without compound **42**[a]

Group	Antitumor effect			Plasma level of F_3dThd[d]			
	N	RTV[b] (mean ± SD)	IR[c] (%)	*N*	C_{max} (μg/mL)	T_{max} (h)	AUC_{0-8} (μg h/mL)
Control	12	11.32 ± 3.59					
F_3dThd alone	6	6.14 ± 4.19	45.7	3	13.0	0.5	8.16
F_3dThd+**42**	6	3.00 ± 1.06	73.5	3	42.5	0.5	38.5

[a] A mixture of F_3dThd (50 mg/kg) and compound **42** (46.3 mg/kg) or F_3dThd (50 mg/kg) alone dissolved in 0.5% HPMC was administered orally once a day for 2 weeks.
[b] RTV (relative tumor volume) was calculated as follows: RTV = (mean tumor volume on day 15)/(mean tumor volume on day 0).
[c] IR (inhibition rate of tumor growth, %) = [1−(mean RTV of drug-treated group)/(mean RTV of control group) × 100].
[d] Blood samples (heparin-plasma) were collected at 0.5, l, 2, 4, and 8 h after oral dosing.

potentiated in vivo antitumor activity of F_3dThd, when administered orally to mice. The excellent properties of **42** are particularly shown in PK experiments in monkeys, where plasma F_3dThd levels were increased very markedly by its co-administration with **42**. These biological results have prompted us to select compound **42** as a candidate for further evaluation of biological profiles. Due to its good oral absorption, we expect compound **42** to additionally inhibit the TP-induced angiogenesis and as a result the process of metastasis in cancer patients.

5. Experimental

5.1. Chemistry

All melting points were determined on a Yanagimoto micromelting point apparatus and were uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a JEOL JNM-EX270 (270 MHz) spectrometer. Chemical shifts are given in δ values (ppm) using tetramethylsilane as the internal standard. All J values are given in hertz. Mass spectra were recorded on a JEOL JMS-SX102A spectrometer. Elemental analyses were carried out with a Yanagimoto C H N Corder MT-5. Reagents and solvents were obtained from commercial sources and used without further purification. Flash column chromatography was performed with Merck silica gel 60 (art. 9385; 230–400 mesh), and reaction progress was monitored by thin-layer chromatography (TLC) analysis on silica gel 60 F_{254} plates (Merck). Visualization was done with UV light (254 nm) or iodine. Yields were based on purified material and were not optimized.

5.1.1. 6-Amino-5-chlorouracil (3). To a suspension of 25.0 g (197 mmol) of 6-aminouracil (**2**) in 155 mL of DMF was added dropwise 16.1 mL (200 mmol) of sulfuryl chloride over 1 h at room temperature. After 3.5 h the reaction mixture was filtered to remove insoluble material. To the filtrate was added ice/water (150 mL), and the precipitate was collected by filtration and recrystallized from water to give 18.5 g (58%) of **3** as white crystals: mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 6.47 (s, 2H), 10.37 (s, 1H), 10.67 (s, 1H). FAB-MS m/z: 160 $(M-H)^-$. Anal. Calcd for $C_4H_4ClN_3O_2 \cdot 0.6H_2O$: C, 27.87; H, 3.04; N, 24.38. Found: C, 27.97; H, 2.74; N, 24.50.

5.1.2. 5-Chloro-6-(chloromethyl)uracil (5). To a suspension of 100 g (623 mmol) of 6-chloromethyluracil (**4**) in 500 mL of AcOH was added 75.0 mL (934 mmol) of sulfuryl chloride at 50 °C. The gas outlet was passed through 4 M aqueous NaOH. After 2.5 h to the reaction mixture was added ice/water (500 mL), and the precipitate was collected by filtration and washed with water and MeOH to give 101 g (83%) of **5** as a white powder: mp 225 °C dec. ^{1}H NMR (DMSO-d_6): δ 4.46 (s, 2H), 11.57 (s, 1H), 11.71 (s, 1H). FAB-MS m/z: 195 $(M+H)^+$. Anal. Calcd for $C_5H_4Cl_2N_2O_2$: C, 30.80; H, 2.07; N, 14.37. Found: C, 30.85; H, 1.99; N, 14.41.

5.1.3. 6-(Aminomethyl)-5-chlorouracil (6). Compound **5** (10.0 g, 51.3 mmol) was suspended in 200 mL of 28% aqueous NH_4OH and stirred for 7 days at room temperature in a sealed tube. An insoluble material was collected by filtration, washed with water and MeOH to give 6.08 g of a purple solid. The crude solid was dissolved in 45 mL of 1 M aqueous HCl at room temperature. The reaction mixture was filtered to remove insoluble material and the filtrate was concentrated under a vacuum. The residue was recrystallized from water and MeOH to give 4.35 g (40%) of a hydrochloride salt of **6** as white crystals: mp 220 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.96 (s, 2H), 8.60 (s, 2H), 11.55 (s, 1H), 11.79 (s, 1H). FAB-MS m/z: 210 $(M-H)^-$, 174 $(M-H-HCl)^-$. Anal. Calcd for $C_5H_6ClN_3O_2 \cdot HCl$: C, 28.32; H, 3.33; N, 19.82. Found: C, 27.98; H, 3.31; N, 19.68. The salt (1.00 g, 4.74 mmol) was treated with 28% aqueous NH_4OH, and the precipitate was collected by filtration and washed with water and MeOH to give 770 mg (93%) of **6** as a white solid: mp 227 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.61 (s, 2H). FAB-MS m/z: 174 $(M-H)^-$. Anal. Calcd for $C_5H_6ClN_3O_2 \cdot H_2O$: C, 31.02; H, 4.17; N, 21.70. Found: C, 31.26; H, 4.16; N, 21.66.

5.1.4. 5-Chloro-6-[(methylamino)methyl]uracil (7). To a suspension of 6.00 g (30.8 mmol) of **5** in 50 mL of water was added 150 mL of 40% aqueous methylamine cooling with an ice-bath and stirred for 4.5 h at room temperature in a sealed tube. The reaction mixture was concentrated under a vacuum. The residue was triturated with MeOH, and the resulting precipitate was collected by filtration and washed with MeOH to give 5.38 g (92%) of **7** as a white powder: mp 197–199 °C. ^{1}H NMR (DMSO-d_6): δ 2.27 (s, 3H), 3.61 (s, 2H). EI-MS (70 eV) m/z: 189 (M^+). Anal. Calcd for $C_6H_8ClN_3O_2$: C, 38.01; H, 4.25; N, 22.16. Found: C, 37.62; H, 4.26; N, 21.94.

5.1.5. 5-Chloro-6-[(ethylamino)methyl]uracil (8). To a suspension of 300 mg (1.54 mmol) of **5** in 3 mL of water was added 5 mL of 70% aqueous ethylamine, cooling with an ice-bath, and stirred for 2.5 h at room temperature in a sealed tube. The reaction mixture was concentrated under a vacuum. The precipitate was triturated with EtOH, and the resulting precipitates were collected by filtration and washed with EtOH to give 162 mg (52%) of **8** as a white powder: mp 189–191 °C. ^{1}H NMR (DMSO-d_6): δ 1.02 (t, 3H, $J = 7.1$ Hz), 2.53 (q, 2H, $J = 7.1$ Hz), 3.64 (s, 2H). FAB-MS m/z: 204 $(M+H)^+$. Anal. Calcd for $C_7H_{10}ClN_3O_2 \cdot 0.2H_2O$: C, 40.57; H, 5.06; N, 20.28. Found: C, 40.60; H, 5.11; N, 20.05.

5.1.6. 5-Chloro-6-[(isopropylamino)methyl]uracil (9). To a solution of 1.50 g (25.4 mmol) of isopropylamine in 5 mL of water was added 500 mg (2.56 mmol) of **5** and stirred for 11 h at room temperature. The reaction

mixture was concentrated under a vacuum. The residue was triturated with $CHCl_3$, and the resulting precipitate was collected by filtration and washed with 50% MeOH to give 35 mg (6%) of **9** as a white solid: mp 185 °C dec. ^{1}H NMR (DMSO-d_6): δ 0.99 (d, 6H, $J = 6.1$ Hz), 2.70 (sept, 1H, $J = 6.1$ Hz), 3.64 (s, 2H). EI-MS (70 eV) m/z: 217 (M^+). Anal. Calcd for $C_8H_{12}ClN_3O_2 \cdot 0.25H_2O$: C, 43.25; H, 5.67; N, 18.91. Found: C, 43.31; H, 5.80; N, 18.86.

5.1.7. 5-Chloro-6-[(propylamino)methyl]uracil (10). To a solution of 1.00 g (16.9 mmol) of propylamine in 5 mL of water was added 300 mg (1.54 mmol) of **5** and stirred for 13 h at room temperature. The reaction mixture was concentrated under a vacuum. The residue was triturated with MeOH, and the resulting precipitate was collected by filtration and washed with MeOH to give 251 mg (75%) of **10** as a white solid: mp 193–195 °C. ^{1}H NMR (DMSO-d_6): δ 0.86 (t, 3H, $J = 7.3$ Hz), 1.41 (tq, 1H, $J = 7.3$, 7.3 Hz), 2.44 (t, 2H, $J = 7.3$ Hz), 3.63 (s, 2H), 8.05 (1H, s). EI-MS (70 eV) m/z: 217 (M^+). Anal. Calcd for $C_8H_{12}ClN_3O_2 \cdot 0.2H_2O$: C, 43.43; H, 5.65; N, 18.99. Found: C, 43.60; H, 5.85; N, 18.98.

5.1.8. 5-Chloro-6-{[(3-hydroxypropyl)amino]methyl}uracil (11). To a solution of 580 mg (7.72 mmol) of 3-amino-1-propanol in 20 mL of water was added 500 mg (2.56 mmol) of **5** and stirred for 19 h at room temperature. The reaction mixture was evaporated under a vacuum. The residue was triturated with MeOH, and the resulting precipitate was collected by filtration. The crude solid was purified by flash column chromatography ($CHCl_3$–MeOH–Et_3N, 20:1:0 to 1000:100:3) and recrystallized from isopropanol to give 70 mg (12%) of **11** as pale yellow needles: mp 167–169 °C. ^{1}H NMR (DMSO-d_6): δ 1.55 (tt, 2H, $J = 6.6$, 6.6 Hz), 2.54 (t, 2H, $J = 6.6$ Hz), 3.44 (t, 2H, $J = 6.6$ Hz), 3.63 (s, 2H). EI-MS (70 eV) m/z: 233 (M^+). Anal. Calcd for $C_8H_{12}ClN_3O_3$: C, 41.12; H, 5.18; N, 17.98. Found: C, 41.12; H, 5.43; N, 18.05.

5.1.9. 5-Chloro-6-{[(4-hydroxybutyl)amino]methyl}uracil (12). Compound **12** was prepared in 2% yield from **5** and 4-amino-1-butanol by a method similar to that described for **11**, as white crystals: mp 164–166 °C. ^{1}H NMR (DMSO-d_6): δ 1.38–1.48 (m, 4H), 2.46–2.51 (m, 2H), 3.38 (t, 2H, $J = 5.6$ Hz), 3.63 (s, 2H). FAB-MS m/z: 248 $(M+H)^+$. Anal. Calcd for $C_9H_{14}ClN_3O_3$: C, 43.64; H, 5.70; N, 16.97. Found: C, 43.57; H, 5.92; N, 16.72.

5.1.10. 6-{[(2-Aminoethyl)amino]methyl}-5-chlorouracil (13). Compound **13** was prepared in 70% yield from **5** and ethylenediamine by a method similar to that described for **10**, as a beige powder: mp 140 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.38–1.48 (m, 4H), 2.46–2.51 (m, 2H), 3.38 (t, 2H, $J = 5.6$ Hz), 3.63 (s, 2H). FAB-MS m/z: 219 $(M+H)^+$. Anal. Calcd for $C_7H_{11}ClN_4O_2 \cdot 0.33H_2O$: C, 37.43; H, 5.23; N, 24.94. Found: C, 37.44; H, 5.47; N, 24.94.

5.1.11. 6-{[(3-Aminopropyl)amino]methyl}-5-chlorouracil (14). To a solution of 2.00 g (27.0 mmol) of trimethylenediamine in 25 mL of water was added 500 mg (2.56 mmol) of **5** and stirred for 6 days at room temperature. The reaction mixture was concentrated under a vacuum. The residue was triturated with EtOH, and the resulting precipitate was collected by filtration and washed with water to give 35 mg (6%) of **14** as a beige powder: mp 135 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.59 (tt, 2H, $J = 6.1$, 6.1 Hz), 2.58 (t, 2H, $J = 6.1$ Hz), 2.83 (t, 2H, $J = 6.1$ Hz), 3.55 (s, 2H). FAB-MS m/z: 233 $(M+H)^+$. Anal. Calcd for $C_8H_{13}ClN_4O_2 \cdot 0.1H_2O$: C, 40.98; H, 5.67; N, 23.89. Found: C, 40.99; H, 5.89; N, 23.56.

5.1.12. 6-[(Dimethylamino)methyl]-5-chlorouracil (15). Compound **15** was prepared in 22% yield from **5** and 50% aqueous dimethylamine by a method similar to that described for **7**, as a yellow powder: mp 130 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.22 (s, 6H), 3.33 (s, 2H), 11.41 (s, 1H). EI-MS (70 eV) m/z: 203 (M^+). Anal. Calcd for $C_7H_{10}ClN_3O_2$: C, 41.29; H, 4.95; N, 20.64. Found: C, 41.25; H, 5.01; N, 20.42.

5.1.13. 6-[(Diethylamino)methyl]-5-chlorouracil (16). Compound **16** was prepared in 33% yield from **5** and diethylamine by a method similar to that described for **8**, as a yellow powder: mp 155 °C dec. ^{1}H NMR (DMSO-d_6): δ 0.97 (t, 6H, $J = 7.1$ Hz), 2.55 (q, 4H, $J = 7.1$ Hz), 3.49 (s, 2H). FAB-MS m/z: 232 $(M+H)^+$. Anal. Calcd for $C_9H_{14}ClN_3O_2$: C, 46.66; H, 6.09; N, 18.14. Found: C, 46.39; H, 6.28; N, 17.95.

5.1.14. 6-(Azetidinylmethyl)-5-chlorouracil (17). To a suspension of 800 mg (4.10 mmol) of **5** in 15 mL of water was added 713 mg (12.5 mmol) of azetidine and stirred for 5 h at room temperature. The reaction mixture was evaporated under a vacuum. The residue was triturated with EtOH, and the resulting precipitate was collected by filtration. The crude solid was purified by flash column chromatography ($CHCl_3$–MeOH, 20:1) and triturated with AcOEt to give 355 mg (40%) of **17** as a white powder: mp 190–191 °C. ^{1}H NMR (DMSO-d_6): δ 1.98 (quintet, 2H, $J = 7.0$ Hz), 3.27 (t, 4H, $J = 7.0$ Hz), 3.46 (s, 2H), 11.23 (s, 1H). FAB-MS m/z: 216 $(M+H)^+$. Anal. Calcd for $C_8H_{10}ClN_3O_2$: C, 44.56; H, 4.67; N, 19.49. Found: C, 44.34; H, 4.72; N, 19.35.

5.1.15. 5-Chloro-6-(pyrrolidinylmethyl)uracil (18). Compound **18** was prepared in 40% yield from **5** and pyrrolidine by a method similar to that described for **10**, as a white powder: mp 207–209 °C. ^{1}H NMR (DMSO-d_6): δ 1.66–1.76 (m, 4H), 2.48–2.60 (m, 4H), 3.52 (s, 2H). EI-MS (70 eV) m/z: 229 (M^+). Anal. Calcd for $C_9H_{12}ClN_3O_2$: C, 47.07; H, 5.27; N, 18.30. Found: C,

46.97; H, 5.36; N, 18.15. Compound **18** (460 mg, 2.00 mmol) was dissolved in 3 mL of 2 M aqueous HCl at 80 °C. To the reaction mixture was added 7 mL of EtOH and allowed to stand at room temperature, and the precipitate was collected by filtration to give 412 mg (77%) of a hydrochloride salt of **18** as colorless needles: mp 255 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.97 (s, 4H), 3.1–3.7 (m, 4H), 4.35 (s, 2H), 11.07 (s, 1H), 11.46 (s, 1H), 11.79 (s, 1H). FAB-MS m/z: 210 $(M+H)^+$, 230 $(M+H-HCl)^+$. Anal. Calcd for $C_9H_{12}ClN_3O_2\cdot HCl$: C, 40.62; H, 4.92; N, 15.79. Found: C, 40.48; H, 4.94; N, 15.50.

5.1.16. 5-Chloro-6-(piperidylmethyl)uracil (19). Compound **19** was prepared in 61% yield from **5** and piperidine by a method similar to that described for **10**, as a white powder: mp 195 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.32–1.56 (m, 6H), 2.34–2.46 (m, 4H), 3.36 (s, 2H), 11.11 (s, 1H). EI-MS (70 eV) m/z: 243 (M^+). Anal. Calcd for $C_{10}H_{14}ClN_3O_2$: C, 49.29; H, 5.79; N, 17.24. Found: C, 49.37; H, 5.83; N, 17.15.

5.1.17. 5-Chloro-6-(hexamethyleneiminylmethyl)uracil (20). To a suspension of 800 mg (4.10 mmol) of **5** in 20 mL of water was added 1.00 g (10.1 mmol) of hexamethyleneimine and stirred for 2.5 h at room temperature. The precipitate was collected by filtration and washed with water to give 495 mg (47%) of **20** as a white powder: mp 200 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.51–1.66 (m, 8H), 2.62–2.70 (m, 4H), 3.54 (s, 2H). FAB-MS m/z: 258 $(M+H)^+$. Anal. Calcd for $C_{11}H_{16}ClN_3O_2$: C, 51.27; H, 6.26; N, 16.30. Found: C, 51.39; H, 6.50; N, 16.37.

5.1.18. 5-Chloro-6-(morpholin-4-ylmethyl)uracil (21). Compound **21** was prepared in 78% yield from **5** and morpholine by a method similar to that described for **20**, as a white solid: mp 245 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.44–2.47 (m, 4H), 3.40 (s, 2H), 3.56–3.60 (m, 4H), 10.84 (s, 1H), 11.53 (s, 1H). EI-MS (70 eV) m/z: 245 (M^+). Anal. Calcd for $C_9H_{12}ClN_3O_3\cdot 0.1H_2O$: C, 43.68; H, 4.97; N, 16.98. Found: C, 43.68; H, 4.81; N, 16.89.

5.1.19. 5-Chloro-6-[(4-methylpiperazinyl)methyl]uracil (22). Compound **22** was prepared in 1% yield from **5** and 1-methylpiperazine by a method similar to that described for **10**, as a beige powder: mp 205 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.14 (s, 3H), 2.35 (s, 4H), 2.50 (s, 4H), 3.33 (s, 2H). FAB-MS m/z: 259 $(M+H)^+$. Anal. Calcd for $C_{10}H_{15}ClN_4O_2$: C, 46.43; H, 5.84; N, 21.66. Found: C, 46.44; H, 6.05; N, 21.53.

5.1.20. 6-[(2,5-Dimethylpyrrolidinyl)methyl]-5-chlorouracil (23). Compound **23** was prepared in 9% yield from **5** and 2,5-dimethylpyrrolidine by a method similar to that described for **20**, as a pale yellow solid: mp 220 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.00 (d, 6H, $J = 5.9$ Hz), 1.38 (m, 2H), 1.83 (m, 2H), 2.67 (m, 2H), 3.59 (s, 2H). FAB-MS m/z: 258 $(M+H)^+$. Anal. Calcd for $C_{11}H_{16}ClN_3O_2$: C, 51.27; H, 6.26; N, 16.30. Found: C, 51.13; H, 6.41; N, 16.34.

5.1.21. 5-Chloro-6-(methoxymethyl)uracil (24). To a solution of 500 mg (2.56 mmol) of **5** in 10 mL of DMF was added 2.00 g of a 28% solution of NaOMe in MeOH and stirred for 8 days at room temperature. The reaction mixture was acidified with 10% HCl in MeOH, and the precipitate was collected by filtration and washed with water to give 25 mg (5%) of **24** as a white powder: mp >300 °C. ^{1}H NMR (DMSO-d_6): δ 3.31 (s, 3H), 4.31 (s, 2H), 11.16 (s, 1H), 11.58 (s, 1H). FAB-MS m/z: 191 $(M+H)^+$. Anal. Calcd for $C_6H_7ClN_2O_3$: C, 37.81; H, 3.70; N, 14.70. Found: C, 37.69; H, 3.69; N, 14.61.

5.1.22. 5-Chloro-6-(chloroethyl)uracil (26). Compound **26** was prepared in 77% yield from **25** by a method similar to that described for **5**, as a white solid: mp 225 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.01 (t, 2H, $J = 6.9$ Hz), 3.88 (t, 2H, $J = 6.9$ Hz), 11.28 (s, 1H), 11.60 (s, 1H). EI-MS (70 eV) m/z: 208 (M^+). Anal. Calcd for $C_6H_6Cl_2N_2O_2\cdot 0.1$AcOH: C, 34.63; H, 3.00; N, 13.03. Found: C, 34.27; H, 3.02; N, 12.75.

5.1.23. 6-[2-(Dimethylamino)ethyl]-5-chlorouracil (27). Compound **27** was prepared in 67% yield from **26** and 50% aqueous dimethylamine by a method similar to that described for **7**, as a white solid: mp 263 °C dec. ^{1}H NMR ($CDCl_3$): δ 2.36 (s, 6H), 2.71–2.82 (m, 4H). FAB-MS m/z: 218 $(M+H)^+$. Anal. Calcd for $C_8H_{12}ClN_3O_2\cdot 0.1H_2O$: C, 43.79; H, 5.60; N, 19.15. Found: C, 43.83; H, 5.79; N, 18.84.

5.1.24. 5-Chloro-6-(2-pyrrolidinylethyl)uracil (28). To a suspension of 210 mg (1.0 mmol) of **26** in 5 mL of water was added 0.42 mL (5.0 mmol) of pyrrolidine and stirred for 7 days at room temperature. The reaction mixture was evaporated under a vacuum. The residue was purified by flash column chromatography ($CHCl_3$–MeOH, 10:1) and recrystallized from MeOH to give 22 mg (9%) of **28** as white crystals: mp 270 °C dec. ^{1}H NMR ($CDCl_3$): δ 1.89 (quintet, 4H, $J = 3.3$ Hz), 2.68 (t, 4H, $J = 3.3$ Hz), 2.82 (m, 2H), 2.93 (m, 2H). FAB-MS m/z: 244 $(M+H)^+$. Anal. Calcd for $C_{10}H_{14}ClN_3O_2$: C, 49.29; H, 5.79; N, 17.24. Found: C, 49.00; H, 6.02; N, 16.90.

5.1.25. 5-Chloro-6-(chloromethyl)-1-methyluracil (30). A solution of 430 mg (2.76 mmol) of 6-(hydroxymethyl)-1-methyluracil (**29**) in 10 mL of thionyl chloride was heated at 60 °C for 2 h. The reaction mixture was evaporated under a vacuum. The precipitate was recrystallized from MeOH to give 270 mg (56%) of 6-(chloromethyl)-1-methyluracil as beige crystals: mp 187–188 °C. ^{1}H NMR (DMSO-d_6): δ 3.30 (s, 3H), 4.69 (s, 2H), 5.82 (s, 1H), 11.40 (s, 1H). FAB-MS m/z: 173 $(M-H)^-$. Anal. Calcd for $C_6H_7ClN_2O_2$: C, 41.28; H,

4.04; N, 16.05. Found: C, 41.29; H, 4.03; N, 16.00. To a solution of 190 mg (1.1 mmol) of 6-(chloromethyl)-1-methyluracil in 1.5 mL of AcOH was added 0.1 mL (1.3 mmol) of sulfuryl chloride and stirred for 1 h at room temperature. The reaction mixture was evaporated under a vacuum. The residue was purified by flash column chromatography ($CHCl_3$–MeOH, 50:1) and recrystallized from MeOH to give 64 mg (28%) of **30** as white crystals: mp 222–224 °C. ^{1}H NMR (DMSO-d_6): δ 3.40 (s, 3H), 4.84 (s, 2H), 12.00 (s, 1H). EI-MS (70 eV) m/z: 208 (M^+). Anal. Calcd for $C_6H_6Cl_2N_2O_2$: C, 34.48; H, 2.89; N, 13.40. Found: C, 34.43; H, 2.83; N, 13.36.

5.1.26. 5-Chloro-1-methyl-6-(pyrrolidinylmethyl)uracil (31). To a suspension of 25 mg (0.12 mmol) of **30** in 0.5 mL of water was added 43 mg (0.60 mmol) of pyrrolidine and stirred for 14 h at room temperature. The pH of the reaction mixture was adjusted to 5 by addition of AcOH, and the precipitate was collected by filtration and recrystallized from MeOH to give 12 mg (41%) of **31** as colorless needles: mp 222–224 °C. ^{1}H NMR (DMSO-d_6): δ 1.70 (s, 4H), 2.57 (s, 4H), 3.43 (s, 3H), 3.74 (s, 2H), 11.81 (s, 1H). EI-MS (70 eV) m/z: 243 (M^+). Anal. Calcd for $C_{10}H_{14}ClN_3O_2$: C, 49.29; H, 5.79; N, 17.24. Found: C, 49.28; H, 5.93; N, 17.26.

5.1.27. 5-Chloro-6-(imidazolylmethyl)uracil hydrochloride (32). A mixture of 5.00 g (25.6 mmol) of **5** and 4.30 g (39.0 mmol) of *N*-acetylimidazole in 100 mL of MeOH was heated under reflux for 2 days. The reaction mixture was allowed to stand at room temperature, and the precipitate was collected by filtration and washed with 30 mL of 10% HCl in MeOH to give 4.32 g (64%) of **32** as a white powder: mp 244–246 °C. ^{1}H NMR (DMSO-d_6): δ 5.40 (s, 2H), 7.74 (d, 1H, $J = 1.3$ Hz), 7.82 (d, 1H, $J = 1.3$ Hz), 9.29 (s, 1H), 11.74 (s, 1H), 11.78 (s, 1H). FAB-MS m/z: 227 $(M+H-HCl)^+$. Anal. Calcd for $C_8H_7ClN_4O_2 \cdot HCl \cdot H_2O$: C, 34.18; H, 3.59; N, 19.93. Found: C, 34.27; H, 3.27; N, 19.85.

5.1.28. 5-Chloro-6-[(2-methylimidazolyl)methyl]uracil hydrochloride (33). A mixture of 500 mg (2.56 mmol) of **5** and 631 mg (7.68 mmol) of 2-methylimidazole in 10 mL of 1 M aqueous KOH was heated at 80 °C for 3 h. The reaction mixture was allowed to stand at room temperature and the pH was adjusted to 4 by addition of 6 M aqueous HCl. The precipitate was collected by filtration and washed with water to give 50 mg (8%) of **33** as a beige powder: mp 240 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.60 (s, 3H), 5.22 (s, 2H), 7.54 (d, 1H, $J = 2.0$ Hz), 7.61 (d, 1H, $J = 2.0$ Hz), 11.77 (s, 1H). FAB-MS m/z: 241 $(M+H-HCl)^+$. Anal. Calcd for $C_9H_9ClN_4O_2 \cdot HCl \cdot 1.25H_2O$: C, 36.08; H, 4.20; N, 18.71. Found: C, 36.03; H, 3.94; N, 18.44.

5.1.29. 5-Chloro-6-[(4-nitroimidazolyl)methyl]uracil (34). Compound **34** was prepared in 12% yield from **5** and 4-nitroimidazole by a method similar to that described for **33**, as a beige solid: mp 155–158 °C. ^{1}H NMR (DMSO-d_6): δ 5.18 (s, 2H), 7.95 (d, 1H, $J = 1.3$ Hz), 8.43 (d, 1H, $J = 1.3$ Hz), 11.50 (s, 1H), 11.70 (s, 1H). FAB-MS m/z: 270 $(M-H)^-$. Anal. Calcd for $C_8H_6ClN_5O_4 \cdot 1.25H_2O$: C, 32.67; H, 2.91; N, 23.81. Found: C, 32.87; H, 2.73; N, 23.60.

5.1.30. 5-Chloro-6-(pyrazolylmethyl)uracil (35). Compound **35** was prepared in 17% yield from **5** and pyrazole by a method similar to that described for **33**, as a yellow solid: mp 220 °C dec. ^{1}H NMR (DMSO-d_6): δ 5.17 (s, 2H), 6.29 (dd, 1H, $J = 2.3$, 1.5 Hz), 7.50 (d, 1H, $J = 1.5$ Hz), 7.86 (d, 1H, $J = 2.3$ Hz), 11.53 (s, 1H), 11.68 (s, 1H). FAB-MS m/z: 227 $(M+H)^+$. Anal. Calcd for $C_8H_7ClN_4O_2$: C, 42.40; H, 3.11; N, 24.72. Found: C, 42.01; H, 3.05; N, 24.39.

5.1.31. 5-Chloro-6-(1,2,4-triazolylmethyl)uracil (36). A mixture of 300 mg (1.54 mmol) of **5** and 327 mg (4.73 mmol) of 1*H*-1,2,4-triazole in 5 mL of 0.75 M aqueous KOH was heated under reflux for 3 h. The reaction mixture was allowed to stand at room temperature and evaporated under a vacuum. The residue was triturated with MeOH, and the resulting precipitate was collected by filtration. The crude solid was purified by flash column chromatography ($CHCl_3$–MeOH, 10:1) and triturated with $CHCl_3$ to give 50 mg (14%) of **36** as a white powder: mp 235 °C dec. ^{1}H NMR (DMSO-d_6): δ 5.26 (s, 2H), 8.03 (s, 1H), 8.64 (s, 1H), 11.57 (s, 1H), 11.70 (s, 1H). FAB-MS m/z: 228 $(M+H)^+$. Anal. Calcd for $C_7H_6ClN_5O_2 \cdot 0.2H_2O$: C, 36.36; H, 2.79; N, 30.29. Found: C, 36.74; H, 2.71; N, 29.96.

5.1.32. 5-Chloro-6-(pyrrolylmethyl)uracil (37). A mixture of 500 mg (2.85 mmol) of **6** and 577 mg (4.37 mmol) of 2,5-dimethoxytetrahydrofuran in 8 mL of AcOH was heated under reflux for 2 h. The reaction mixture was allowed to room temperature and evaporated under a vacuum. The residue was purified by flash column chromatography ($CHCl_3$–MeOH, 50:1) and triturated with *i*-PrOH to give 155 mg (24%) of **37** as a beige powder: mp 210 °C dec. ^{1}H NMR (DMSO-d_6): δ 4.92 (s, 2H), 6.04 (d, 2H, $J = 1.8$ Hz), 6.87 (d, 2H, $J = 1.8$ Hz), 11.61 (s, 2H). FAB-MS m/z: 226 $(M+H)^+$. Anal. Calcd for $C_9H_8ClN_3O_2 \cdot 0.25H_2O$: C, 46.97; H, 3.72; N, 18.26. Found: C, 47.26; H, 3.68; N, 17.87.

5.1.33. 5-Bromo-6-(chloromethyl)uracil (38). To a solution of 100 g (623 mmol) of 6-chloromethyluracil (**4**) in 1.0 L of DMF was added 122 g (685 mmol) of *N*-bromosuccinimide cooling with an ice-bath and stirred for 1 h. To the reaction mixture was added ice/water (1.0 L), and the precipitate was collected by filtration and washed with AcOH and water to give 126 g (84%) of **38** as a white powder: mp 245 °C dec. ^{1}H NMR (DMSO-d_6): δ 4.47 (s, 2H), 11.61 (s, 1H), 11.66 (s, 1H). EI-MS (70 eV) m/z: 240 (M^+). Anal. Calcd for $C_5H_4BrClN_2O_2$: C, 25.08; H, 1.68; N, 11.70. Found: C, 24.81; H, 1.67; N, 11.57.

5.1.34. 5-Bromo-6-(pyrrolidinylmethyl)uracil (42). To a solution of 178 g (2.50 mol) of pyrrolidine in 1.0 L of water was added 120 g (0.501 mol) of **38** and stirred for 45 min at 35 °C. The pH of the reaction mixture was adjusted to 7 by addition of 120 g (2.0 mol) of AcOH, and the precipitate was collected by filtration and washed with water and MeOH to give 112 g (82%) of **42** as a white powder: mp 213–215 °C. ^{1}H NMR (DMSO-d_6): δ 1.64–1.79 (m, 4H), 2.52–2.63 (m, 4H), 3.55 (s, 2H). FAB-MS m/z: 274 $(M+H)^+$. Anal. Calcd for $C_9H_{12}BrN_3O_2$: C, 39.44; H, 4.41; N, 15.33. Found: C, 39.54; H, 4.44; N, 15.49. Compound **42** (10.0 g, 36.5 mmol) was dissolved in 60 mL of 2 M aqueous HCl at 80 °C. To the reaction mixture was added 150 mL of EtOH and allowed to stand at room temperature, and the precipitate was collected by filtration to give 9.90 g (87%) of a hydrochloride salt of **42** as colorless needles: mp 255 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.7–2.2 (m, 4H), 3.2–3.7 (m, 4H), 4.34 (s, 2H), 10.91 (s, 1H), 11.48 (s, 1H), 11.73 (s, 1H). FAB-MS m/z: 274 $(M+H-HCl)^+$. Anal. Calcd for $C_9H_{12}BrN_3O_2\cdot HCl$: C, 34.81; H, 4.22; N, 13.53. Found: C, 34.76; H, 4.19; N, 13.30.

5.1.35. 6-(Chloromethyl)-5-iodouracil (39). Compound **39** was prepared in 92% yield from **4** and *N*-iodosuccinimide by a method similar to that described for **38**, as a white powder: mp 225 °C dec. ^{1}H NMR (DMSO-d_6): δ 4.49 (s, 2H), 11.52 (s, 1H), 11.58 (s, 1H). FAB-MS m/z: 285 $(M-H)^-$. Anal. Calcd for $C_5H_4ClIN_2O_2$: C, 20.96; H, 1.41; N, 9.78. Found: C, 21.10; H, 1.36; N, 9.87.

5.1.36. 5-Iodo-6-(pyrrolidinylmethyl)uracil (43). To a solution of 740 mg (10.5 mol) of pyrrolidine in 10 mL of water was added 1.0 g (3.5 mmol) of **39** and stirred for 20 h at room temperature. The reaction mixture was evaporated under a vacuum, and the precipitate was recrystallized from MeOH to give 195 mg (17%) of **43** as pale yellow needles: mp 178 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.64–1.79 (m, 4H), 2.49–2.57 (m, 4H), 3.57 (s, 2H), 11.36 (s, 1H). FAB-MS m/z: 322 $(M+H)^+$. Anal. Calcd for $C_9H_{12}IN_3O_2$: C, 33.66; H, 3.77; N, 13.09. Found: C, 33.73; H, 3.89; N, 13.05.

5.1.37. 6-(Pyrrolidinylmethyl)uracil (41). Compound **41** was prepared in 9% yield from **4** and pyrrolidine by a method similar to that described for **43**, as pale yellow crystals: mp 177–179 °C. ^{1}H NMR (DMSO-d_6): δ 1.68–1.76 (m, 4H), 2.42–2.55 (m, 4H), 3.28 (s, 2H), 5.44 (s, 1H), 10.68 (s, 2H). FAB-MS m/z: 196 $(M+H)^+$. Anal. Calcd for $C_9H_{13}N_3O_2\cdot 0.8H_2O$: C, 51.57; H, 7.02; N, 20.04. Found: C, 51.59; H, 6.94; N, 19.73.

5.1.38. 6-(Pyrrolidinylmethyl)thymine (44). Compound **44** was prepared in 25% yield from **40** and pyrrolidine by a method similar to that described for **43**, as white crystals: mp 196–198 °C. ^{1}H NMR (DMSO-d_6): δ 1.67–1.75 (m, 4H), 1.78 (s, 3H), 2.45–2.55 (m, 4H), 3.38 (s, 2H). EI-MS (70 eV) m/z: 209 (M^+). Anal. Calcd for $C_{10}H_{15}N_3O_2\cdot 0.2H_2O$: C, 56.43; H, 7.29; N, 19.74. Found: C, 56.39; H, 7.36; N, 19.62.

5.2. Water solubility

Solubility of the compounds was determined in water after shaking for 24 h at 23 °C. The water-soluble concentration was determined by the HPLC method after separation of soluble fraction.

5.3. Biology

5.3.1. Assay of human TP activity. TP protein was partially purified from human placenta, and TP activity was measured by using [6-^{3}H]dThd as the substrate according to a previously described method.[16] The reaction mixture consisted of a total volume of 0.125 mL containing 100 mM potassium phosphate buffer (pH 7.4), 0.6 mM of [6-^{3}H]dThd containing 9.25 kBq of radiolabeled compound, TP, and inhibitor solution, and the mixture was incubated at 37 °C for 5 min. The reaction was terminated by heating at 100 °C for 2 min in boiling water, and the reaction mixture was centrifuged at 1900*g* for 5 min. A 10 μL sample of the supernatant was spotted on a silica gel TLC plate and developed with a mixture of $CHCl_3$, MeOH, and AcOH (17:3:1 by vol). A mixture of 5 mM dThd and thymine was applied to the plate as a visible marker before loading of the test samples. The thymine spot was separated into a vial and extracted with 4 M HCl (0.1 mL). After adding 10 mL of scintillator, the radioactivity was measured.

5.3.2. Oral absorption in mice. Compound **3** (300 mg/kg) suspended in 0.5% hydroxypropylmethylcellulose (HPMC) was orally administered to BALB/c mice (male, 9 weeks old, $n = 3$). Compounds **18** (38.8 or 116.4 mg/kg), **32** (46.4 or 139.3 mg/kg), and **42** (46.3 or 231.4 mg/kg) with F_3dThd (50 mg/kg) dissolved in 0.5% HPMC were orally administered to ICR mice (male, 6 weeks old, $n = 3$). Blood samples (heparinized plasma) were collected at 0.5, l, 2, 4, and 8 h after administration. The extraction of plasma samples was performed using 50% acetonitrile, 75% MeOH, or 10% trichloroacetic acid added to the plasma (0.2 mL), and the precipitated plasma proteins were removed by centrifugation (3000 rpm, 10 min). These protein precipitating solvents were used depending on the compound tested. The compound concentrations in the supernatant were measured by reverse-phase HPLC using a Chemcosorb 300-5C18 column (4.6 mm × 250 mm, Chemco Co., Ltd) under the following chromatographic conditions: mobile phase, 6% acetonitrile–potassium phosphate buffer (pH = 7.2); flow rate, 1.0 mL/min; detection, 272 or 300 nm; column temperature, room temperature.

5.3.3. Oral absorption in cynomolgus monkeys. Compound **42** (9.25 mg/kg) and F_3dThd (10 mg/kg) dissolved in 0.5% HPMC were orally administered to cynomolgus monkeys (male and female, 2.90–3.65 kg, $n = 3$). Blood

samples (heparinized plasma) were collected from a forearm vein 0.5, l, 2, 4, and 8 h after administration. The plasma concentrations of F_3dThd were measured in the same manner as previously described.[16]

5.3.4. Oral absorption and in vivo antitumor effect on human gastric cancer (AZ-521) bearing nude mice. Approximate 2×2 mm fragments of AZ-521 tumor were implanted into right axillae of 8 weeks-old male nude mice. When the tumor volume reached about 200 mm^3 (day 0), a mixture of F_3dThd (50 mg/kg) and compound **42** (46.3 mg/kg) or F_3dThd (50 mg/kg) alone dissolved in 0.5% HPMC was administered orally once a day for 2 weeks. Relative tumor volume (RTV) was calculated as follows: RTV = (mean tumor volume on day 15)/(mean tumor volume on day 0). The antitumor effects of F_3dThd were estimated by the following equation: inhibition rate of tumor growth (IR, %) = [1−(mean RTV of drug-treated group)/(mean RTV of control group)×100]. Blood samples (heparin-plasma) were collected at 0.5, l, 2, 4, and 8 h after administration. The plasma concentrations of F_3dThd were measured in the same manner as previously described.[16]

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