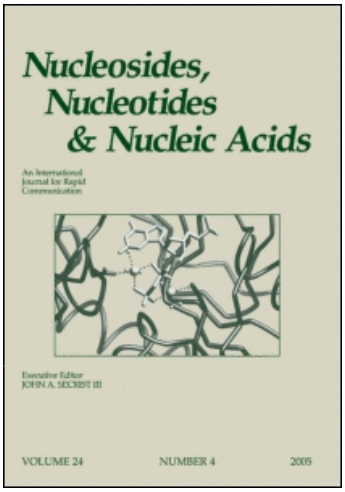

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Inhibitory Potency of 5-Benzyluracil, 5-Phenylcytosine and 5-Phenylpyrimidin-2-one Nucleosides Against Uridine Phosphorylase from Mouse Leukemic L1210 Cells

Ivan Votruba[a]; Marcela Krečmerová[a]; Hubert Hřebabecký[a]; Antonín Holý[a]
[a] Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Praha, (Czech Republic)

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NUCLEOSIDES & NUCLEOTIDES, 18(11&12), 2551–2564 (1999)

INHIBITORY POTENCY OF 5-BENZYLURACIL, 5-PHENYLCYTOSINE AND 5-PHENYLPYRIMIDIN-2-ONE NUCLEOSIDES AGAINST URIDINE PHOSPHORYLASE FROM MOUSE LEUKEMIC L1210 CELLS

Ivan Votruba*, Marcela Krečmerová, Hubert Hřebabecký and Antonín Holý

Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, 16610 Praha (Czech Republic).

ABSTRACT: The inhibitory activity of a series of novel sugar-modified nucleosides derived from 5-benzyluracil, 5-phenylcytosine and 5-phenylpyrimidin-2-one against uridine phosphorylase purified from mouse leukemic L-1210 cells was investigated. Significant activity was encountered with $O^{2,2'}$-anhydro-5-benzylcytidine hydrochloride, 2',3'-dideoxy-5-benzyluridine, 2',3'-dideoxy-4-thiouridine and α- and β-anomers of 5-benzyl-1-(2-deoxy-D-*arabino*-hexopyranosyl)uracil.

Introduction

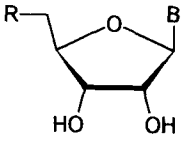
Uridine phosphorylase (EC 2.4.2.3) plays a crucial role in the degradation of pyrimidine nucleosides as well as in the salvage pathways which supply precursors for the nucleic acids synthesis *de novo*. This double role in cell metabolism is strategic particularly in neoplastic tissues wherein the enzyme level can be increased by the action of cytokines and growth factors.[1] On one hand, uridine phosphorylase causes an undesirable phosphorolysis of pyrimidine nucleoside analogues used in cancer chemotherapy; thus, its inhibitors can be useful in promoting such therapy. On the other hand, the inhibition of uridine phosphorylase delays the disappearance of uridine from the cell pool and enhances the therapeutic effects of 5-fluorouracil in transformed cells by lowering its toxicity in normal tissues.[2] Thus, the rational design of uridine phosphorylase inhibitors has two aspects: (a) development of novel pyrimidine or pyrimidine nucleoside analogs with cytostatic activity and/or (b) potentiation of the pharmacological effect of known pyrimidine antimetabolites. One of the established

inhibitors, benzylacyclouridine (BAU, IND 039655) in combination with 5-fluorouracil, reached the clinical Phase II treatment of cancer patients.

It was also demonstrated that uridine phosphorylase has a key role in the metabolism of pathogenic protozoal parasites, e.g. *Trypanosoma* sp.,[3] *Toxoplasma* sp.[4] and/or *Schizostoma* sp.[5] These enzymes seem to have lower restrictions for the inhibitor structure which might lead to development of inhibitors specific for the protozoal enzyme and not affecting the host cell metabolism, with a potential chemotherapeutic use.

Various structural types of uridine phosphorylase inhibitors (modified pyrimidine bases, pyrimidine nucleosides or pyrimidine acyclic derivatives) have been synthesized (for review, see ref. 6). Some of them are very specific and powerful.[7] TdG (thymine 2'-deoxyglucoside) for example is able to distinguish between uridine phosphorylase and the frequently accompanying related enzyme, thymidine phosphorylase. The latter enzyme is an important angiogenic factor taking part in the tumor growth[8] and its level is particularly high in certain forms of cancer (breast, ovary, bladder, colon).[9] However, it also is the key-enzyme for tumor-specific liberation of 5-fluorouracil from its prodrugs tegafur,[9,10] 5'-deoxy-5-fluorouridine (doxifluridine, Furtulon)[11] and capecitabine (5'-deoxy-5-fluoro-N^4-pentyloxycarbonylcytidine)[12] used in cancer treatment.

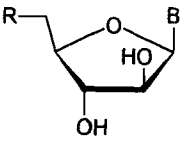
5-Substituted pyrimidines or pyrimidine nucleosides containing a hydrophobic group (benzyl, benzyloxybenzyl) occupy an important position among the most potent inhibitors of uridine phosphorylases. Most attention has focused on 5-benzyluracil derivatives.[13] In our project on uridine phosphorylase inhibitors we have synthesized four classes of base-modified nucleosides: 5-benzyluracil and 5-benzyl-4-thiouracil derivatives,[14] 5-phenylcytosine derivatives[15] and 5-phenylpyrimidin-2-one derivatives.[16] The alteration of the sugar part of the nucleoside molecule involved the β-D-ribofuranosides (**1**), 5-chloro-5-deoxy-β-D-ribo- (**2**) and 5-chloro-5-deoxy-β-D-arabino-nucleosides (**4**), 5-deoxy-β-D-ribo- (**3**) and 5-deoxy-β-D-arabinofuranosides (**5**), β-D-arabinofuranosides (**6**), β-D-xylofuranoside (**7**), anomeric 2-deoxy-D-ribonucleosides (**8, 9**), 2,3-dideoxy-β-D-ribonucleosides (**10**), 2,3-dideoxy-2,3-didehydro-β-D-ribofuranosides (**11, 12**), anomeric 3-azido-2,3-dideoxy-β-D-ribonucleosides (**13, 14**), anomeric 2-deoxy-β-D-*arabino*-hexopyranosides (**15, 16**) and anomeric D-*arabino*-2-hexeno-



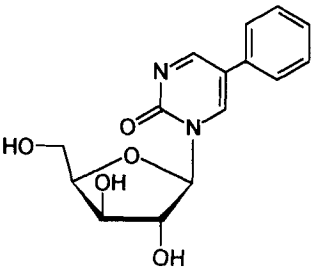
1 a B=BnU, R=OH
1 b B=BnCyt, R=OH
1 c B=BnthioU, R=OH
1 d B=BnMeSU, R=OH

2 a B=BnC, R=Cl
2 b B=PheC, R=Cl

3 a B=BnC, R=H
3 b B=PheC, R=H



4 B=PheC, R=Cl
5 B=PheC, R=H
6 a B=PheC, R=OH
6 b B=BnC, R=OH



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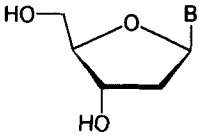
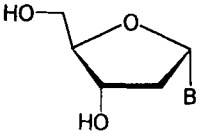
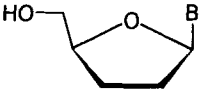


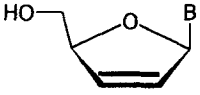
8 a B=BnC
8 b B=PhePym



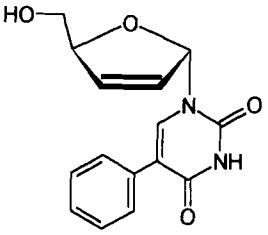
9 a B=BnC
9 b B=PhePym



10 a B=BnU
10 b B=BnthioU
10 c B=BnC
10 d B=PheC
10 e B=BnPym
10 f B= PhePym



11 a B=PheU
11 b B=PheC



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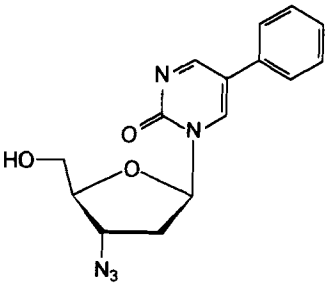

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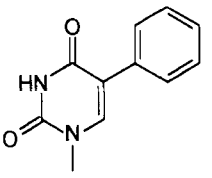
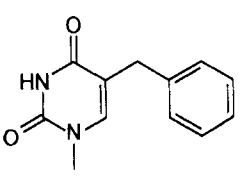
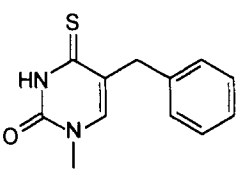


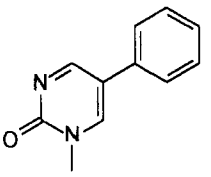
PheU



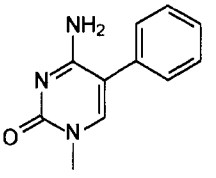
BnU



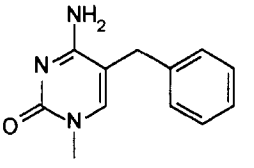
BnthioU



PhePym



PheC



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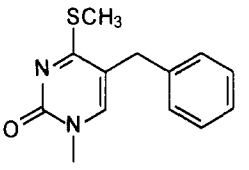
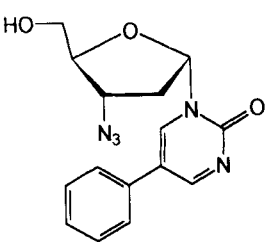


BnMeSU

pyranosides (**21, 22**). Furthermore, the series encompassed 2,2'-anhydronucleosides (**17**) and other related compounds. In this paper, we are presenting the data on the inhibitory activity of these compounds on uridine phosphorylase purified from mouse leukemic L-1210 cells, in comparison with established inhibitors TdG (**23**), BAU [5-benzyl-1-(2-hydroxyethoxymethyl)uracil, (**24**)] and 2,2'-anhydro-5-benzyluridine (**17d**).

Results

Chemistry. Most of the compounds were described in our previous communications.[14-16] We report here the synthesis of the hitherto unknown α- and β-anomers of 2',3'-dideoxy-2',3'-didehydro-5-phenyluridine (Scheme 1). Methyl 5-*O*-benzoyl-2-deoxy-3-*O*-mesylribofuranoside (**25**)[16b] gave on reaction with 5-phenyl-2,4-bis-(trimethylsilyloxy)pyrimidine in the presence of trimethylsilyl trifluoromethanesulfonate, two anomeric mesylates **26** and **27.** On treatment with 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile, the α-anomer **27** afforded the 5'-O-



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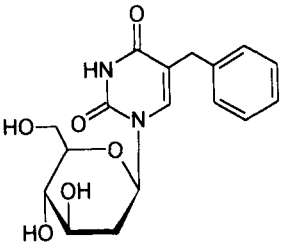

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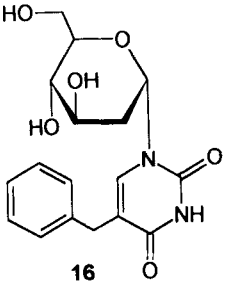

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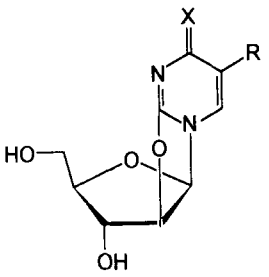
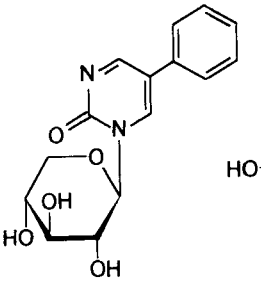


17a R=H, X=$NH_2^+Cl^-$
b R=Phe, X=$NH_2^+Cl^-$
c R=Bn, X=$NH_2^+Cl^-$
d R=Bn, X=O



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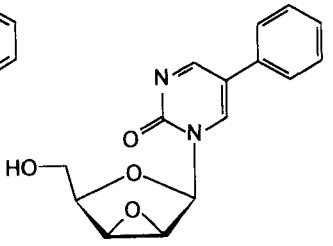

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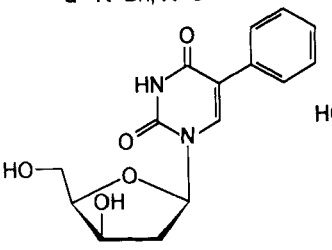

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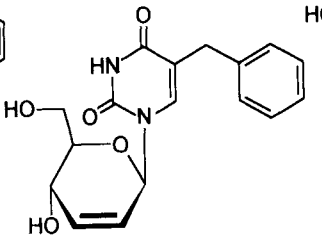

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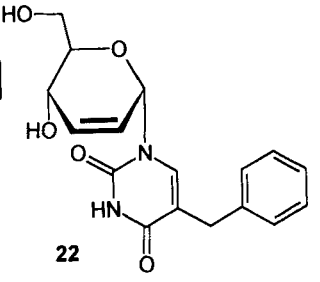

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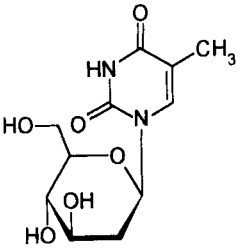

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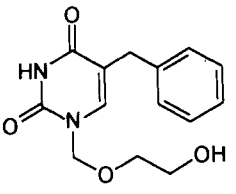


24

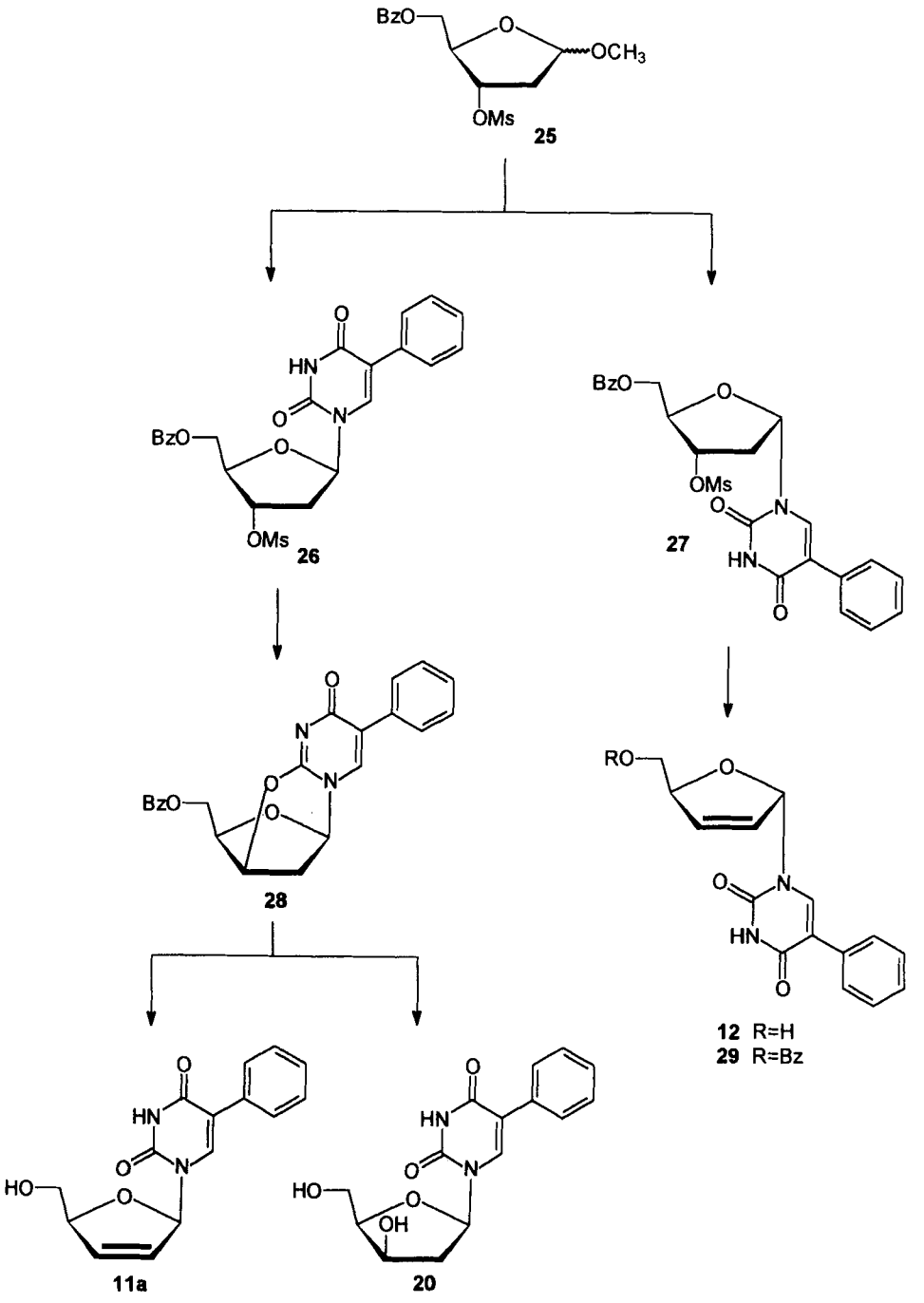
benzoate **29** which gave on methanolysis the elimination product **12,** whilst the same reaction of the β-anomer **26** resulted in the formation of the 3',2-anhydronucleoside **28**. By the action of sodium in HMPTA/THF followed by methanolysis this compound afforded 2,3-dideoxy-2,3-didehydro derivative **11a** together with the 2-deoxy-β-D-*threo*-pentofuranoside **20.** The structure of the ultimate products **11a, 12** and **20** was confirmed by their ^{1}H-NMR spectra.

Enzyme assay. The reaction mixture contained in a final volume of 100 μl: 50 mM potassium phosphate pH 7.4, 50μM [^{14}C-U-uracil]uridine (1 MBq μmol^{-1}), 15 μg of enzyme protein and 10 μM compound tested. Reaction mixtures were incubated for 10 min at 37 °C, and appropriate aliquots were withdrawn, spotted on W3MM paper and chromatographed in 1-butanol–acetic acid–water (10 : 1 : 3) overnight. The corresponding uracil and uridine spots were cut out and the radioactivity was determined by liquid scintillation counting. The inhibitory effect was expressed as v_i/v_o ratio at a constant concentration of the substrate (50 μM uridine) and/or compound tested (10 μM) (Table 1).

Kinetic experiments. Kinetic constants (K_m , K_i, V_{max}) were determined from the both Lineweaver-Burk and Dixon plots using five concentrations of the tested compound (including zero point) and four concentrations of the corresponding substrate (uridine). Data were evaluated by the non-linear regression method from four independent experiments. The concentration of the enzyme used was 150 μg/ml. Reactions were carried out at 37 °C for 10 min. The results are summarized in Table 2.

Discussion

Our data (Tables 1, 2) identify six new inhibitors with the inhibitory potency which decreases in the order **15** > **17c** > **16** > **10b** > **10a** > **1b**. The activity of compound **15** relates to TdG (**23**) and its increased inhibitory activity in relation to the parent compound may be interpreted simply by the replacement of 5-benzyluracil for thymine. Both anomeric 2-deoxyhexopyranosides (**15** and **16**) are effective inhibitors of the enzyme: however, they differ by the mechanism of inhibition: while the β-anomer **15** interferes with uridine phosphorolysis *via* mixed-noncompetitive mechanism, the α-anomer **16** acts *via* competitive inhibition of the enzyme, similarly as TdG (**23**).[17]



Scheme 1

TABLE 1. Inhibition of uridine phosphorylase from L1210 cells.

Compound	v_i/v_o	Reference	Compound	v_i/v_o	Reference
1a	0.83	14	11a	1.14	*a*
1b	0.33	14	11b	1.08	15
1c	1.07	14	12	0.94	*a*
1d	1.00	14	13	0.79	16b
2a	1.20	14	14	1.16	16b
2b	1.31	15	15	0.02	14
3a	1.13	14	16	0.14	14
3b	0.91	15	17a	1.04	20
4	1.19	15	17b	1.17	15
5	1.03	15	17c	0.03	14
6a	1.01	15	17d	0.05	18
6b	0.87	14	18	0.92	16a
7	0.78	16a	19	0.94	16a
8a	0.99	14	20	1.21	*a*
8b	0.82	16a	21	0.93	14
9a	1.15	14	22	0.95	14
9b	0.84	16a	23	0.16	21
10a	0.12	14	24	0.06	19
10b	0.13	14			
10c	1.05	14			
10d	0.91	15			
10e	1.20	14			
10f	1.23	16b			

a This paper.

The second most active inhibitor of the series, compound **17c** belongs to the uridine phosphorylase inhibitors with the 2,2'-anhydronucleoside structure. Neither 2,2'-anhydrouridine nor 2,2'-anhydrocytidine (**17a**) inhibit the enzyme, however, 2,2'-anhydro-5-benzyluridine (**17d**) is a powerful inhibitor.[18] Contrary to the 5-benzyluracil nucleosides which regularly exhibit inhibitory activity disregarding the character of the sugar residue, none of their 5-benzylcytosine counterparts was active. Compound **17c** is the only exception. Comparison of the inhibitory activity of **17c** with the activity of 2,2'-anhydro-5-benzyluridine (**17d**) on uridine phosphorylase from L-1210 cells showed that the uridine derivative **17d** is approximately one order of magnitude more potent compared to its 5-benzylcytidine congener (Table 2). However, it cannot be excluded that compound **17c** might behave *in vivo* as a prodrug liberating 2,2'-anhydro-5-benzyl-

TABLE 2. Kinetic characteristics of uridine phosphorylase (L1210 cells) inhibitors [a]

Compound	K_i (μM)	K_i/K_m
[b] 1b	2.27 ± 0.14	0.051
10b	0.478 ± 0.062	0.011
10a	0.575 ± 0.035	0.013
[c] 15	0.097 ± 0.011	0.002
16	0.401 ± 0.031	0.009
17c	0.152 ± 0.012	0.003
17d	0.0124 ± 0.0018	0.0003
23	0.579 ± 0.044	0.013
[d] 24	0.113 ± 0.009	0.003

[a] K_m for uridine: 44.3 ± 1.4 μM, V_{max} = 72.6 ± 2.6 μmol/min/mg. [b] Compound **1b** is a substrate for uridine phosphorylase, its phosphorolysis was confirmed by HPLC analysis. [c] Mixed-noncompetitive type of inhibition. [d] Partially-competitive type of inhibition.

uridine (**17d**). It might be worthwhile to examine its influence on uridine levels in the cellular pool and in plasma following its application in an animal study

The comparatively high inhibitory activity of the two 2',3'-dideoxynucleosides **10** is interesting from the viewpoint of their relation to the antiretroviral dideoxynucleosides. The uracil derivative **10a** has been already described[19] and found inactive against HIV, however, in our hands it turned out to be a potent inhibitor of uridine phosphorylase.

Also the related 4-thiouracil compound **10b** is an interesting structural lead: Another 4-thiouracil derivative, 5-benzyl-4-thiouridine (**1b**) inhibits the enzyme as well, furthermore, it is a substrate for uridine phosphorylase (at 50 μM concentration, 57% of **1b** is phosphorolyzed by the addition of 0.5 mg/ml of the enzyme). Comparison with 5-substituted 2-pyrimidinone derivatives shows that the 4-oxo (thioxo) group at the pyrimidine ring is indispensable for the inhibition of uridine phosphorylase and that the replacement of the 5-benzyl group by 5-phenyl results in the loss of activity.

Experimental

Melting points were determined on a Kofler block and are uncorrected. ^{1}H NMR spectra (δ, ppm; *J*, Hz) were recorded with a Varian UNITY 500 instrument in hexadeuteriodimethyl sulfoxide with tetramethylsilane as internal standard. Column chrom-

atography was performed on 30–60 μm silica gel (Service Laboratories of the Institute) and thin-layer chromatography (TLC) on Silufol UV254 foils (Kavalier, Votice). If not stated otherwise, the solutions were evaporated at 2 kPa and bath temperature 30–60 °C; compounds were dried at 13 Pa.

1-(5-*O*-Benzoyl-2-deoxy-3-*O*-methanesulfonyl-β-D-*erythro*-pentofuranosyl)-5-phenyluracil (26) and 1-(5-*O*-benzoyl-2-deoxy-3-*O*-methanesulfonyl-α-D-*erythro*-pentofuranosyl)-5-phenyluracil (27). A suspension of 5-phenyluracil (2.63 g, 14 mmol) and ammonium sulfate (20 mg) in hexamethyldisilazane (100 ml) was heated at 150 °C until it dissolved, and then for additional 2 h. The solvent was evaporated and the residue was codistilled with xylene (100 ml), leaving the silyl derivative. Trimethylsilyl trifluoromethanesulfonate (4.5 ml) was added to a solution of the silyl derivative and methyl 5-O-benzoyl-2-deoxy-3-O-methanesulfonyl-α-L-*erythro*-pentofuranoside (**25**)[16b] (4.59 g, 13.9 mmol) in acetonitrile (100 ml) cooled to 0 °C. The mixture was stirred at 0 °C for 20 min and the reaction was quenched with saturated aq. $NaHCO_3$ (100 ml). After stirring at room temperature for 10 min, the product was taken up in ethyl acetate (3 x 300 ml). The organic layer was dried over Mg_2SO_4, the solvent was evaporated and the residue was chromatographed on a column of silica gel (500 g) in toluene–ethyl acetate (1 : 2) to give 2.09 g (31%) of the β-anomer **26** and 0.67 g (10%) of α-anomer **27** as solid foams.

β-anomer **26**: ^{1}H NMR: δ 2.62 (ddd, 1 H, $J_{2a',1'}$ = 5.2, $J_{2a',2b'}$ = 14.6, $J_{2a',3'}$ = 2.6, H-2a'), 2.77 (ddd, 1 H, $J_{2b',1'}$ = 7.8, $J_{2b',3'}$ = 6.9, H-2b'), 3.33 (s, 3 H, OSO_2CH_3), 4.51 (dd, 1 H, $J_{5a',4'}$ = 5.1, $J_{5a',5b'}$ = 14.2, H-5a'), 4.52 (m, 1 H, H-4'), 4.59 (dd, 1 H, $J_{5b',4'}$ = 8.3, H-5b'), 5.84 (dt, 1H, $J_{3',4'}$ = 2.6, H-3'), 6.27 (dd, 1 H, H-1'), 7.24, 7.39, 7.47, 7.65, 7.95 (H-arom.), 7.75 (s, 1 H, H-6), 11.65 (s, 1 H, H-3). Anal Calcd for $C_{23}H_{22}N_2O_8S$ (486.5): C 56.78; H 4.56, N 5.76; S 6.59; Found: C 56.99; H 4.78; N 5.53; S 6.39.

α-anomer **27**: ^{1}H NMR: δ 2.60 (brdt, 1 H, $J_{2a',1'}$ = 2.5, $J_{2a',2b'}$ = 15.4, H-2a'), 3.00 (brdt, 1 H, $J_{2b',1'}$ = 6.8, H-2b'), 3.29 (s, 3 H, OSO_2CH_3), 4.39 (dd, 1 H, $J_{5a',4'}$ = 5.2, $J_{5a',5b'}$ = 12.2, H-5a'), 4.42 (dd, 1 H, $J_{5b',4'}$ = 4.5, H-5b'), 5.03 (brtd, 1 H, $J_{4',3'}$ = 1.4, H-4'), 5.49 (ddd, 1 H, $J_{3',2a'}$ = 2.2, $J_{3',2b'}$ = 6.3, H-3'), 6.32 (dd, 1 H, H-1'), 7.31 (7.37, 7.56, 7.69, 8.10, H-arom.), 7.79 (s, 1 H, H-6), 11.60 (s, 1 H, H-3). Anal Calcd for $C_{23}H_{22}N_2O_8S$ (486.5.: C 56.78; H 4.56; N 5.76; S 6.59. Found: C 57.02; H 4.67; N 5.49; S, 6.34.

2,3'-Anhydro-1-(5-O-benzoyl-2-deoxy-β-D-*threo*-pentofuranosyl)-5-phenyluracil (28). A solution of the 3'-mesyl derivative **26** (973 mg, 2 mmol) and 1,8-diazabicyclo-[5.4.0]undec-7-ene (0.45 ml, 3 mmol) in acetonitrile (45 ml) was refluxed for 40 min. The solution was cooled and taken down *in vacuo*. Chromatography of the residue on a silica gel column (120 g) in ethyl acetate–acetone–ethanol–water (18 : 3 : 2 : 1) afforded 500 mg (64%) of crystalline anhydro derivative **28**, m.p. 228.5–230 °C; ^{1}H NMR: δ 2.60 (ddd, 1 H, $J_{2a',1'}$ = 4.0, $J_{2a',2b'}$ = 12.9, $J_{2a',3'}$ = 3.0, H-2a'), 2.71 (brdd, 1 H, $J_{2b',1'}$ = 0.5, $J_{2b',3'}$ = 1.5, H-2b'), 4.44 (dd, 1 H, $J_{5a',4'}$ = 5.6, $J_{5a',5b'}$ = 11.6, H-5a'), 4.60 (dd, 1 H, $J_{5b',4'}$ = 5.1, H-5b'), 4.64 (brtd, 1 H, $J_{4',3'}$ = 2.2, H-4'), 5.49 m, 1 H, H-3'), 6.06 (brd, 1 H, H-1'), 7.31, 7.37, 7.47, 7.57, 7.64, 7.92, (H-arom.), 8.01 (s, 1 H, H-6). Anal Calcd for $C_{22}H_{18}N_2O_5$ (390.40): C 67.68; H 4.65; N 7.18% N; Found: C 67.52; H 4.67; N 7.39.

1-(2,3-Dideoxy-β-D-*glycero*-pent-2-enofuranosyl)-5-phenyluracil (11a) and 1-(2-deoxy-β-D-*threo*-pentofuranosyl)-5-phenyluracil (20). Tetrahydrofuran (20 ml) and anhydro derivative **28** (390 mg, 1 mmol) were added to a solution of sodium (46 mg, 2 mmol) in hexamethylphosphorous triamide, HMPTA) (1 ml) and the mixture was refluxed for 4 h. Then another portion of sodium solution (46 mg) in HMPTA (1 ml) was added and the mixture was refluxed for 3 h. The mixture was cooled, diluted with methanol (20 ml) and stirred for 20 h. The solution was neutralized with Dowex 50, H^+), the resin was filtered off and solvents were evaporated. The residue was chromatographed on a silica gel column (100 g) in ethyl acetate–acetone–ethanol–water (36 : 6 : 1 : 1) to give 150 mg (36%) of compound **11a** and 115 mg (26%) of compound **20**.

Compound **11a**: M.p. 312.5–314.5 °C. ^{1}H NMR: δ 3.65 (dd, 2 H, $J_{5',4'}$ = 2.5, $J_{5',OH}$ = 5.0, 2 x H-5'), 4.83 (m, 1 H, H-4'), 5.13 t, 1 H, 5'-OH), 5.98 (dt, 1 H, $J_{3',1'}$ = $J_{3',4'}$ = 1.5, $J_{3',2'}$ = 6.0, H-3'), 6.41 (dt, 1 H, $J_{2',1'}$ = $J_{2',4'}$ = 1.5, H-2'), 6.90 (dt, 1 H, $J_{1',4'}$ = 3.2, H-1'), 7.28, 7.34,7.52, H-arom.), 8.10 (s, 1 H, H-6), 11.50 (s, 1 H, H-3). Anal Calcd for $C_{15}H_{14}N_2O_4$ (286.3): C 62.93; H 4.93; N 9.79; Found: C 62.70; H 4.93; N 9.87.

Compound **20**: M.p. 205 - 206.5 °C; ^{1}H NMR: δ 2.00 (brd, 1 H, $J_{2a',3'}$ = 1.0, $J_{2a',2b'}$ = 13.9, H-2a'), 2.60 (ddd, 1 H, $J_{2b',1'}$ = 8.3, $J_{2b',3'}$ = 5.1, H-2b'), 3.64 (brdt, 1 H, $J_{5a',4'}$ = 6.8, $J_{5a',5b'}$ = 11.7, H-5a'), 3.73 (brdt, 1H, $J_{5b',4'}$ = 4.9, H-5b'), 3.87 (m, 1 H, H-4'), 4.27 (m, 1 H, H-3'), 4.74 (t, 1 H, $J_{OH,5'}$ = 5.6, 5'-OH), 5.37 (d, 1 H, $J_{3',OH}$ = 3.0, 3'-OH),

6.16 (dd, 1 H, $J_{1',2a'}$ = 1.5, H-1'), 7.30 9, 7.38, 7.52, H-arom.), 8.29 (s, 1 H, H-6), 11.47 (s, 1 H, H-3). Anal Calcd for $C_{15}H_{16}N_2O_5$ (304.3): C 59.21; H 5.30; N 9.21; Found: C 59.10; H 5.34; N 9.12.

1-(2,3-Dideoxy-α-D-*glycero*-pent-2-enofuranosyl)-5-phenyluracil (12). 1,8-Diazabicyclo[5.4.0]undec-7-ene (0.23 ml, 1.5 mmol) was added to a solution of mesyl deriv-ative **27** (487 mg, 1 mmol) in acetonitrile (15 ml) and the solution was refluxed for 12 h. The solvent was evaporated and the residue was partitioned between ethyl acetate (15 ml) and water (5 ml). The organic layer was washed with water (5 ml), dried (Mg_2SO_4) and the solvent evaporated. A solution of the residue (**29**) in methanolic ammonia (30 ml) was set aside at room temperature for 2 days and then taken down *in vacuo*. Chromato-graphy of the residue on silica gel (50 g) in toluene–acetone–triethylamine (15 : 15 : 1) afforded 170 mg (59%) of compound **12** as solid foam. ^{1}H NMR: δ 3.46 (dd, 1 H, $J_{5a',4'}$ = 4.6, $J_{5a',5b'}$ = 11.5, H-5a'), 3.51 (dd, 1 H, $J_{5b',4'}$ = 4.4, H-5b'), 4.85 (brs, 1 H (5'-OH), 5.10 m, 1 H, H-4'), 6.02 (dt, 1 H, $J_{3',1'}$ = $J_{3',4'}$ = 1.7, $J_{3',2'}$ = 6.0, H-3'), 6.45 dt, 1 H, $J_{2',1'}$ = $J_{2',4'}$ = 1.7, H-2'), 6.93 (dt, 1 H, $J_{1',4'}$ = 5.2, H-1'), 7.31, 7.37, 7.47, H-arom.), 7.46 (s, 1 H, H-6), 11.(s, 1 H, H-3).) Anal Calcd for $C_{15}H_{14}N_2O_4$ (286.3.: C 62.93; H 4.93; N 9.79; Found: C 63.05; H 5.11; N 9.66.

Enzyme source. Mouse leukemic L1210 cells were inoculated (i.p., 10^5 cells per animal) in DBA/2 inbred female mice (25 g) and after 8 days ascites were released from peritoneum and washed twice in phosphate-buffered saline (PBS).

Purification of uridine phosphorylase (2.4.2.3). Cells were resuspended in 20 mM potassium phosphate pH 8.0 supplemented with 1 mM DTT, 1 mM EDTA (buffer A) and protease inhibitors (Sigma, 40 μg/ml bestatin, 2 μg/ml pepstatin, 2 μg/ml leupeptin and 2 μg/ml aprotinin). The cell suspension was then freeze-thawed (three times) and homogenized in a Dounce tissue grinder (Wheaton, pestle A, 15 strokes). The crude extract was centrifuged at 100 000 g for 100 min and the supernatant was fractionated by ammonium sulfate. The enzyme proteins were salted out between 25 - 55% of $(NH_4)_2SO_4$ saturation, dissolved in buffer A and desalted on Sephadex PD-10 columns (Pharmacia LKB) in the same buffer. The resulting eluent was then applied onto the column (4.5 x 2.1 cm) of DEAE-Sephacel (Pharmacia LKB, equilibrated in buffer A) and chromatography was performed in a concentration gradient of KCl (0 - 0.25 M) in

buffer A. The enzyme activity was detected as a single peak between 0.1 - 0.25 M KCl. The most active fractions were then pooled and used as a partially purified uridine phosphorylase.

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