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Bioorganic & Medicinal Chemistry
Bioorganic & Medicinal Chemistry 12 (2004) 3443–3450

Synthesis and evaluation of 6-methylene-bridged uracil derivatives. Part 2: Optimization of inhibitors of human thymidine phosphorylase and their selectivity with uridine phosphorylase

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Received 5 March 2004; revised 28 April 2004; accepted 29 April 2004

Abstract—A series of novel 6-methylene-bridged uracil derivatives have been optimized for clinical use as the inhibitors of human thymidine phosphorylase (TP). We describe their synthesis and evaluation. Introduction of a guanidino or an amidino group enhanced the in vitro inhibitory activity of TP comparing with formerly reported inhibitor **1**. Their selectivity for TP based on uridine phosphorylase inhibitory activity was also evaluated. Compound **2** (TPI) has been selected for clinical evaluation based on its strong TP inhibition and excellent modulation of 2′-deoxy-5-(trifluoromethyl)uridine (F_3dThd) pharmacokinetics. As a result, TAS-102 (a combination of F_3dThd and TPI) is currently in phase 1 clinical studies.

1. Introduction

2′-Deoxy-5-(trifluoromethyl)uridine (F_3dThd) is known to have strong in vitro and in vivo anti-tumor activities.[1,2] In addition, F_3dThd was expected to be effective against cancer cells resistant to 5-fluorouracil and/or 2′-deoxy-5-fluorouridine, characterized by amplification of thymidylate synthase and/or deletion of orotate phosphoribosyltransferase.[3] However, F_3dThd is known to be quickly cleaved into an inactive compound [5-(trifluoromethyl)uracil] both in vitro and in vivo by pyrimidine nucleoside phosphorylases.[4–6] Therefore, it did not provide satisfactory clinical anti-tumor effects.[7] The cleavage of the glycosyl linkage of F_3dThd is mediated by one of two types of pyrimidine nucleoside phosphorylases, uridine phosphorylase (UP) or thymidine phosphorylase (TP). The former, UP, mainly occurs in rodents such as mice and rats, and the latter, TP, is a principal F_3dThd cleaving enzyme in human.[8,9] Therefore, to improve the anti-tumor effects of F_3dThd in human, there is necessity to design an inhibitor directed toward TP rather than an inhibitor of UP.

In our preceding report,[10] human TP was revealed to possess the tight limitation at the active site to fit 6-methylene-bridged uracil derivatives. Compound **1** was selected for further in vivo evaluation because of the superior pharmacokinetic (PK) profile and the inhibitory activity of human TP (Fig. 1).

In this paper, we describe the synthesis and biological evaluation of a series of novel 6-methylene-bridged

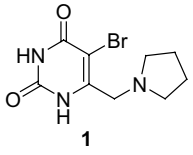

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Figure 1.

Keywords: Thymidine phosphorylase inhibitor; TPI; 2′-Deoxy-5-(trifluoromethyl)uridine (F_3dThd); TAS-102.
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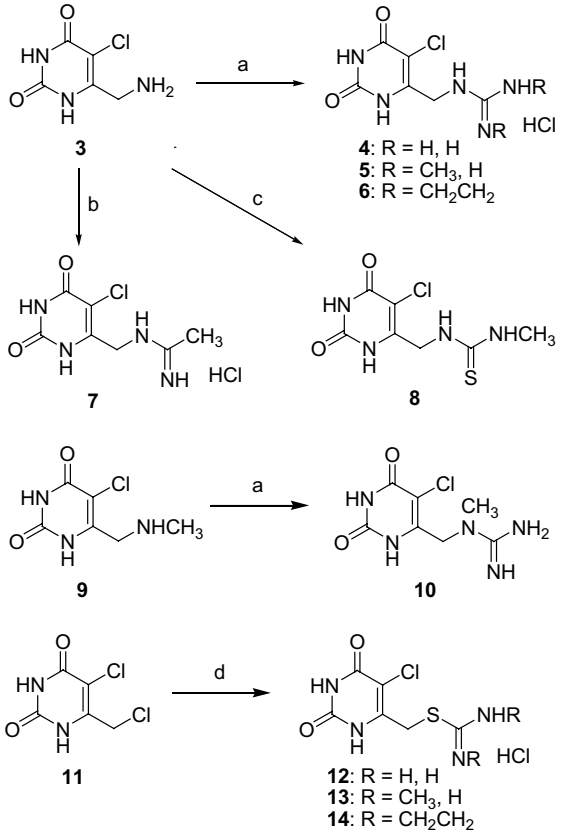
doi:10.1016/j.bmc.2004.04.046

uracil derivatives possessing a guanidino or an amidino group. Several derivatives have been synthesized and showed the enhanced in vitro TP inhibitory activity. The selectivity of inhibitors to TP and UP is also discussed.

2. Chemistry

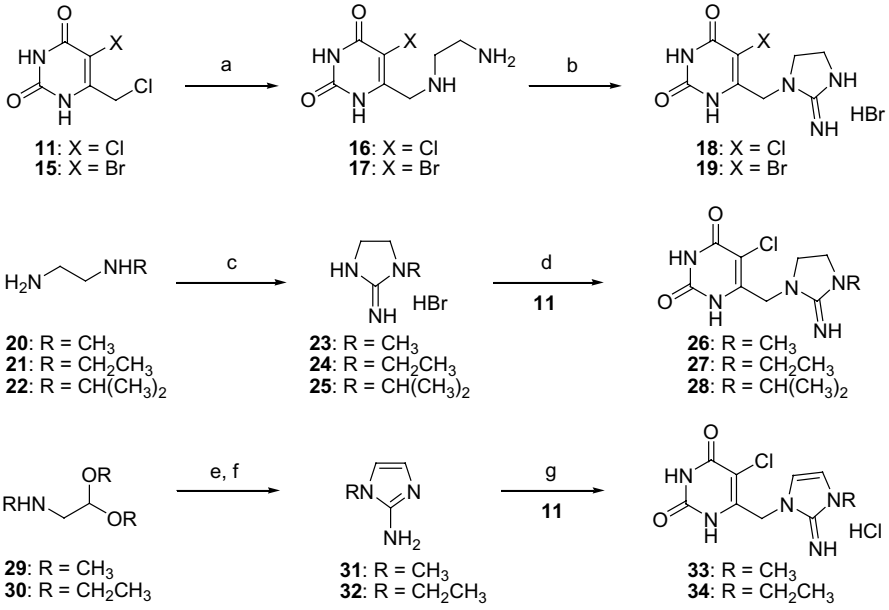
Scheme 1 illustrates the synthesis of 6-methylene-bridged uracil derivatives possessing a guanidino, an amidino, or a thioureido group. Compounds **3**, **9**, and **11** were prepared as previously described.[10] Compounds **4**–**6** and **10** were prepared by treating **3** or **9** with appropriate methylisothioureas in water.[11] Compound **7** was prepared by treating **3** with 1-ethoxyethanimine hydrochloride in DMF. Thiourea analogue **8** was prepared by heating **3** with methyl isothiocyanate in DMF. Isothiourea analogues **12**–**14** were prepared by heating **11** with appropriate thioureas in EtOH.

Scheme 2 illustrates the synthesis of 6-methylene-bridged uracil derivatives possessing cyclic guanidines. The synthesis of compounds **15** and **16** was previously reported.[10] Compound **17** was prepared by treating **15** with a large excess of ethylenediamine in water. Treatment of diamines **16** and **17** with cyanogen bromide[12] in water resulted in the formation of 2-iminoimidazolidine derivatives **18** and **19**, respectively. Cyclization of *N*-alkylethylendiamines **20**–**22** with cyanogen bromide in benzene,[12] followed by alkylation of the resulting 1-alkyl-2-iminoimidazolidines **23**–**25** with **11** using NaOEt in DMF,[13] gave the desired 3-alkyl-2-iminoimidazolidine derivatives **26**–**28**. 1-Alkyl-2-aminoimidazoles **31** and **32**, prepared as free bases from **29** and **30** by the Lawson's method,[14] were alkylated with **11** in water to result in the formation of the desired 3-alkyl-2-iminoimidazoline derivatives **33** and **34**.[13]

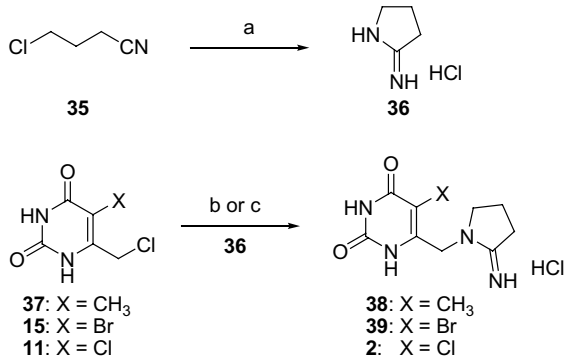


Scheme 1. Reagents and conditions: (a) $[CH_3SC(=NH)NH_2]_2{\cdot}H_2SO_4$, $CH_3SC(=NH)NHCH_3{\cdot}HI$, or 2-methylthio-2-imidazoline hydroiodide, KOH, H_2O; (b) $CH_3C(=NH)OCH_2CH_3{\cdot}HCl$, DMF; (c) CH_3NCS, DMF; (d) $NH_2C(=S)NH_2$, $CH_3NHC(=S)NH_2$, or 2-imidazolidinethione, EtOH.

Scheme 3 illustrates the synthesis of the compounds possessing a 2-iminopyrrolidyl group. 2-Iminopyrrol-



Scheme 2. Reagents and conditions: (a) ethylenediamine, H_2O; (b) BrCN, H_2O; (c) BrCN, benzene; (d) NaOEt, DMF; (e) cyanamide, aq AcOH; (f) concd HCl; (g) H_2O.



Scheme 3. Reagents and conditions: (a) NH_3, MeOH; (b) NaOEt, DMF; (c) DBU, MeOH.

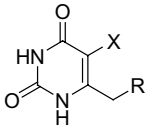
idine hydrochloride (**36**) was prepared from **35** in a previously described manner.[15] 6-(Chloromethyl)thymine (**37**) was prepared according to the published method.[16] Alkylation of **36** with **37**, **15**, and **11** in the presence of NaOEt in DMF resulted in the desired 2-iminopyrrolidine derivatives **38**, **39**, and **2**, with chemical yields of 22%, 13%, and 38%, respectively. The reaction conditions were not satisfactory for a large-scale preparation. The application of an alkylation with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in MeOH led to the improvement of chemical yield (68% for compound **2**) as well as the ease of handling and simplicity of the workup procedure because of no formation of inorganic byproducts.

3. Results and discussion

In our previous report,[10] compound **1** has been selected for further in vivo evaluation. Careful evaluation of compound **1** revealed a high death rate (4/6) and body weight loss (−13.9%, $n = 2$) in ICR mice (male, 5 weeks old) given high doses (2000 mg/kg, po.) for 14 days. Body weight loss (−6.9%, $n = 6$) was also observed in ICR mice (male, 5 weeks old) at lower doses (1000 mg/kg, po.). These primarily disappointing results prompted us to search for a new type of chemical class of compounds. Hence, we focused our study on the design of new inhibitors with strong TP inhibitory activity and possessing negligible toxicity.

In Table 1, we show the inhibitory effect of 6-methylene-bridged uracil derivatives on human TP. We found that the potency of guanidine derivative **4** was close to that of

Table 1. Inhibitory effect of 6-methylene-bridged uracil derivatives on human TP and rat UP



Compd	R	X	TP IC_{50}[a,b] (μM)	UP IC_{50}[a,c] (μM)
1	*N*-Pyrrolidinyl	Br	0.51	14
4	$NHC(=NH)NH_2$	Cl	0.27	390
5	$NHC(=NH)NHCH_3$	Cl	0.087	230
6	2-Imidazolin-2-ylamino	Cl	31	NT[d]
7	$NHC(=NH)CH_3$	Cl	1.5	>100
8	$NHC(=S)NHCH_3$	Cl	>100	NT[d]
10	$NCH_3C(=NH)NH_2$	Cl	0.031	630
12	$SC(=NH)NH_2$	Cl	0.35	610
13	$SC(=NH)NHCH_3$	Cl	0.15	NT[d]
14	2-Imidazolin-2-ylthio	Cl	25	NT[d]
18	*N*-(2-Iminoimidazolidinyl)	Cl	0.013	>100
19	*N*-(2-Iminoimidazolidinyl)	Br	0.030	>100
26	*N*-(2-Imino-3-methylimidazolidinyl)	Cl	0.046	>100
27	*N*-(3-Ethyl-2-iminoimidazolidinyl)	Cl	0.36	>100
28	*N*-(2-Imino-3-isopropylimidazolidinyl)	Cl	4.0	>100
33	*N*-(2-Imino-3-methylimidazolinyl)	Cl	0.24	NT[d]
34	*N*-(3-Ethyl-2-iminoimidazolinyl)	Cl	6.9	NT[d]
2 (TPI)	*N*-(2-Iminopyrrolidinyl)	Cl	0.035[e]	>100
38	*N*-(2-Iminopyrrolidinyl)	CH_3	0.12	NT[d]
39	*N*-(2-Iminopyrrolidinyl)	Br	0.032	NT[d]
9	$NHCH_3$	Cl	12	7.8
40	$NHCH_2CH_3$	Cl	20	50
41	*N*-Pyrrolidinyl	Cl	2.2	8.6
42	*N*-Imidazolyl	Cl	1.0	93

[a] Inhibitory effect is reported as an IC_{50} value, indicating the concentration of test compound required to inhibit enzyme activity by 50%. This assay tests at least three concentrations of each compound.

[b] A 0.6 mM concentration of [6-^{3}H]dThd is used as the substrate for the TP reactions.

[c] A 0.6 mM concentration of [5-^{3}H]Urd is used as the substrate for the UP reactions.

[d] Not tested.

[e] The K_i value for TPI determined by using recombinant human TP was 0.017 μM.[17]

the formerly reported candidate **1**. Furthermore, compound **5** as a *N*-methyl derivative of **4** showed more potent activity. The substitution of the end of guanidine with imidazoline function (Compound **6**) significantly abolished the inhibitory effects. This may indicate that a nonsubstituted imino group was probably necessary to enhance the inhibitory effect. After introduction of the smallest amidino group, methylamidino group, the potency was not restored (compound **7**) up to the level of compound **4**. With replacement of imine by thione, the resulting thiourea derivative (compound **8**) dramatically diminished the inhibitory potency. Notably compound **10** showed close inhibitory activity to its isomer **5**. Alkyl substitution at the amino group, containing the nonsubstituted imino-group in guanidine, could be important in enhancing the inhibitory activity. Evaluation of corresponding isothiourea derivatives (**12**–**14**) of guanidine derivatives (**4**–**6**) showed close inhibitory activity, however the isothiourea derivatives appeared to be less stable in acidic or basic conditions. The above results indicated that compounds containing the guanidine group seemed to play essential roles in the development of clinically important chemical entities.

In our preceding report,[10] we have found that pyrrolidine and imidazole best matched the active site of human TP. In addition, the best biological results were observed, when bromide was substituted at the 5-position of the uracil molecule. Among the iminoimidazolidinyl derivatives (**18**, **19**, and **26**–**28**), compound **18** showed the best inhibitory effect in vitro. Unexpectedly the bromide substituted derivative **19** did not show more potent activity. It was suggested that the conformation of the inhibitors would be changed the fitting to the region that surround the active site of human TP. *N*-alkyl derivatives of **18** were evaluated because they were expected to improve the inhibitory activity based on the effects of compounds **4** and **5**. Methyl derivative **26** showed diminished potency. Furthermore, ethyl and isopropyl derivatives (**27** and **28**) showed much diminished inhibitory activity, probably caused by too large molecular size. The iminoimidazolinyl derivatives (**33** and **34**) appeared to be almost 10-fold weaker than the corresponding imidazolidinyl derivatives (compounds **26** and **27**). *N*-Alkyl substitution appeared to be not effective in enhancing the inhibitory activity of TP, because of tight limitation of the active site of human TP as we described previously.[10] Furthermore, introduction of a double bond in the 5-membered heterocycle lead to the reduction of the inhibitory activity, therefore the compounds without a double bond appeared to be important to maximize the activity. Based on these results, the 2-iminopyrrolidinyl derivatives were evaluated. Although compound **7** showed only moderate activity, compound **2** possessed strong inhibitory activity, indicating the 2-iminopyrrolidinyl group matching well at the active site of human TP. Methyl derivative **38** showed weaker activity than **2**. The potency of bromo derivative **39**, which has a similar structure to that of the former clinical candidate **1**, was close to that of **2**.

Since compound **1** had toxic effects, the compounds with more diverse chemical structured were preferred for further in vivo biological evaluation. Therefore, the PK studies of F_3dThd given with compounds (**2**, **4**, **5**, **12**, **18**, **19**, **26**, and **27**) were performed in mice after oral administration. The maximum plasma concentration (C_{max}) and an AUC value of each compound are shown in Table 2. To improve the anti-tumor effect of F_3dThd, the clinical candidate should have a similar ratio of the C_{max} and the IC_{50} values like compound **1**. Considering the IC_{50} value of compound **1** against human TP, the C_{max} and AUC_{0-8} values of compounds **2**, **18**, and **19** should have been similar to those of compound **1** to exert similar pharmacological effect. Linear guanidine or amidine derivatives (**4**, **5**, and **12**) showed moderate values of C_{max} and AUC, but they did not satisfy our criteria. In contrast, the C_{max} and AUC_{0-8} values of cyclic guanidine derivative **18** were higher than those of linear compounds. Considering its IC_{50} value, one may expect this compound to be worth of further pharmacological evaluation. Bromo and alkyl derivatives of **18** (compounds **19**, **26**, and **27**) were also evaluated, however, their values were not superior to those of compound **18**. The synthesis of compound **18** required a highly toxic reagent, cyanogen bromide. In order not to violate the safety of workers, the development of this compound should not be enforced. Compound **2** (TPI) was finally evaluated, since its values of C_{max} and AUC met our criteria of a potent pharmacological agent. In addition, its synthesis was characterized by a good yield and safer synthetic procedure than that of **18**. To confirm its in vivo potency, TPI (9.42 mg/kg) was administered orally with an equimolar amount of F_3dThd (10 mg/kg) to monkeys ($n = 3$) to evaluate its ability to elevate the plasma concentration of F_3dThd. Plasma F_3dThd levels were increased very markedly, with a T_{max} of 1 h, a C_{max} of 15.2 μg/mL, and an AUC value of 28.5 μg h/mL, compared with F_3dThd administration alone, with a T_{max} of 1 h, a C_{max} of 0.23 μg/mL, and an AUC value of 0.28 μg h/mL.[17] In addition, the combined oral administration of TPI significantly potentiated the anti-tumor activity of F_3dThd against AZ-521 human stomach cancer xenografts in nude mice.[17]

Table 2. Oral absorption of compounds **1**, **2**, **4**, **5**, **12**, **18**, **19**, **26**, and **27** in mice[a]

Compd	C_{max}[b] (μM)	T_{max}[c] (h)	AUC_{0-8}[d] (μM h)
1	80	0.25	130
2 (TPI)	11	0.5	17
4	3.1	0.5	7.1
5	5.0	0.5	5.1
12	7.4	0.5	12
18	12	0.5	26
19	6.9	0.5	5.8
26	3.1	0.5	3.4
27	1.8	0.5	1.3

[a] Compounds **1**, **2**, **4**, **5**, **12**, or **18** (0.169 mmol/kg) with F_3dThd (0.169 mmol/kg) dissolved in 0.5% hydroxypropylmethylcellulose (HPMC) were orally administered to ICR mice (male, 6 weeks old, $n = 3$). Compounds **19**, **26**, or **27** (0.169 mmol/kg) with F_3dThd (0.169 mmol/kg) dissolved in 0.5% HPMC was orally administered to BALB/c nu/nu nude mice (male, 6 weeks old, $n = 3$).

[b] Maximum plasma concentration after oral dosing.

[c] Time to C_{max}.

[d] Area under the concentration–time curve for 0–8 h after oral dosing.

Despite the presence of strong TP inhibitory effects and good PK profile, one more factor seemed to be important for the selection of therapeutically important compound. This additional factor was selectivity, in other words, the compound should have a weak inhibitory effect to UP.

Table 1 shows the inhibitory effect of typical compounds on rat UP and comparison with those on human TP. Compound **1** had moderate activity against UP. However, it was about 30-fold weaker than TP. In contrast to the case of TP, introduction of an imino group did not enhance the inhibitory effect of UP (compounds **4**, **5**, **7**, **10**, **12**, **18**, **19**, **26**, **27**, **28**, and **2**). All the compounds showed diminished inhibitory activity when compared with **1**. It appeared that an amidino or a guanidino group at the 6-position did not match at the active site of UP. In contrast, compounds with no imino group (**9**,**41**)[10] had close inhibitory effect on UP when compared with compound **1**. Compounds **40** and **42**[10] showed weaker activity than **1**. Three types of compounds were found among the 6-methylene-bridged uracil derivatives. Typically, compounds **9**, **40**, and **41** were dual inhibitors of TP and UP, compounds **1** and **42** were selective inhibitors of TP, and compound **2** was a specific inhibitor of TP. Compound **1** showed a high enough C_{max} value in mice to exercise the inhibition of UP in vivo. In contrast, specific TP inhibitor **2** should not affect the function of UP in vivo. To avoid unexpected adverse effects, it was clear that selection of the specific TP inhibitor was a better choice. Therefore, TPI has been chosen as a candidate being able to provide an excellent pharmacological profile when clinically used.

4. Conclusion

In conclusion, we evaluated novel 6-methylene-bridged uracil derivatives with an amidino or a guanidino group as potent human TP inhibitors. The introduction of an imino group has created more potent human TP inhibitors than former pre-clinical candidate **1**. The specific inhibitors of TP were prepared as a result of a mismatch with the active site of UP.

Compound **2** (TPI) enhanced plasma concentration of F_3dThd in monkeys and potentiated the in vivo antitumor activity of F_3dThd in mice after combined oral administration. Furthermore, neither significant body weight loss nor animal death was observed in ICR mice (male, 5 weeks old, $n = 6$) even after administration of TPI at high dose (2000 mg/kg, po.). Based on these biological results, TPI has been selected as a candidate for clinical trials. Furthermore, human TP has been found to be identical to Platelet Derived Endothelial Cell Growth Factor (PD-ECGF), known as an endogenous angiogenic factor.[18] The results of biological studies on TPI indicated that TPI may also inhibit the process of angiogenesis, which is closely associated with malignancy of solid tumors.[19,20] Since the process of angiogenesis is closely related to metastatic ability, TPI may have a significant impact on the treatment of patients with advanced cancers. TAS-102 (a combination of F_3dThd and TPI) is currently in the phase 1 clinical study for colorectal and breast cancer patients in the USA.

5. Experimental

5.1. Chemistry

All melting points were determined on a Yanagimoto micromelting point apparatus and are 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 are based on purified material and were not optimized.

5.1.1. Amino{[5-chloro-2,6-dioxo(1,3-dihydropyrimidin-4-yl)]methyl}carboxamidine hydrochloride (4). A mixture of 600 mg (3.42 mmol) of **3** and 455 mg (3.27 mmol) of methylisothiourea sulfate in 33 mL of 0.1 M aqueous KOH was heated at 75 °C for 2 h. The reaction mixture was allowed to stand at room temperature. An insoluble material was collected by filtration and suspended in 5 mL of 2 M aqueous HCl at room temperature for 2 h. The precipitate was collected by filtration to give 287 mg (33%) of **4** as a brown powder: mp 255 °C dec. ^{1}H NMR (DMSO-d_6): δ 4.29 (d, 2H, $J = 5.0$ Hz), 7.45 (s, 3H), 7.99 (t, 1H, $J = 5.0$ Hz), 11.45 (s, 1H), 11.64 (s, 1H). FAB-MS m/z: 218 $(M+H-HCl)^+$. Anal. Calcd for $C_6H_8ClN_5O_2 \cdot HCl \cdot 0.1H_2O$: C, 28.16; H, 3.62; N, 27.37. Found: C, 28.37; H, 3.67; N, 27.13.

5.1.2. 5-Chloro-6-({[imino(methylamino)methyl]amino}-methyl)uracil hydrochloride (5). A mixture of 500 mg (2.85 mmol) of **3** and 1.32 g (5.70 mmol) of 1,2-dimethyl-2-thiopseudourea hydroiodide in 19 mL of 0.3 M aqueous KOH was heated at 40 °C for 3 days. An insoluble material was collected by filtration and dissolved in 20 mL of 0.2 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 320 mg (42%) of **5** as yellow crystals: mp 172 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.75 (d, 3H, $J = 4.3$ Hz), 4.30 (d, 2H, $J = 5.6$ Hz), 7.60 (s, 2H), 7.74–7.82 (m, 2H), 11.35 (s, 1H), 11.67 (s, 1H). FAB-MS m/z: 232 $(M+H)^+$. Anal. Calcd for $C_7H_{10}ClN_5O_2 \cdot HCl \cdot H_2O$: C, 29.65; H, 4.23; N, 24.17. Found: C, 29.39; H, 4.58; N, 24.48.

5.1.3. 5-Chloro-6-[(2-imidazolin-2-ylamino)methyl]uracil hydrochloride (6). Compound **6** was prepared in 25% yield from **3** and 2-methylthio-2-imidazoline hydroiodide by a method similar to that described for **5**, as brown crystals: mp 235 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.62 (s, 4H), 4.38 (d, 2H, $J = 5.6$ Hz), 8.45 (s, 1H), 8.69 (t, 1H, $J = 5.6$ Hz), 11.44 (s, 1H), 11.67 (s, 1H). FAB-MS m/z: 244 $(M+H-HCl)^+$. Anal. Calcd for $C_8H_{10}ClN_5O_2 \cdot HCl \cdot 0.5H_2O$: C, 33.23; H, 4.18; N, 24.22. Found: C, 33.26; H, 4.09; N, 24.13.

5.1.4. 5-Chloro-6-{[(iminoethyl)amino]methyl}uracil hydrochloride (7). A mixture of 500 mg (2.85 mmol) of **3** and 705 mg (5.70 mmol) of ethyl acetimidate hydrochloride in 12 mL of DMF was stirred at room temperature for 14 h. The precipitate was collected by filtration and washed with 10% HCl in MeOH to give 190 mg (26%) of **7** as a white powder: mp 220 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.19 (s, 3H), 4.40 (d, 2H, $J = 5.0$ Hz), 9.13 (s, 1H), 9.63 (s, 1H), 9.86 (t, 1H, $J = 5.0$ Hz), 11.53 (s, 1H), 11.73 (s, 1H). FAB-MS m/z: 217 $(M+H-HCl)^+$. Anal. Calcd for $C_7H_9ClN_4O_2 \cdot HCl \cdot 0.6H_2O$: C, 31.86; H, 4.28; N, 21.23. Found: C, 31.68; H, 4.13; N, 21.49.

5.1.5. 5-Chloro-6-({[(methylamino)thioxomethyl]amino}methyl)uracil (8). A mixture of 500 mg (2.85 mmol) of **3** and 219 mg (3.00 mmol) of methyl isothiocyanate in DMF was heated at 70 °C for 2 h. The reaction mixture was allowed to stand at room temperature and diluted with 50 mL of water. The precipitate was collected by filtration and washed with water and MeOH to give 435 mg (61%) of **8** as a red powder: mp 195 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.82 (s, 3H), 4.52 (s, 2H), 7.86 (s, 2H), 10.90 (s, 1H), 11.60 (s, 1H). FAB-MS m/z: 247 $(M-H)^-$. Anal. Calcd for $C_7H_9ClN_4O_2S \cdot 0.4H_2O$: C, 32.86; H, 3.86; N, 21.89. Found: C, 32.81; H, 3.58; N, 21.83.

5.1.6. Amino{[5-chloro-2,6-dioxo(1,3-dihydropyrimidin-4-yl)]methyl}methylcarboxamidine (10). A mixture of 300 mg (1.58 mmol) of **9** and 661 mg (4.75 mmol) of methylisothiourea sulfate in 10 mL of 0.4 M aqueous KOH was stirred at room temperature for 3 days. An insoluble material was collected by filtration and suspended in 5 mL of 28% aqueous NH_4OH at room temperature for 0.5 h. The precipitate was collected by filtration to give 281 mg (77%) of **4** as a white powder: mp 200 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.00 (s, 3H), 4.25 (s, 2H). FAB-MS m/z: 232 $(M+H)^+$. Anal. Calcd for $C_7H_{10}ClN_5O_2 \cdot H_2O$: C, 33.68; H, 4.84; N, 28.05. Found: C, 33.96; H, 4.97; N, 27.92.

5.1.7. {[5-Chloro-2,6-dioxo(1,3-dihydropyrimidin-4-yl)]methyl}thiocarboxamidine hydrochloride (12). A mixture of 300 mg (1.54 mmol) of **11** and 140 mg (1.84 mmol) of thiourea in EtOH was heated under reflux for 6 h. The precipitate was collected by filtration and washed with EtOH to give 337 mg (81%) of **12** as a white powder: mp 220 °C dec. ^{1}H NMR (DMSO-d_6): δ 4.35 (s, 2H), 9.46 (s, 4H), 11.57 (s, 1H), 11.70 (1H, s). FAB-MS m/z: 235 $(M+H-HCl)^+$. Anal. Calcd for $C_6H_7ClN_4O_2S \cdot HCl$: C, 26.58; H, 2.97; N, 20.66. Found: C, 26.93; H, 3.05; N, 20.31.

5.1.8. 5-Chloro-6-({[(methylamino)thioxomethyl]amino}methyl)uracil hydrochloride (13). Compound **13** was prepared in 93% yield from **11** and *N*-methylthiourea by a method similar to that described for **12**, as a white powder: mp 205 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.90 (s, 3H), 4.32 (s, 2H), 9.40 (s, 1H), 9.77 (s, 1H) 10.20 (s, 1H), 11.51 (s, 1H), 11.67 (s, 1H). FAB-MS m/z: 249 $(M+H-HCl)^+$. Anal. Calcd for $C_7H_9ClN_4O_2S \cdot HCl$: C, 29.49; H, 3.53; N, 19.65. Found: C, 29.61; H, 3.60; N, 19.70.

5.1.9. 5-Chloro-6-(2-imidazolin-2-ylthiomethyl)uracil hydrochloride (14). Compound **14** was prepared in 80% yield from **11** and 2-imidazolidinetaahione by a method similar to that described for **12**, as a white powder: mp 220 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.34 (s, 4H), 4.41 (s, 2H), 10.60 (s, 1H), 11.70 (s, 1H). FAB-MS m/z: 261 $(M+H-HCl)^+$. Anal. Calcd for $C_8H_9ClN_4O_2S \cdot HCl$: C, 32.34; H, 3.39; N, 18.85. Found: C, 32.46; H, 3.40; N, 18.98.

5.1.10. 6-{[2-(Aminoethyl)amino]methyl}-5-bromouracil (17). A mixture of 45.0 g (0.188 mol) of **15** and 56.4 g (0.940 mol) of ethylenediamine in 188 mL of water was stirred at room temperature for 2 h. The precipitate was collected by filtration and washed with water and MeOH to give 22.9 g (46%) of **17** as a white powder: mp 168 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.63–2.75 (m, 4H), 3.58 (s, 2H). FAB-MS m/z: 263, 265 $(M+H)^+$. Anal. Calcd for $C_7H_{11}BrN_4O_2$: C, 31.96; H, 4.21; N, 21.30. Found: C, 31.73; H, 4.31; N, 21.32.

5.1.11. 5-Chloro-6-[(2-iminoimidazolidinyl)methyl]uracil hydrobromide (18). To a solution of 3.42 g (32.3 mmol) of cyanogen bromide in 32 mL of water was added 7.00 g (32.0 mmol) of **16** and stirred for 3 h at room temperature. The reaction mixture was cooled with an ice-bath. The precipitate was collected by filtration and washed with water, DMF, and MeOH to give 3.58 g (34%) of **18** as a beige powder: mp 242 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.49–3.63 (m, 4H), 4.45 (s, 2H), 8.03 (s, 1H), 8.07 (s, 2H), 11.31 (s, 1H), 11.67 (s, 1H). FAB-MS m/z: 244 $(M+H-HBr)^+$. Anal. Calcd for $C_8H_{10}ClN_5O_2 \cdot HBr$: C, 29.61; H, 3.42; N, 21.58. Found: C, 29.67; H, 3.51; N, 21.62.

5.1.12. 5-Bromo-6-[(2-iminoimidazolidinyl)methyl]uracil hydrobromide (19). Compound **19** was prepared in 25% yield from **17** and cyanogen bromide by a method similar to that described for **18**, as a white powder: mp 248 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.50–3.61 (m, 4H), 4.42 (s, 2H), 8.02 (s, 1H), 8.05 (s, 2H), 11.31 (s, 1H), 11.62 (s, 1H). FAB-MS m/z: 288, 290 $(M+H-HBr)^+$.

Anal. Calcd for $C_8H_{10}BrN_5O_2 \cdot HBr$: C, 26.04; H, 3.00; N, 18.98. Found: C, 25.97; H, 3.04; N, 18.91.

5.1.13. 2-Imino-1-methylimidazolidine hydrobromide (23). To a solution of 10.0 g (135 mmol) of **25** in 80 mL of benzene cooling with an ice-bath was added a solution of 15.8 g (149 mmol) of cyanogen bromide in 20 mL of benzene and stirred for 18 h at room temperature. The precipitate was collected by filtration and washed with benzene to give 23.0 g (95%) of **23** as a white powder. ^{1}H NMR (DMSO-d_6): δ 2.90 (s, 3H), 3.45–3.64 (m, 4H), 7.74 (s, 1H), 7.91 (s, 2H). EI-MS (70 eV) m/z: 99 (M^+).

5.1.14. 1-Ethyl-2-iminoimidazolidine hydrobromide (24). Compound **24** was prepared in 99% yield from **21** and cyanogen bromide by a method similar to that described for **23**, as a white powder. ^{1}H NMR (DMSO-d_6): δ 1.09 (t, 3H, $J = 7.3$ Hz), 3.33 (q, 2H, $J = 7.3$ Hz), 3.47–3.66 (m, 4H), 7.73 (s, 1H), 7.91 (s, 2H). EI-MS (70 eV) m/z: 113 (M^+).

5.1.15. 2-Imino-1-isopropylimidazolidine hydrobromide (25). Compound **25** was prepared in 96% yield from **22** and cyanogen bromide by a method similar to that described for **23**, as a beige powder. ^{1}H NMR (DMSO-d_6): δ 1.14 (d, 6H, $J = 6.6$ Hz), 3.45–3.61 (m, 4H), 4.03 (sept, 1H, $J = 6.6$ Hz), 7.70 (s, 1H), 7.91 (s, 2H). EI-MS (70 eV) m/z: 127 (M^+).

5.1.16. 5-Chloro-6-[(2-imino-3-methylimidazolidinyl)-methyl]uracil (26). To a suspension of 1.84 g (10.2 mmol) of **23** in 20 mL of DMF was added 1.04 g (15.4 mmol) of NaOEt and stirred for 0.5 h at room temperature. To the reaction mixture was added 1.00 g (5.12 mmol) of **11** and stirred for 18 h at room temperature. The reaction mixture was diluted with 100 mL of MeOH. The precipitate was collected by filtration and washed with 0.1% aqueous AcOH and MeOH to give 290 mg (22%) of **26** as a brown solid: mp 215 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.92 (s, 3H), 3.57 (s, 4H), 4.27 (s, 2H). FAB-MS m/z: 258 $(M+H)^+$. Anal. Calcd for $C_9H_{12}ClN_5O_2$: C, 41.95; H, 4.69; N, 27.18. Found: C, 41.64; H, 4.75; N, 26.80.

5.1.17. 5-Chloro-6-[(3-ethyl-2-iminoimidazolidinyl)-methyl]uracil (27). Compound **27** was prepared in 11% yield from **24** and **11** by a method similar to that described for **26**, as a beige solid: mp 205 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.11 (t, 3H, $J = 7.3$ Hz), 3.35 (q, 2H, $J = 7.3$ Hz), 3.59 (s, 4H), 4.26 (s, 2H), 9.76 (s, 1H). FAB-MS m/z: 272 $(M+H)^+$. Anal. Calcd for $C_{10}H_{14}ClN_5O_2 \cdot 0.2H_2O$: C, 43.63; H, 5.27; N, 25.44. Found: C, 43.69; H, 5.23; N, 25.51.

5.1.18. 5-Chloro-6-[(2-imino-3-isopropylimidazolidinyl)-methyl]uracil (28). Compound **28** was prepared in 15% yield from **25** and **11** by a method similar to that described for **26**, as a white solid: mp 220 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.15 (d, 6H, $J = 6.3$ Hz), 3.48–3.62 (m, 4H), 4.04 (sept, 1H, $J = 6.3$ Hz), 4.26 (s, 2H). FAB-MS m/z: 286 $(M+H)^+$. Anal. Calcd for $C_{11}H_{16}ClN_5O_2 \cdot 0.8H_2O$: C, 44.02; H, 5.91; N, 23.33. Found: C, 44.07; H, 5.90; N, 23.37.

5.1.19. 2-Amino-1-methylimidazole (31). To a solution of 5.20 g (124 mmol) of cyanamide in 40 mL of 50% aqueous AcOH was added 5.00 g (42.0 mmol) of **29** and heated at 100 °C for 1 h. The reaction mixture was evaporated under a vacuum. The residue was dissolved in 20 mL of concd HCl and heated at 100 °C for 0.25 h. The pH of the reaction mixture was adjusted to 11 by addition of KOH. The reaction mixture was extracted with $CHCl_3$, dried over $MgSO_4$, and evaporated under a vacuum to give 687 mg (17%) of **31** as a yellow oil. ^{1}H NMR (DMSO-d_6): δ 3.29 (s, 3H), 5.20 (s, 2H), 6.31 (d, 1H, $J = 2.6$ Hz), 6.50 (d, 1H, $J = 2.6$ Hz). EI-MS (70 eV) m/z: 97 (M^+).

5.1.20. 2-Amino-1-ethylimidazole (32). Compound **32** was prepared in 41% yield from **30** and cyanamide by a method similar to that described for **31**, as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.33 (t, 3H, $J = 7.3$ Hz), 3.73 (q, 2H, $J = 7.3$ Hz), 6.53 (d, 1H, $J = 2.6$ Hz), 6.65 (d, 1H, $J = 2.6$ Hz). EI-MS (70 eV) m/z: 111 (M^+).

5.1.21. 5-Chloro-6-{[2-imino-3-methyl(4-imidazolinyl)]-methyl}uracil hydrochloride (33). A mixture of 250 mg (1.28 mmol) of **11** and 350 mg (3.61 mmol) of **31** in 10 mL of water was heated at 100 °C for 1 h. The precipitate was collected by filtration and extracted with 2 M aqueous HCl. An insoluble material was filtered out and the filtrate was evaporated under a vacuum. The residue was washed with MeOH to give 80 mg (24%) of **33** as a beige powder: mp 250 °C dec. ^{1}H NMR (DMSO-d_6): δ 3.46 (s, 3H), 5.04 (s, 2H), 7.04 (d, 1H, $J = 2.6$ Hz), 7.08 (d, 1H, $J = 2.6$ Hz), 8.11 (s, 2H), 11.38 (s, 1H), 11.70 (s, 1H). EI-MS (70 eV) m/z: 255 (M^+). Anal. Calcd for $C_9H_{10}ClN_5O_2 \cdot 0.25H_2O$: C, 36.44; H, 3.91; N, 23.61. Found: C, 36.41; H, 3.86; N, 23.55.

5.1.22. 5-Chloro-6-{[3-ethyl-2-imino(4-imidazolinyl)]-methyl}uracil hydrochloride (34). Compound **34** was prepared in 7% yield from **32** and **11** by a method similar to that described for **33**, as a beige powder: mp 235 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.26 (t, 3H, $J = 7.3$ Hz), 3.89 (q, 2H, $J = 7.3$ Hz), 5.06 (s, 2H), 7.07 (d, 1H, $J = 2.6$ Hz), 7.07 (d, 1H, $J = 2.6$ Hz), 8.19 (s, 2H), 11.47 (s, 1H), 11.72 (s, 1H). FAB-MS m/z: 270 $(M+H-HCl)^+$. Anal. Calcd for $C_{10}H_{12}ClN_5O_2 \cdot H_2O$: C, 37.05; H, 4.66; N, 21.60. Found: C, 37.33; H, 4.33; N, 21.52.

5.1.23. 6-[(2-Iminopyrrolidinyl)methyl]thymine hydrochloride (38). To a suspension of 1.00 g (5.73 mmol) of **35** in 10 mL of DMF was added 1.16 g (17.0 mmol) of NaOEt and stirred for 0.5 h at room temperature. To the

reaction mixture was added 1.00 g (5.73 mmol) of **36** and stirred for 16 h at room temperature. The precipitate was collected by filtration, washed with water and MeOH, and extracted with 2 M aqueous HCl. An insoluble material was filtered out and the filtrate was evaporated under a vacuum. The residue was washed with EtOH to give 320 mg (22%) of **38** as a white powder: mp 250 °C dec. ^{1}H NMR (DMSO-d_6): δ 1.76 (s, 3H), 2.02 (quintet, 2H, $J = 7.6$ Hz), 2.84 (t, 2H, $J = 7.6$ Hz), 3.51 (t, 2H, $J = 7.6$ Hz), 4.55 (s, 2H). FAB-MS m/z: 223 $(M+H-HCl)^+$. Anal. Calcd for $C_{10}H_{14}N_4O_2 \cdot HCl \cdot 0.5H_2O$: C, 44.86; H, 6.02; N, 20.93. Found: C, 44.55; H, 6.14; N, 20.72.

5.1.24. 5-Bromo-6-[(2-iminopyrrolidinyl)methyl]uracil hydrochloride (39). Compound **39** was prepared in 13% yield from **15** and **36** by a method similar to that described for **38**, as a white powder: mp 180 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.05 (quintet, 2H, $J = 7.4$ Hz), 2.86 (t, 2H, $J = 7.4$ Hz), 3.59 (t, 2H, $J = 7.4$ Hz), 4.63 (s, 2H), 9.29 (s, 1H), 9.68 (s, 1H), 11.44 (s, 1H), 11.69 (s, 1H). FAB-MS m/z: 287, 289 $(M+H-HCl)^+$. Anal. Calcd for $C_9H_{11}BrN_4O_2 \cdot HCl \cdot 1.25H_2O$: C, 31.23; H, 4.22; N, 16.19. Found: C, 31.23; H, 4.31; N, 16.16.

5.1.25. 5-Chloro-6-[(2-iminopyrrolidinyl)methyl]uracil hydrochloride (2). To a solution of 116 g (0.962 mol) of **35** and 293 g (1.92 mol) of DBU in 1.25 L of MeOH was added 125 g (0.641 mol) of **11** and heated under reflux for 2 h. The precipitate was collected by filtration, washed with MeOH, and dissolved in 0.4 L of 2 M aqueous HCl at 90 °C. To the reaction mixture was added 1.6 L of EtOH and allowed to stand at room temperature, and the precipitate was collected by filtration to give 121.5 g (68%) of **2** as white crystals: mp 245 °C dec. ^{1}H NMR (DMSO-d_6): δ 2.04 (quintet, 2H, $J = 7.6$ Hz), 2.87 (t, 2H, $J = 7.6$ Hz), 3.59 (t, 2H, $J = 7.6$ Hz), 4.69 (s, 2H), 9.40 (s, 1H), 9.75 (s, 1H), 11.46 (s, 1H), 11.73 (s, 1H). FAB-MS m/z: 243 $(M+H-HCl)^+$. Anal. Calcd for $C_9H_{11}ClN_4O_2 \cdot HCl$: C, 38.73; H, 4.33; N, 20.07. Found: C, 38.76; H, 4.38; N, 20.01.

5.2. Biology

5.2.1. Assay of human TP activity. TP was extracted from human placenta. This study was performed as previously described.[17]

5.2.2. Assay of rat UP activity. UP was extracted from rat liver. This study was performed as previously described.[17]

5.2.3. Oral absorption in mice. This study was performed as previously described.[10]

Acknowledgements

We are grateful to Prof. Kei Takeda of Hiroshima University for giving us helpful advice.

We thank Dr. Kazuhiro Kobayashi, Mr. Tomio Yamazaki, and Mr. Toshiharu Mutoh for performing mass spectra and elemental analysis studies.

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