Discovery of 5-Substituted-6-chlorouracils as Efficient Inhibitors of Human Thymidine Phosphorylase

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Thymidine phosphorylase plays an important role in angiogenesis, which is an attractive target for therapy of cancer and other diseases. In our continuous effort to develop novel inhibitors of thymidine phosphorylase, we have discovered that 6-halouracils substituted at position C5 by certain hydrophobic groups exhibit significant inhibitory activity against this enzyme. The most potent compounds bear a five- or six-membered cyclic substituent containing a π-electron system at C5 and a chlorine atom attached at C6. 6-Chloro-5-cyclopent-1-en-1-yluracil **7a** is the most efficient derivative in this study, with $K_i = 0.20 \pm 0.03$ μM ($K_i/^{dThd}K_m = 0.0017$) for thymidine phosphorylase expressed in V79 cells and $K_i = 0.29 \pm 0.04$ μM ($K_i/^{dThd}K_m = 0.0024$) for the enzyme purified from placenta.

Introduction

Angiogenesis is the complex process in which new blood vessels are formed from existing vasculature. The proliferation of a network of blood vessels occurs physiologically during embryogenesis, female menstrual cycle, and wound healing. Nevertheless, angiogenesis is relatively infrequent event in a healthy adult. Thus, increased angiogenic activity could be a promising drug target for several pathological processes, including solid tumor growth and metastasis, rheumatoid arthritis, psoriasis, diabetic retinopathy, and hyperproliferation of vasa vasorum in atherosclerosis. Thymidine phosphorylase (TP)[a] was identified as one of the enzymes that play an important role in this process.[1−3]

TP (identical to platelet-derived endothelial cell growth factor PD-ECGF and gliostatin) was discovered 50 years ago as an enzyme participating in the thymidine salvage pathway. TP catalyzes the cleavage of thymidine into thymine and 2-deoxy-D-ribose-1-phosphate, which is subsequently dephosphorylated to give 2-deoxy-D-ribose. This simple monosaccharide has recently been proved to possess significant chemotactic activity for endothelial cells and to be one of the angiogenesis-inducing factors. The expression of TP is considerably elevated in various solid tumors, as well as in diverse chronic inflammatory diseases, and corresponds roughly with the level of produced 2-deoxy-D-ribose and, thus, with tumor vascularisation or progression of angioproliferative disorders.[1,4] In addition, TP seems to inhibit tumor cell apoptosis.[5] Also, it is involved in degradation of pyrimidine nucleoside analogue drugs such as BVDU [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine] and 2′-deoxy-5-(trifluoromethyl)uridine.[6] Therefore, the search for inhibitors of human TP has attracted considerable attention in recent years.

In the past few years, numerous potential inhibitors of human TP have been identified. Nevertheless, only few structural types can be distinguished. The most potent inhibitors are uracil derivatives bearing a small group at C5 (usually an atom of chlorine or bromine) and an amino or methylene amino group at C6.[7−9] TPI [5-chloro-6-(2-iminopyrrolidin-1-yl)methyl-2,4-(1*H*,3*H*)-pyrimidine] **1** (Figure 1) developed by Taiho Pharmaceutical Co. seems to be the most promising compound, so far.[8]

Other interesting TP inhibitor types include 7-deazaxanthine[10] and 5′-*O*-tritylinosine[11] analogues. Also, specific compounds containing phosphonate group have shown significant inhibitory activity against TP from physiological human tissues or rat SD-lymphoma.[12,13] However, none of the above-mentioned compounds has passed clinical trials, yet.

The positive influence of a hydrophobic group on the inhibitory activity of several uracil derivatives against TP has been known for almost 40 years.[14,15] On the other hand, the hydrophobic substitution at C5 was underestimated and the main attention focused on functionalization at C6 is probably due to the fact that 5-phenyluracil and its homologues showed very modest activity against mammalian TP in early studies.[14] However, practically no attention was paid to the potency of 6-halouracils.

In our study, we report the synthesis and evaluation of a novel class of human TP inhibitors based on 6-chlorouracil having a hydrophobic substituent at C5. An exploration of other functional groups at position C6 was also carried out.

Chemistry

The description of the synthesis of the title compounds was categorized in accordance with whether the change of substituent was accomplished at C5 or C6. The first part was dedicated to

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[a] Abbreviations: %FA, fraction absorption; Ac, acetyl; BisTris, 2-(bis-(2-hydroxyethyl)amino)-2-(hydroxymethyl)propane-1,3-diol; Bn, benzyl; *n*-BuLi, *n*-butyllithium; BVDU, [(*E*)-5-(2-bromovinyl)-2′-deoxyuridine]; DMF, dimethylformamide; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; Et, ethyl; K_i, dissociation equilibrium constant of the inhibitor/enzyme complex; log $D_{6.5}$, decimal logarithm of distribution coefficient at pH 6.5; log *P*, decimal logarithm of partition coefficient; log S_{cal}, calculated decimal logarithm of solubility in mol/L; log S_{exp}, experimentally determined decimal logarithm of solubility in mol/L; $Pd(PPh_3)_4$, tetrakis(triphenylphosphine)palladium(0); PD-ECGF, platelet-derived endothelial cell growth factor; Ph, phenyl; pK_a, minus the decimal logarithm of acid dissociation constants; QM, quantum mechanics; QSAR, quantitative structure-activity relationship; SD-lymphoma, Sprague−Dawley rats lymphoma; THF, tetrahydrofuran; $^{dTmd}K_m$, dissociation equilibrium constant of the thymidine/enzyme complex; TMS, trimethylsilane; TMSI, iodotrimethylsilane; TP, thymidine phosphorylase; TPI, [5-chloro-6-(2-iminopyrrolidin-1-yl)methyl-2,4(1*H*,3*H*)-pyrimidine]; TPSA, topological polar surface area.

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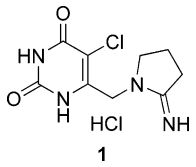

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Figure 1. The structure of TPI.

the 6-chlorouracil derivatives bearing the hydrophobic group at C5, and the second to 5-phenyluracils with diverse functionality at C6.

5-Substituted-6-chlorouracils were prepared by four synthetic routes. All of them started from commercially available 4-chloro-2,6-dimethoxypyrimidine **2** by direct ortho-lithiation with butyllithium at −78 °C.

5-Alkyl-4-chloro-2,6-dimethoxypyrimidines **4a**−**g** were obtained by reaction of lithiated intermediate **3** with appropriate alkyl halide in yields varying from 74 to 92%. The reaction conditions were similar to those described by Cushman and coworkers.[16]

The crucial part of the synthesis of the compounds **5a**−**g** was the hydrolysis of methoxy groups. After screening a variety conditions, e.g., TMSI in CH_2Cl_2, BBr_3 in CH_2Cl_2, and aqueous HBr in AcOH, the best results were obtained by employing 35% aqueous hydrochloric acid either in acetic acid or in a mixture of H_2O/THF/dioxane. Under these conditions, the yields were relatively high and the products were obtained simply by crystallization (Scheme 1).

Indeed, compounds **5a**,[17] **5b**,[17] **5c**,[18] and **5g**[18] have been already reported by different synthetic methods, but their inhibitory potency remained undiscovered. These compounds were included in our study to comprehensively illustrate the structure−activity relationship.

The intermediate alcohols **6a**−**d** were synthesized simply by quenching of the lithiated reaction mixture with the appropriate ketone followed by a slow rise in temperature. By this synthetic procedure we have obtained satisfactory yields of products from cyclohexanone (**6b**) and acetone (**6c**).

However, the results obtained in reactions with cyclopentanone (preparation of **6a**) and 3-pentanone (preparation of **6d**) were rather modest, probably due to increased enolization. With cyclopentanone, this drawback can be partially overcome by addition of cerium(III) chloride. The deprotection step was accomplished again by refluxing derivatives **6a**−**d** with hydrochloric acid either in THF/dioxane or in acetic acid (Scheme 2).

The lithiated intermediate **3** was readily transmetalated with zinc chloride in THF at room temperature to give the corresponding organozinc reagent **8**. Subsequent Negishi couplings, with the use of aryl halides and $Pd(PPh_3)_4$ as the catalyst, were used for syntheses of derivatives bearing aromatic substituents at C5. Compounds **9a**−**f** were obtained in high yields (62−97%) and deprotected to give the final products **10a**−**f** (Scheme 3).

The initial step in the synthesis of 5-[(1*E*)-alk-1-en-1-yl]-4-chloro-2,6-dimethoxypyrimidines **12a**−**c** was the preparation of aldehyde **11**. The previously reported protocol[19] was altered by addition of DMF instead of ethyl formate in order to simplify the removal of the side products by column chromatography. The resulting carbonyl compound **11** reacted smoothly with phosphorus ylides to give the mixtures of *E*/*Z* isomers. The *E*/*Z* ratio was determined by GC (47:53 for **12a**:*Z*-isomer, 47:53 for **12b**:*Z*-isomer, 49:51 for **12c**:*Z*-isomer). The desired *E*-isomers **12a**−**c** were purified by preparative reversed-phase chromatography and finally deprotected by concentrated hydrochloric acid in acetic acid to obtain **13a**−**c** (Scheme 4).

The second part of our work was devoted to the 5-phenyluracil derivatives substituted at C6 by various groups. The uracil bearing the phenyl group at C5 was chosen because of its high stability under diverse chemical conditions and high inhibitory activities against human TPs in the preliminary assay. The 6-methyl analogue was prepared from 5-bromo-2,4-dimethoxy-6-methylpyrimidine.[20] The transformation was started by halogen−lithium exchange by *n*-butyllithium at −78 °C, followed by lithium/zinc transmetalation at room temperature. Subsequent Negishi cross-coupling with iodobenzene was carried out using $Pd(PPh_3)_4$ as catalyst at 60 °C in THF.

The deprotection step accomplished by concentrated hydrochloric acid in THF/dioxane at reflux provided the desired product in a good yield (Scheme 5). Our effort to synthesize 6-fluoro derivative by a route similar that used for preparation of 6-chloro derivative was unsuccessful. Therefore, this product was achieved in a rather classical way, as shown in Scheme 6. In the first step of the sequence, 5-phenylbarbituric acid **17** was chlorinated with $POCl_3$ to afford 2,4,6-trichloro-5-phenylpyrimidine **18** by the procedure introduced by Hendry and coworkers.[21] Further fluorination was performed by potassium fluoride in sulfolane in the presence of 18-crown-6 at 160 °C.

The resulting trifluoro derivative **19** was treated with 2 equiv of lithium benzylate and subsequently deprotected by BBr_3 in dichloromethane to give the desired 6-fluoro-5-phenyluracil **21**. 6-Bromo analogue **23** was obtained by bromination of the barbituric acid **17** followed by basic hydrolysis.

The potential of aromatic nucleophilic substitution of the chlorine atom at C6 was demonstrated by the synthesis of 5-phenyl-6-pyrrolidin-1-yluracil **24** and 6-[(2-aminoethyl)-amino]-5-phenyluracil **25** (Scheme 7). In these cases, pyrrolidine and ethane-1,2-diamine were used as the nucleophiles.

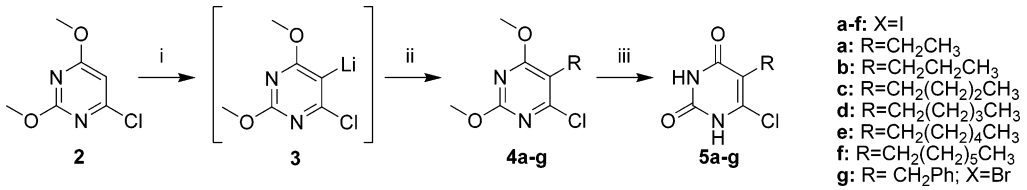
Results and Discussion

All the prepared compounds were evaluated for inhibitory activity against recombinant human TP expressed in V79 Chinese hamster cells and TP purified from human placenta. As shown in the Table 1, most of the presented derivatives possess significant activity against both of the enzymes. Also, the agreement of the results in both enzymatic assays is noteworthy.

The first stage of exploring the impact of the substitution consisted of introducing alkyl groups of various lengths to position C5 of 6-chlorouracil. It is evident that the activity of the homologues rapidly increases from the ethyl to the butyl derivative. Interestingly, when pentyl is attached, remarkable setback is observed. The inhibitory potency is recovered by further elongation of the side chain to heptyl. We assume that one of the possible reasons for such fluctuation could be the limited space in the hydrophobic pocket of the enzyme compensated by more convenient distortion of the side chain in the case of heptyl analogue **5f**.

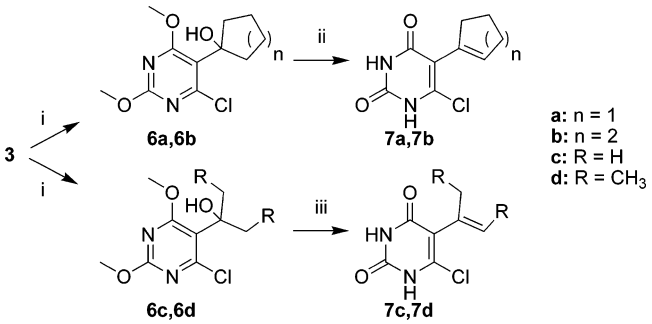
Generally, introduction of the double bond into the side chain results in significant increase of potency. All compounds bearing a linear alkenyl group instead of the corresponding aliphatic chain exhibit dramatically higher inhibitory efficacy. Furthermore, replacement of the linear chain by a cyclic alkenyl ring leads to even more potent derivatives. 6-Chloro-5-cyclopent-1-en-1-yluracil (**7a**) exhibits the highest inhibitory activity against both TPs in this group as well as in the whole study. Apparently, the cyclopentenyl group suitably accommodates the hydrophobic pocket of the enzyme. In contrast, compound **7d**,

Scheme 1[a]



[a] Conditions: (i) *n*-BuLi, THF, −78 °C; (ii) R−X, −78 °C; (iii) concd aq HCl, AcOH, reflux.

Scheme 2[a]



[a] Conditions: (i) ketone, −78 °C to rt; (ii) concd HCl, THF, dioxane, reflux; (iii) concd HCl, AcOH, reflux.

with a branched side chain that closely resembles an "open" cyclopentene, shows at least 2-fold lower activity.

Also, high affinities toward the enzyme (although lower than for **7a**) were shown for compounds **10a** and **10e**, bearing phenyl and thiophenyl substituent at C5, respectively. Any further substitution of the phenyl ring, replacement by a bulkier aromatic group, or insertion of the methylene bridge between uracil and phenyl moiety causes significant loss of activity. The reduction of the potency may be owing to the unfavorable steric interaction with the enzyme. As can be expected, introduction of a more polar, electron-deficient, heteroaromatic group is also unfavorable.

All modifications performed at position C6 resulted in a decrease of inhibitory activity in both tested lines. 6-Fluoro and 6-bromo derivatives exhibit activity 2- and 5-fold lower compared to their 6-chloro counterpart, respectively. The substitution of the chlorine atom by a methyl group causes complete loss of affinity. Surprisingly, introduction of a substituent containing one or two amino functionalities, which was shown to enhance significantly the inhibitory potency of 5-bromouracil derivatives in our previous study,[7] exerts no activity at all.

We have shown that the potency of the presented compounds strongly depends on the hydrophobicity of the substituent at position C5. We have used in silico techniques for better understanding of this hydrophobic interaction. The structure of human TP is now well-known.[24−26] We have used the X-ray structure of human TP in complex with TPI[24] for our docking study. For the first approximation, the docking studies were done using Chem3D Ultra 10.0 software.[27] We have used the implemented dock algorithm with exported distances between uracil moiety and its binding amino acids (H116, K221, S217, R202) of human TP from the X-ray structure of human TP in complex with TPI.[24]

A strong contribution to the tight interactions between human TP and 5-substituted-6-chloruracil comes from the hydrophobic substituent at position C5, which is held in the huge hydrophobic pocked formed by V208, L211, L213, I214, I218, and L251 of the α domain and L75, I78, L148, V232, V234, V241, F242, and L255 of the α/β domain (Figure 2). This multiple hydrophobic interaction explains the high affinity of 5-substituted-6-chloruracils for human TP.

This docking study shows a large hydrophobic pocket (which is theoretically able to accept substituent with 26 carbon atoms), which offers a design opportunity for further development of existing human TP inhibitors based on uracil moiety. QSAR and QM studies are now under way and will be presented soon.

During our study, we have also measured the water solubility and dissociation properties of the leading compound **7a** to predict its possible parenteral applicability. The experimentally determined value log $S_{exp} = -2.46$ is in excellent agreement with the value calculated by ALOGPS 2.1[28,29] software (log $S_{cal} = -2.42$). The acid dissociation constant, pK_a, was determined by UV spectroscopy to be 5.80 ± 0.10. The data show that the compound possesses acceptable water solubility, which can be easily increased by salt formation.[30]

Moreover, we have calculated several descriptors for estimation of the intestinal absorption, an important component of oral bioavailability. Topological polar surface area, TPSA, was calculated by method described by Ertl et al.[31] to be 53.72 Å. The distribution coefficient at pH = 6.5, log $D_{6.5} = 0.40 \pm 0.38$, was predicted from log P calculated by ACD/LogP Freeware 10.05[32] (log $P = 1.18 \pm 0.30$) and the experimental pK_a value by the method of Scherrer and Howard.[33] Hydrogen-bond-donor count is 2, hydrogen-bond-acceptor count is 4, and the number of rotatable bonds is 1.[34] Also, the Lipinski's rule-of-five[35] is not violated for compound **7a**. According to the recent models of Hou et al.[36] and on the basis of our calculations, we assume that the fraction absorption of our compound **7a**, %FA, (defined as total mass of drug absorbed divided by the given dose of drug),[36] will be with high probability more than 30%.

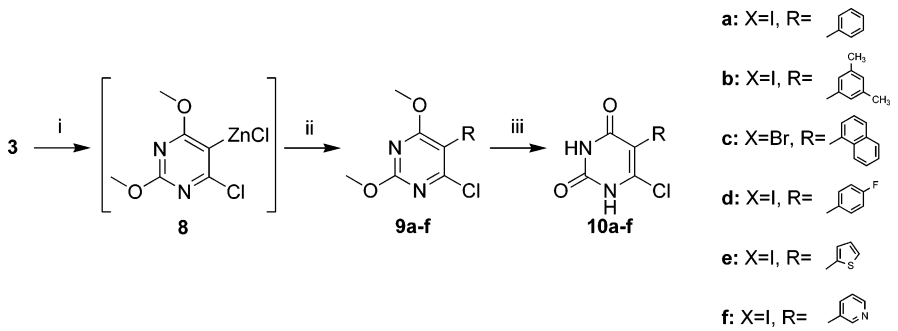
Conclusion

In conclusion, a series of 5-substituted-6-chlorouracil derivatives has been identified to possess significant activity against human thymidine phosporylases. The most effective inhibitor is compound **7a**, which inhibits the enzyme expressed in V79 cells competitively with $K_i = 0.20 \pm 0.03$ μM ($K_i/^{dThd}K_m = 0.0017$) and the enzyme purified from placenta with $K_i = 0.29 \pm 0.04$ μM ($K_i/^{dThd}K_m = 0.0024$). Obviously, the potency of the presented compounds strongly depends on the nature of the substituent at position C5. A cyclic group with a π-electron system seems to be essential for ideal inhibitory activity. Furthermore, our results demonstrate that the efficacy of inhibition of uracil derivatives does not require an amino or related group at position C6. In this manner, our study changes the traditional view on uracil-based TP inhibitors and provides a novel lead for further research.

Experimental Section

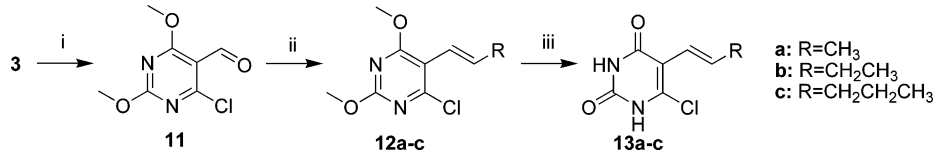
Melting points are uncorrected and were determined on a Kofler block or on a Büchi B-540 melting point apparatus. NMR spectra were recorded on Bruker Avance 500 (^{1}H at 500 MHz, ^{13}C at 125.8 MHz) and Bruker Avance 400 (^{1}H at 400 MHz, ^{13}C at 100.6 MHz)

Scheme 3[a]



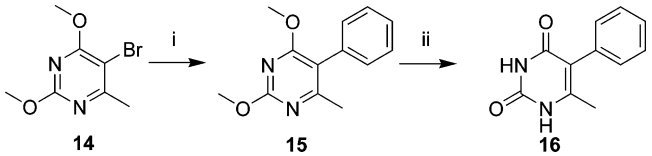
[a] Conditions: (i) $ZnCl_2$, THF, rt; (ii) R−X, $Pd(PPh_3)_4$, THF, 60 °C; (iii) concd HCl, THF, dioxane, reflux.

Scheme 4[a]



[a] Conditions: (i) DMF, −78 °C; (ii) $Ph_3P{=}CH{-}R$, THF, 0 °C; (iii) concd HCl, AcOH, reflux.

Scheme 5[a]



[a] Conditions: (i) (a) *n*-BuLi, THF, −78 °C; (b) $ZnCl_2$, THF, rt; (c) iodobenzene, $Pd(PPh_3)_4$, THF, 60 °C; (ii) concd HCl, THF, dioxane, reflux.

spectrometers with TMS as internal standart or referenced to the residual solvent signal. Mass spectra were measured on a ZAB-EQ (VG Analytical) spectrometer. The chemicals were obtained from commercial sources (Sigma-Aldrich) or prepared according to the published procedures. 4-Chloro-2,6-dimethoxypyrimidine was sublimed under reduced pressure before use. THF was distilled from sodium benzophenone ketyl under argon.

5-Alkyl-4-chloro-2,6-dimethoxypyrimidines, 4a−g. To a solution of 4-chloro-2,6-dimethoxypyrimidine (2 g, 11.5 mmol) in anhydrous THF (30 mL) was slowly added *n*-BuLi (8.5 mL, 13.6 mmol, 1.6 M solution in hexanes) at −78 °C under an argon atmosphere. Stirring was continued for 0.5 h at the same temperature. Alkyl iodide (14 mmol) was then added neat. Temperature was allowed to rise spontaneously to room temperature and stirring was continued for another 16 h. The resulting mixture was hydrolyzed with saturated aqueous NH_4Cl (50 mL) and extracted with Et_2O (3 × 100 mL). The combined organic extract were washed with water (40 mL), dried (Na_2SO_4), and evaporated. Crude products were purified by column chromatography on silica gel or flash chromatography.

2-(4-Chloro-2,6-dimethoxypyrimidin-5-yl)alcohols, 6a−d. Method A. To a solution of 4-chloro-2,6-dimethoxypyrimidine (5.35 mmol) in anhydrous THF (20 mL) was added *n*-BuLi (4 mL, 6.41 mmol, 1.6 M solution in hexanes) at −78 °C under argon atmosphere. The mixture was stirred for 0.5 h, ketone was added via syringe, and stirring was continued for 0.75 h at −78 °C, 2 h at −20 °C, and 2 h at 0 °C. The mixture was partitioned between saturated aqueous NH_4Cl solution (30 mL) and ether (3 × 60 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by chromatography on a silica gel column.

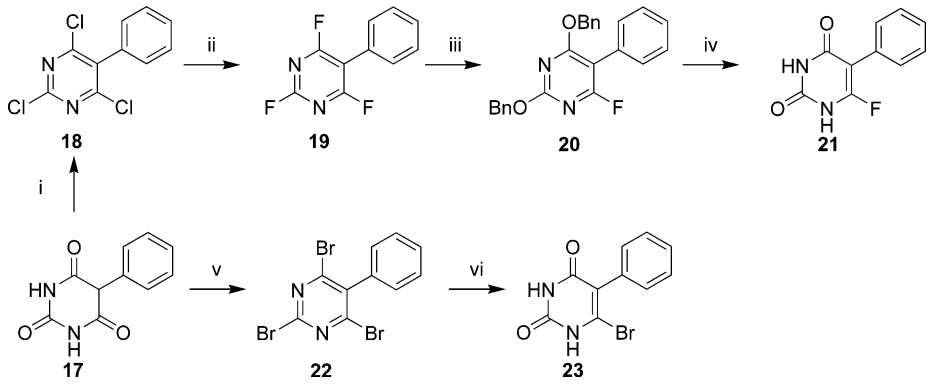
Method B. This method was similar to method A, except that the slow increase of temperature after addition of ketone was followed by additional stirring for 16 h at room temperature.

Method C. $CeCl_3{\cdot}7H_2O$ (6.4 g, 17.2 mmol) was dried for 3 h at 120−130 °C under high vacuum. The reaction flask was evacuated and refilled with argon three times. Anhydrous THF (35 mL) was added followed, after several minutes, by a ketone (11.5 mmol). The resulting mixture was stirred for 15 min and cooled to −78 °C. Concurrently, a solution of 4-chloro-2,6-dimethoxypyrimidine (2 g, 11.5 mmol) in dry THF (27 mL) was treated with *n*-BuLi (8.2 mL, 13.1 mmol, 1.6 M solution in hexanes) for 0.5 h at −78 °C under argon atmosphere and this mixture was subsequently transferred to the flask containing the ketone and $CeCl_3$. This slurry solution was stirred for 1 h at −78 °C, 2 h at −20 °C, 1.5 h at 0 °C, and 16 h at room temperature. The reaction was quenched by saturated aqueous solution of NH_4Cl (50 mL) and extracted with Et_2O (3 × 150 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, and evaporated to dryness. The residue was purified by column chromatography on silica gel.

5-Aryl-4-chloro-2,6-dimethoxypyrimidines, 9a−f. A solution of 4-chloro-2,6-dimethoxypyrimidine (0.7 g, 4 mmol) in anhydrous THF (20 mL) was cooled to −78 °C and treated with *n*-BuLi (3 mL, 4.8 mmol, 1.6 M solution in hexanes). After stirring for 30 min at −78 °C, a solution of dry $ZnCl_2$ (1.36 g, 10 mmol) in THF (10 mL) was added, the dry ice bath was removed, and the mixture was allowed to warm to room temperature and stirred for further 1 h. Then, a degassed solution of $Pd[(C_6H_5)_3P]_4$ (115 mg, 0.1 mmol) and aryl iodide (4.5 mmol) in THF (10 mL) was added via syringe and the resulting mixture was heated to 65 °C for 16 h. The reaction mixture was partitioned between NH_4Cl (50 mL) and Et_2O (3 × 100 mL). The organic layer was washed with a saturated solution of EDTA (40 mL) and water (50 mL), dried (Na_2SO_4), and concentrated in vacuo. Products were purified by silica gel chromatography.

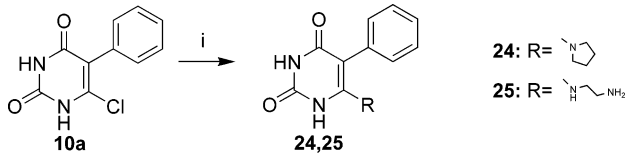
4-Chloro-2,6-dimethoxypyrimidine-5-carbaldehyde, 11. An oven-dried flask was charged with 4-chloro-2,6-dimethoxypyrimidine (1.9 g, 10.9 mmol) and then evacuated and backfilled with argon. Anhydrous THF (40 mL) was added through a rubber septum. The mixture was cooled to −78 °C and a 1.6 M solution of *n*-BuLi in hexanes (7.5 mL, 12 mmol) was added dropwise. The mixture was stirred for an additional 0.5 h. Then, DMF (2 mL, 26 mmol) was added and stirring was continued for 2 h at the same temperature. The reaction was quenched by addition of aqueous HCl (1.6 M, 50 mL) and the mixture was extracted with ether (3 × 70 mL). The combined organic layers were washed with aqueous HCl (1.6 M, 50 mL) and water (40 mL), dried (Na_2SO_4), and evaporated to dryness. The residue was purified by column chromatography on silica gel (ethyl acetate−toluene, 1:6) to afford the pure product (1.5 g, yield 70%). The characterizations of the product fully agreed with those previously reported.[19]

Scheme 6[a]



[a] Conditions: (i) $POCl_3$, *N*,*N*-dimethylaniline, reflux; (ii) KF, sulfolane, 18-crown-6, 160 °C; (iii) BnOLi, THF, −78 °C to rt; (iv) BBr_3, CH_2Cl_2, −78 °C to rt; (v) $POBr_3$, *N*,*N*-dimethylaniline, toluene, reflux; (vi) NaOH, H_2O.

Scheme 7[a]



[a] Conditions: (i) R−H, reflux.

Table 1. Inhibitory Activities of Selected Derivatives against Human TPs

	K_i (μM)[a]	
compd	human TP, expressed in V79 cells	human TP, purified from placenta
5a[b]	>20	>20
5b[b]	5.81 ± 0.82	4.02 ± 0.83
5c[c]	1.03 ± 0.10	1.65 ± 0.34
5d	3.67 ± 1.25	3.09 ± 0.42
5e	1.83 ± 0.39	1.79 ± 0.26
5f	1.06 ± 0.20	1.60 ± 0.25
5g[c]	4.65 ± 0.89	4.55 ± 0.56
7a	0.20 ± 0.03	0.29 ± 0.04
7b	0.42 ± 0.07	0.75 ± 0.10
7c	3.34 ± 0.45	5.10 ± 0.88
7d	0.49 ± 0.06	0.91 ± 0.14
10a	0.40 ± 0.04	0.43 ± 0.09
10b	1.74 ± 0.11	1.27 ± 0.22
10c	4.59 ± 0.99	3.94 ± 0.49
10d	0.71 ± 0.11	0.97 ± 0.18
10e	0.28 ± 0.06	0.54 ± 0.10
10f	3.01 ± 0.59	3.99 ± 0.74
13a	1.17 ± 0.19	1.21 ± 0.20
13b	0.63 ± 0.09	0.92 ± 0.10
13c	0.41 ± 0.06	0.91 ± 0.14
16	>20	>20
21	0.50 ± 0.08	0.47 ± 0.10
23	1.21 ± 0.16	1.21 ± 0.19
24	>20	>20
25	>20	>20
TPI[d]	0.0013 ± 0.00017	0.0021 ± 0.00023

[a] K_m(thymidine) = 121 ± 19 μmol L^{-1}. [b] Reference 17. [c] Reference 18. [d] Reference 8.

5-(Alk-1-en-1-yl)-4-chloro-2,6-dimethoxypyrimidines, 12a−c. The mixture of alkyltriphenylphosphonium bromide (0.64 mmol) and *n*-BuLi (0.4 mL, 0.64 mmol, 1.6 M in hexanes) in THF (5 mL) was stirred for 30 min at 0 °C. Then, the solution of **11** (100 mg, 0.49 mmol) in THF was added and this mixture was stirred for an additional 2 h at the same temperature. The reaction was quenched by addition of aqueous NH_4Cl (5 mL). Extraction with ether (3 × 15 mL) and drying of organic layer over Na_2SO_4 was followed by evaporation to dryness. Chromatography on silica gel (petroleum ether−ethyl acetate, 10:1) afforded mixtures of *E* and *Z* isomers. The *E* isomers were isolated by reversed-phase HPLC.

General Methods for Deprotection of Final Uracil Derivatives 5a−g, 7a−d, 10a−f, and 13a−c. Method I. A mixture of the appropriate dimethoxypyrimidine (1 mmol) in concentrated hydrochloric acid (2 mL) and of acetic acid (2 mL) was refluxed for 2 h. The cooled slurry was treated with water (2 mL), heated to the reflux for 1−2 min, and then refrigiated for 2 h. The resulting precipitate was filtered and washed with water and ether.

Method II. A mixture of the appropriate dimethoxypyrimidine (1 mmol) in concentrated hydrochloric acid (2 mL), THF (1.5 mL), and dioxane (1.5 mL) was heated to reflux for 2 h. The resulting mixture was cooled and half of the volume was removed by evaporation. Water (2 mL) was then added. The mixture was boiled for 1−2 min, and after refrigerating for 2 h, the crystals were filtered and washed with water and ether.

6-Chloro-5-ethylpyrimidine-2,4(1*H*,3*H*)-dione, 5a, was prepared by method I: yield 82%, white crystals; mp 216 °C (lit.[17] mp 227−228 °C); ^{1}H NMR (DMSO-d_6) δ 0.96 (t, 3H, J = 7.4 Hz), 2.30 (q, 2H), 11.30 (s, 1H), 11.80 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 12.75 (C2′), 18.78 (C1′), 111.55 (C5), 140.81(C6), 149.80 (C2), 162.87(C4). Anal. ($C_6H_7ClN_2O_2$) C, H, N.

6-Chloro-5-propylpyrimidine-2,4(1*H*,3*H*)-dione, 5b, was prepared by method I: yield 84%, white crystals; mp 221 °C (lit.[17] mp 238−240 °C); ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, J = 7.4 Hz), 1.40 (sext, 2H, J = 7.5 Hz), 2.26 (bt, 2H, J = 7.5 Hz), 11.30 (s, 1H), 11.80 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 13.78 (C3′), 21.18 (C2′), 27.19 (C1′), 110.14 (C5), 141.22 (C6), 149.83 (C2), 163.09-(C4). Anal. ($C_7H_9ClN_2O_2$) C, H, N.

5-Butyl-6-chloropyrimidine-2,4(1*H*,3*H*)-dione, 5c, was prepared by method I: yield 88%, white crystals; mp 194−196 °C (lit.[18] mp 197−198 °C); ^{1}H NMR (DMSO-d_6) δ 0.86 (t, 3H, J = 7.4 Hz), 1.28 (m, 2H), 1.35 (m, 2H), 2.29 (bt, 2H, J = 7.6 Hz), 11.29 (s, 1H), 11.80 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 13.96 (C4′), 22.04 (C3′), 24.97 (C2′), 30.07 (C1′), 110.32 (C5), 141.15 (C6), 149.85 (C2), 163.07 (C4). Anal. ($C_8H_{11}ClN_2O_2$) C, H, N.

6-Chloro-5-pentylpyrimidine-2,4(1*H*,3*H*)-dione, 5d, was prepared by method I: yield 94%, white crystals; mp 191−192 °C (water); ^{1}H NMR (DMSO-d_6) δ 0.87 (t, 3H, J = 7.5 Hz), 1.25 (m, 4H), 1.37 (m, 2H), 2.28 (bt, 2H, J = 7.5 Hz), 11.30 (s, 1H), 11.80 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 14.06 (C5′), 22.10 (C4′), 25.18 (C3′), 27.51 (C2′), 31.06 (C1′), 110.39 (C5), 141.05 (C6), 149.82 (C2), 163.06 (C4). Anal. ($C_9H_{13}ClN_2O_2$) C, H, N.

6-Chloro-5-hexylpyrimidine-2,4(1*H*,3*H*)-dione. 5e, was prepared by method I: yield 89%, white crystals; mp 186 °C (water); ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 7.0 Hz), 1.25 (m, 6H), 1.36 (m, 2H), 2.28 (t, 2H, J = 7.6 Hz), 11.29 (s, 1H), 11.80 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 14.11 (C6′), 22.20 (C5′), 25.23 (C4′), 27.80 (C3′), 28.52 (C2′), 31.06 (C1′), 110.34 (C5), 141.11 (C6), 149.825 (C2), 163.05 (C4). Anal. ($C_{10}H_{15}ClN_2O_2$) C, H, N.

6-Chloro-5-heptylpyrimidine-2,4(1*H*,3*H*)-dione, 5f, was prepared by method I: yield 92%, white crystals; mp 178−179 °C (water); ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, J = 7.4 Hz), 1.25 (m, 8H), 1.36 (m, 2H), 2.28 (bt, 2H, J = 7.6 Hz), 11.30 (s, 1H), 11.80

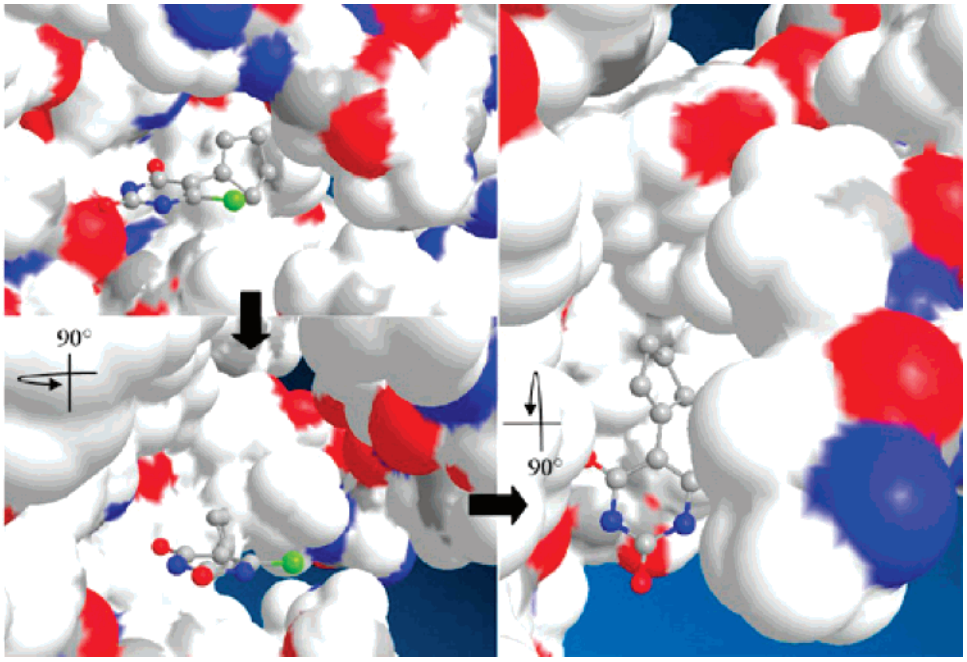


Figure 2. Docking model of **7a** to the active site of human TP; molecular surface of human TP (H, white; C, gray; N, blue; O, red) and **7a** in ball and stick model (C, gray; N, blue; O, red; Cl, green).

(bs, 1H); ^{13}C NMR (DMSO-d_6) δ 14.15 (C7′), 22.26 (C6′), 25.24 (C5′), 27.85 (C4′), 28.67 (C3′), 28.84 (C2′), 31.41 (C1′), 110.34 (C5), 141.16 (C6), 149.86 (C2), 163.07 (C4). Anal. ($C_{11}H_{17}ClN_2O_2$) C, H, N.

5-Benzyl-6-chloropyrimidine-2,4(1*H*,3*H*)-dione, 5g, was prepared by method II: yield 97%, white crystals; mp 247 °C (lit.[18] mp 235−236 °C); ^{1}H NMR (DMSO-d_6) δ 3.65 (s, 2H), 7.20 (m, 3H), 7.25 (m, 2H), 11.42 (s, 1H), 11.95 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 31.05 (CH_2), 109.76 (C5), 126.59 (C4′), 128.42 (2C, C3′), 128.78 (2C, C2′), 139.34 (C1′), 142.81 (C6), 150.08 (C2), 163.45 (C4). Anal. ($C_{11}H_9ClN_2O_2$) C, H, N.

6-Chloro-5-cyclopent-1-en-1-ylpyrimidine-2,4(1*H*,3*H*)-dione, 7a, was prepared by method II: yield 49%, pale orange crystals; mp 276−277 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 1.86 (pent, 2H, J = 7.4 Hz), 2.39 (m, 2H), 2.47 (m, 2H), 5.89 (pent, 1H, J = 2.2 Hz), 11.46 (s, 1H), 12.06 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 23.09 (C4′), 32.71 (C5′), 34.86 (C3′), 109.08 (C5), 133.00 (C2′), 134.11 (C1′), 140.93 (C6), 149.59 (C2), 161.99 (C4). Anal. ($C_9H_9ClN_2O_2$) C, H, N.

6-Chloro-5-cyclohex-1-en-1-ylpyrimidine-2,4(1*H*,3*H*)-dione, 7b, was prepared by method II: yield 76%, white crystals; mp 279 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 1.54 (m, 2H), 1.61 (m, 2H), 2.00 (m, 2H), 2.06 (m, 2H), 5.57 (sept, 1H, J = 1.7 Hz), 11.30 (s, 1H), 11.87 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 21.65(C5′), 22.44 (C4′), 25.03 (C3′), 27.62 (C6′), 114.02 (C5), 129.61 (C2′), 129.96 (C1′), 140.98 (C6), 149.79 (C2), 162.23 (C4). Anal. ($C_{10}H_{11}ClN_2O_2$) C, H, N.

6-Chloro-5-(1-methylethenyl)pyrimidine-2,4(1*H*,3*H*)-dione, 7c, was prepared by method II: yield 44%, white crystals; mp 256 °C (water); ^{1}H NMR (DMSO-d_6) δ 1.84 (bt, 3H, J = 1.3 Hz), 4.90 (dq, 1H, J = 1.0 Hz, J_{gem}= 2.0 Hz), 5.26 (bpent, 1H, $J \sim J_{gem}$ = 2.0 Hz), 5.57 (sept, 1H, J = 1.7 Hz), 11.30 (s, 1H), 11.87 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 22.26 (CH_3), 113.44 (C5), 119.12 (=CH_2), 136.73 (*C*=), 140.85 (C6), 149.77 (C2), 161.88 (C4). Anal. ($C_7H_7ClN_2O_2$) C, H, N.

6-Chloro-5-[(1*E*)-1-ethylprop-1-en-1-yl]pyrimidine-2,4(1*H*,3*H*)-dione, 7d, was prepared by method II: yield 40%, white crystals; mp 226−229 °C (water); ^{1}H NMR (DMSO-d_6) δ 0.84 (t, 3H, J = 7.6 Hz), 1.67 (d, 3H, J = 6.8 Hz), 2.21 (bq, 2H, J = 7.6 Hz), 5.33 (qt, 1H, $J \leq$ 1.0 Hz), 11.29 (s, 1H), 11.87 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 12.46, 13.39, 22.68, 113.76 (C5), 126.93 (=CH), 133.74 (*C*=), 141.71 (C6), 149.86 (C2), 162.47 (C4). Anal. ($C_9H_{11}ClN_2O_2$) C, H, N.

6-Chloro-5-phenylpyrimidine-2,4(1*H*,3*H*)-dione, 10a, was prepared by method I (yield 77%) or method II (yield 91%), white crystals; mp 310 °C (water); ^{1}H NMR (DMSO-d_6) δ 7.27 (m, 2H), 7.38 (m, 3H), 11.49 (s, 1H), 12.10 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 112.04 (C5), 127.94, 128.09 (2C), 130.92 (2C), 131.95, 142.32 (C6), 149.82 (C2), 162.49 (C4). Anal. ($C_{10}H_7ClN_2O_2$) C, H, N.

6-Chloro-5-(3,5-dimethylphenyl)pyrimidine-2,4(1*H*,3*H*)-dione, 10b, was prepared by method II: yield 93%, white crystals; mp 288 °C (water); ^{1}H NMR (DMSO-d_6) δ 2.26 (q, 6H, J = 0.8 Hz), 6.84 (dt, 2H, J = 0.8 Hz, J = 1.7 Hz), 6.96 (sept, 1H, J = 0.8 Hz), 11.45 (s, 1H), 12.07 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 21.04 (2C) 112.25 (C5), 128.46 (2C), 129.41, 131.80, 137.05 (2C), 142.11 (C6), 149.81 (C2), 162.51 (C4). Anal. ($C_{12}H_{11}ClN_2O_2$) C, H, N.

6-Chloro-5-(2-naphthyl)pyrimidine-2,4(1*H*,3*H*)-dione, 10c, was prepared by method II: yield 82%, white crystals; mp >325 °C (water); ^{1}H NMR (DMSO-d_6) δ 7.37 (m, 1H), 7.52 (m, 3H), 7.73 (m, 1H), 7.96 (m, 2H), 11.53 (s, 1H), 12.25 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 110.45 (C5), 125.35, 125.68, 126.13, 126. 52, 128.39, 128.68, 129.15, 130.07, 132.08, 133.40, 143.64 (C6), 150.22 (C2), 162.66 (C4). Anal. ($C_{14}H_9ClN_2O_2$) C, H, N.

6-Chloro-5-(4-fluorophenyl)pyrimidine-2,4(1*H*,3*H*)-dione, 10d, was prepared by method II: yield 89%, white crystals; mp 298−299 °C (water); ^{1}H NMR (DMSO-d_6) δ 7.21 (m, 2H), 7.32 (m, 2H), 11.51 (s, 1H), 12.15 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 111.13 (C5), 115.03 (d, 2C, J_{CF} = 21.5 Hz, C3′), 128.02 (d, J_{CF} = 3.2 Hz, C1′), 133.07 (d, 2C, J_{CF} = 8.3 Hz, C2′), 142.48 (C6), 149.77 (C2), 161.83 (d, J_{CF} = 3.2 Hz, C4′), 162.47 (C4). Anal. ($C_{10}H_6ClFN_2O_2$) C, H, N.

6-Chloro-5-(2-thienyl)pyrimidine-2,4(1*H*,3*H*)-dione, 10e, was prepared by method II: yield 70%, white crystals; mp 291−292 °C (water, EtOAc/EtOH); ^{1}H NMR (DMSO-d_6) δ 7.19 (dd, 1H, J = 3.6 Hz, J = 5.2 Hz), 7.34 (dd, 1H, J = 1.2 Hz, J = 3.6 Hz), 7.71 (dd, 1H, J = 1.2 Hz, J = 5.2 Hz), 11.72 (s, 1H), 12.39 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 105.82 (C5), 126.47 (C5′), 127.43 (C4′), 129.22 (C3′), 131.72 (C2′), 142.88 (C6), 149.29 (C2), 161.97 (C4). Anal. ($C_8H_5ClN_2O_2S$) C, H, N.

6-Chloro-5-pyridin-3-ylpyrimidine-2,4(1*H*,3*H*)-dione Hydrochloride, 10f. The compound was prepared by method I, except the 2 mL of water was not added after refluxing and the crystalline product was filtered directly from the cooled reaction mixture and washed with EtOH and ether: yield 76%, white crystals; mp >325 °C; ^{1}H NMR (DMSO-d_6) δ 8.02 (dd, 1H, J = 5.0 Hz, J = 7.8 Hz), 8.44 (d, 1H, J = 7.8 Hz), 8.85 (m, 2H), 11.80 (bs, 1H),

12.00 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 106.94 (C5), 126.28 (C5′), 130.96 (C3′), 142.53 (C4′), 144.36 (C6), 144.78 (C6′), 146.34 (C2′), 149.60 (C2), 161.99 (C4). Anal. ($C_9H_6ClN_3O_2$. HCl) C, H, N.

6-Chloro-5-[(1*E*)-prop-1-en-1-yl]pyrimidine-2,4(1*H*,3*H*)-dione, 13a, was prepared by method I: yield 43%, pale beige crystals; mp 268 °C (water); ^{1}H NMR (DMSO-d_6) δ 1.79 (dd, 3H, J = 1.7 Hz, J = 6.8 Hz,), 6.09 (dq, 1H, J = 1.7 Hz, J = 15.7 Hz), 6.73 (dq, 1H, J = 6.8 Hz, J = 15.7 Hz), 11.38 (s, 1H), 12.04 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 19.55 (C3′), 107.12 (C5), 120.74 (C2′), 130.11 (C1′), 140.93 (C6), 149.05 (C2), 162.09 (C4). Anal. ($C_7H_7ClN_2O_2$) C, H, N.

5-[(1*E*)-But-1-en-1-yl]-6-chloropyrimidine-2,4(1*H*,3*H*)-dione, 13b, was prepared by method I: yield 59%, beige crystals; mp 248 °C (water); ^{1}H NMR (DMSO-d_6) δ 0.99 (t, 3H, J = 1.7 Hz), 1.43 (m, 2H), 6.07 (dt, 1H, J = 1.2 Hz, J = 15.8 Hz), 6.79 (dt, 1H, J = 6.7 Hz, J = 15.8 Hz), 11.38 (bs, 1H), 12.05 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 13.72 (C4′), 26.77 (C3′), 107.06 (C5), 118.68 (C2′), 136.667 (C1′), 141.14 (C6), 149.04 (C2), 162.08 (C4). Anal. ($C_8H_9ClN_2O_2$) C, H, N.

6-Chloro-5-[(1*E*)-pent-1-en-1-yl]pyrimidine-2,4(1*H*,3*H*)-dione, 13c, was prepared by method I: yield 55%, white crystals; mp 231 °C (water); ^{1}H NMR (DMSO-d_6) δ 0.88 (t, 3H, J = 7.3 Hz), 1.40 (sext, 2H, J = 7.3 Hz), 2.10 (qd, 2H, J = 1.5 Hz, J = 7.2 Hz), 6.08 (dt, 1H, J = 1.5 Hz, J = 15.7 Hz), 6.74 (dt, 1H, J = 7.2 Hz, J = 15.7 Hz), 11.38 (s, 1H), 12.05 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 13.75 (C5′), 22.21 (C4′), 35.84 (C3′), 107.015 (C5), 119.71 (C2′), 135.02 (C1′), 141.11 (C6), 149.03 (C2), 162.07 (C4). Anal. ($C_9H_{11}ClN_2O_2$) C, H, N.

2,4-Dimethoxy-6-methyl-5-phenylpyrimidine, 15. In a flame-dried flask, a 1.6 M solution of *n*-BuLi in hexanes (4.3 mL, 6.9 mmol) was added dropwise to a solution of **14**[20] (1.34 g, 5.57 mmol) in THF (30 mL) at −78 °C under argon atmosphere. The mixture was stirred for 30 min. Then a solution of $ZnCl_2$ (1.8 g, 13.2 mmol) in THF (10 mL) was added, and the mixture was allowed to warm to room temperature and stirred for an additional 1 h. The resulting mixture was treated with a solution of phenyl iodide (1.4 g, 0.78 mL, 6.9 mmol) and $Pd(PPh_3)_4$ (133 mg, 0.11 mmol) in THF (10 mL) at 60 °C for 16 h. The mixture was diluted saturated aqueous NH_4Cl (25 mL) and extracted with ether (3 × 75 mL). The extract was washed with brine (45 mL) and concentrated under reduced pressure. Chromatography on silica gel (petroleum ether−ethyl acetate, 10:1) afforded **15** (356 mg, 28%) as white crystals; mp 92 °C (cyclohexane). The analytical sample was sublimed under reduced pressure.

6-Methyl-5-phenylpyrimidine-2,4(1*H*,3*H*)-dione, 16. The mixture of **15** (184 mg, 0.8 mmol), THF (1 mL), dioxane (1 mL), and concentrated aqueous HCl (1.3 mL) was heated to reflux for 3.5 h, cooled down, and refrigerated for 30 min. The precipitate was collected by filtration, washed with water, and ether/petroleum ether mixture and dried to give 131 mg of analytically pure white crystals: yield 81%; mp 323−325 °C; ^{1}H NMR (DMSO-d_6) δ 1.93 (s, 3H), 7.20 (d, 2H), 7.30 (t, 1H), 7.36 (t, 2H) 10.93 (bs, 1H), 11.10 (bs, 1H); ^{13}C NMR (DMSO-d_6) δ 22.40 (CH_3), 111.77 (C5), 127.50, 128.31 (2C), 131.33 (2C), 133.83, 149.53 (C6), 151.19 (C2), 163.98 (C4). Anal. ($C_{11}H_{10}N_2O_2$) C, H, N.

2,4,6-Trifluoro-5-phenylpyrimidine, 19. Under reduced pressure (5 mBar) at 140 °C, the solvent (2 mL) was removed from a mixture of KF (1.47 g, 25.30 mmol) and sulfolane (22 mL). Subsequently, compound **18**[21] (2 g, 7.7 mmol) and 18-crown-6 (150 mg) were added. The resulting mixture was heated to 160 °C for 5 h, cooled down, and partitioned between water (75 mL) and petroleum ether (100 mL). Aqueous phase was extracted with ether (2 × 200 mL). The combined organic phases were washed with water (3 × 400 mL), dried over Na_2SO_4, and evaporated. Chromatography on silica gel (hexanes−toluene, 20:3) afforded **19** as colorless oil (720 mg, 44%).

2,4-Bis(benzyloxy)-6-fluoro-5-phenylpyrimidine, 20. The mixture of benzyl alcohol (432 mg, 3.99 mmol), THF (5 mL), and 1.6 M *n*-BuLi in hexanes (2.5 mL, 3.99 mmol) prepared at −78 °C was added to a solution of **19** (400 mg, 1.9 mmol) in THF (10 mL) over 40 min at the same temperature. The resulting mixture was stirred for 2 h at −78 °C and 16 h at room temperature. The reaction was quenched with saturated NH_4Cl (25 mL) and extracted with ethyl acetate (3 × 75 mL). The organic phase was dried (Na_2SO_4), evaporated in vacuo, and chromatographed on silica gel (petroleum ether−EtOAc, 20:1). Crystallization from cyclohexane gave 528 mg of **20** as white needles (yield 72%; mp 87−88 °C).

6-Fluoro-5-phenylpyrimidine-2,4(1*H*,3*H*)-dione, 21. A solution of BBr_3 (3 mL, 1 M in CH_2Cl_2) was added to a solution of **20** (180 mg, 0.47 mmol) in CH_2Cl_2 (5 mL) at −78 °C. The mixture was stirred overnight at room temperature and partitioned between aqueous saturated $NaHCO_3$ (50 mL) and ethyl acetate (75 mL). Aqueous phase was acidified by diluted aqueous HCl and extracted with ethyl acetate (3 × 75 mL). The organic layers were combined, dried (Na_2SO_4), and evaporated under reduced pressure. Chromatography on silica gel (EtOAc−acetone−EtOH−H_2O, 17:3:3:2) afforded 30 mg of product **21**: yield 31%; mp 282 °C (water); ^{1}H NMR (DMSO-d_6) δ 7.35 (m, 5H) 11.40 (bs, 2H); ^{13}C NMR (DMSO-d_6) δ 94.55 (d, J_{CF} = 10.9 Hz, C5), 127.58, 128.48 (d, J_{CF} = 26.0 Hz), 130.33 (2C), 148.94 (d, J_{CF} = 11.4 Hz, C2), 159.30 (d, J_{CF} = 266.4 Hz, C6), 164.00 (d, J_{CF} = 15.1 Hz, C4). Anal. ($C_{10}H_7FN_2O_2$) C, H, N.

2,4,6-Tribromo-5-phenylpyrimidine, 22. In a flame-dried flask flushed with argon, the 5-phenylbarbituric acid **17**[21] (2.4 g, 11.75 mmol) and $POBr_3$ (13.45 g, 47 mmol) were mixed with toluene (23 mL, freshly distilled from sodium) and *N*,*N*-dimethylaniline (2.7 mL, 21.3 mmol), and the mixture was refluxed for 6 h. The mixture was poured onto crushed ice and extracted with ether (3 × 100 mL). The organic phase was dried (Na_2SO_4) and evaporated to dryness. Crystallization from ethyl acetate afforded 2.21 g of **22** as pale yellow crystals (yield 48%; mp 208 °C). Further product was recovered from mother liquors by chromatography on silica gel (petroleum ether−ethyl acetate, 20:1) (0.51 g, 11%).

6-Bromo-5-phenylpyrimidine-2,4(1*H*,3*H*)-dione, 23. The finely powdered compound **22** (500 mg, 1.27 mmol) was refluxed with NaOH (0.21 g, 5.25 mmol) in H_2O (3.5 mL) for 1.5 h. The resulting solution was cooled, neutralized with diluted hydrochloric acid, and adsorbed onto silica gel. Chromatography on silica gel (ethyl acetate−toluene, 20:3) and subsequent crystallization from aqueous ethanol afforded 73 mg of **23** as white crystals: yield 22%; mp 308−309 °C; ^{1}H NMR (DMSO-d_6) δ 7.23 (m, 2H), 7.33 (m, 1H), 7.38 (m, 2H), 11.46 (s, 1H), 12.02 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 115.17 (C5), 127.88 (C4′), 128.05 (2C, C3′), 130.93 (2C, C2′), 133.89 (C1′), 133.98 (C6), 150.13 (C2), 161.86 (C4). Anal. ($C_{10}H_7BrN_2O_2$) C, H, N.

5-Phenyl-6-pyrrolidin-1-ylpyrimidine-2,4(1*H*,3*H*)-dione, 24. The mixture of **10a** (676 mg, 3.03 mmol) and pyrrolidine (10 mL) was refluxed for 6 h. After evaporation to dryness, ethyl acetate (15 mL) was added and the suspension was heated to reflux for 1 min. Then, the mixture was diluted by EtOH (5 mL) and the heating was repeated. The precipitate was filtrated, suspended in water (5 mL), and boiled for 1 min. The crystalline product was collected and recrystallized from methanol to give 647 mg of **24** as white crystals: yield 83%; mp 311 °C; ^{1}H NMR (DMSO-d_6) δ 1.62 (m, 8H), 2.96 (m, 4H), 7.15 (m, 3H), 7.24 (m, 2H); ^{13}C NMR (DMSO-d_6) δ 89.71 (C5), 125.54 (C1′), 127.08 (2C, C3′), 132.50 (2C, C2′), 136.52 (C1′), 151.86 (C2), 152.74 (C6), 164.17 (C4). Anal. ($C_{14}H_{15}N_3O_2$) C, H, N.

6-[(2-Aminoethyl)amino]-5-phenylpyrimidine-2,4(1*H*,3*H*)-dione, 25. A mixture of **10a** (500 mg, 2.25 mmol) and ethylenediamine (6 mL) was heated to reflux for 5 h. The mixture was left to stand overnight at room temperature. The precipitate was collected by filtration, washed with EtOH, and recrystallized from water to give 467 mg of **25** as white crystals: yield 84%; mp 251 °C; ^{1}H NMR (DMSO-d_6) δ 2.26 (m, 2H), 3.15 (m, 2H), 5.97 (bs, 2H), 7.20 (m, 1H), 7.22 (m, 2H), 7.32 (m, 2H), 9.60−10.20 (bs, 3H); ^{13}C NMR (DMSO-d_6) δ 41.69, 44.68, 88.03 (C5), 125.97, 128.31 (2C), 131.74 (2C), 134.37 (C1), 152.61 (C2), 154.92 (C6), 163.21 (C4). Anal. ($C_{12}H_{14}N_4O_2 \cdot H_2O$) C, H, N.

Thymidine Phosphorylases. The recombinant human thymidine phosphorylase (V79TP) expressed in V79 Chinese hamster cells

was a commercial product (Sigma, T-9319) and the enzyme from human placenta (hpTP) was purified using a combination of described procedures.[22,23]

Enzyme Assay. The standard reaction mixture (50 μL) contained 20 mM BisTris-HCl (pH 6.4), 1 mM EDTA, 2 mM DTT, 200 μM potassium phosphate (pH 6.7), various concentration of [^{3}H-methyl]-thymidine, and tested compounds. The reaction was started by the addition of 44 nU of enzyme, incubated at 37 °C for 8 min, and stopped by spotting a 2 μL aliquot onto a silica gel 60 F_{254} plate that had been prespotted with 0.01 μmol of each thymine and thymidine. The plate was developed in the nonaqueous phase of the solvent system ethyl acetate−water−formic acid (60:35:5). The spots were visualized under UV light (254 nm) and cut out for radioactivity determination in toluene-based scintillation cocktail.

Kinetic constants K_m and K_i were determined from the Lineweaver−Burk and Dixon plots. Data based on results from at least four independent experiments were evaluated by the nonlinear regression method (GOSA, Bio-Log).

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Supporting Information Available: Spectroscopic data for new compounds and elemental analysis results. This material is available free of charge via the Internet at http://pubs.acs.org.

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