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Structural diversity of nucleoside phosphonic acids as a key factor in the discovery of potent inhibitors of rat T-cell lymphoma thymidine phosphorylase

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ABSTRACT

Structurally diverse, sugar-modified, thymine-containing nucleoside phosphonic acids were evaluated for their ability to inhibit thymidine phosphorylase (TP, EC 2.4.2.4) purified from spontaneous T-cell lymphomas of an inbred Sprague-Dawley rat strain. From a large set of tested compounds, among them a number of pyrrolidine-based derivatives, 10 nucleotide analogues with IC_{50} values below 1 μM were selected. Out of them, four compounds strongly inhibited the enzyme with IC_{50} values lying in a range of 11–45 nM. These most potent compounds might be bi-substrate analogues.

Thymidine phosphorylase (TP), the catabolic enzyme cleaving thymidine by a phosphorolytic mechanism (Fig. 1), has attracted the attention of many research groups.[1–8]

The enzyme, identical to the platelet-derived endothelial cell growth factor (PDECGF),[9–12] is involved in angiogenesis and chemotaxis in human tumors,[10,11,13,14] and thus can be considered as a target in cancer treatment. Elevated levels of TP found in colorectal, ovarian, pancreatic, and breast tumors,[4,15] and at other hyperproliferative disease states such as rheumatoid arthritis[16] and psoriasis,[17] correlate with increased hypoxia. The inhibition of TP may result in reduction of tumor growth and metastasis,[18–25] and it also potentiates[26] the antiproliferative effect of nucleoside drugs such as 5-(*E*)-(bromovinyl)-2′-deoxyuridine, 2′-deoxy-5-trifluoromethyluridine, 2′-deoxy-5-iodouridine, and 5-fluoro-2′-deoxyuridine which are substrates of TP.[27] As follows from the survey of literature, the inhibition of TP represents a promising target in cancer chemotherapy.[28–32]

One of the most potent inhibitors of human TP is 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]uracil hydrochloride **1**[21] (TPI, Fig. 2) which inhibits the enzyme competitively with $K_i = 17$ nM.[29] In vivo, TPI increases the proportion of apoptotic cancer cells in TP-positive tumors and suppresses the growth of the tumors in mouse model.[21]

A recently published study on the inhibition of TP, purified from rat T-cell lymphomas of Sprague-Dawley rat strain, by phosphonomethoxyalkyl derivatives of thymine (Fig. 2) **2a** (FPMPT), **2b** (HPMPT), **2c** (PMPT), and **2d** (PMET) showed that these compounds were competitive inhibitors of both thymidine and inorganic phosphate.[6] It suggests that these phosphonic acids are bi-substrate

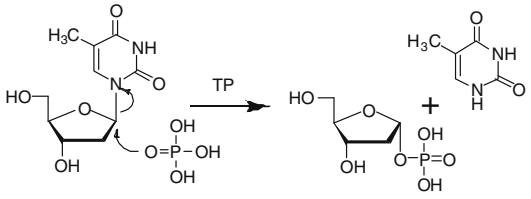

Figure 1. TP-catalysed thymidine cleavage.

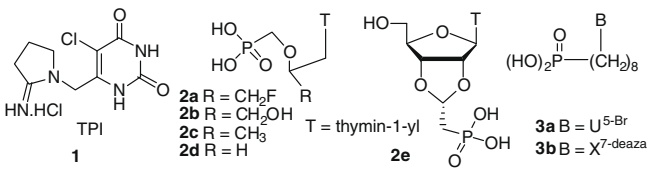

Figure 2. Examples of TP inhibitors.

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analogues. They possess considerable inhibitory potency towards T-cell lymphoma TP but not to rat liver, *Escherichia coli*, and human TPs. The competitive type of inhibition of compounds **2a–c** towards thymidine and inorganic phosphate resembles the character of inhibition of 8-phosphonooctyl derivatives **3a** and **3b** towards *E. coli* TP published by Perez-Perez and co-workers [33] In contrast to compounds **2a–d** and **3a, b**, the nucleoside phosphonic acid **2e** (Fig. 2) was reported to be a potent inhibitor of human recombinant TP with K_i = 236 nM.[34,35]

These findings prompted us to undertake a structure–activity study on the inhibition of TP, purified from spontaneous T-cell lymphomas of an inbred Sprague-Dawley rat strain,[6] with structurally diverse thymine-containing nucleoside phosphonic acids.

About 200 compounds that were prepared during our studies on various types of phosphonate analogues of nucleotides over last years were tested as potential inhibitors of rat T-cell lymphoma TP.[6] Out of this large pool of compounds, we selected those that reduced TP activity to at least 40% at the 1:10 ratio of the inhibitor versus thymidine as substrate (Table 1). In order to select most potent inhibitors, we measured the IC_{50} values of eleven promising nucleoside phosphonates and uncovered 10 submicromolar-level inhibitors. Among them, four very potent ones exhibited IC_{50} values within 11–45 nM range at 100 μM concentration of thymidine

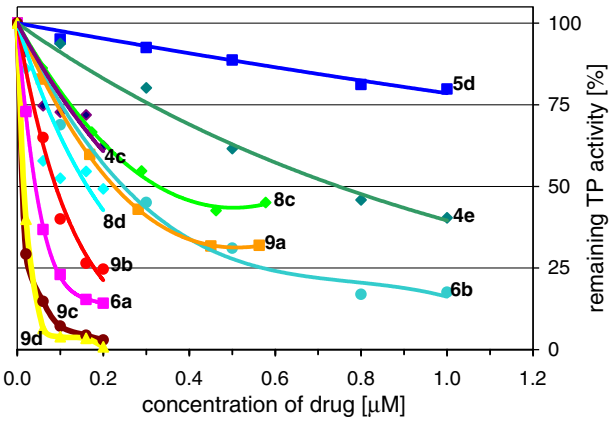
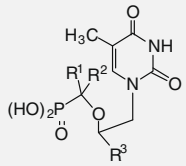
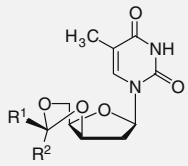
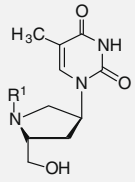
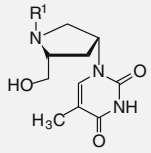
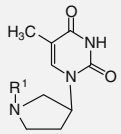
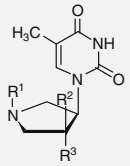


Figure 3. T-lymphoma TP inhibition by selected phosphonates: determination of IC_{50} values (for experimental conditions, see Table 1).

Table 1
Nucleoside phosphonic acids-based inhibitors of T-cell lymphoma thymidine phosphorylase

Inhibitor					Residual activity of TP[a] (%)	IC_{50}[a,b] (nM)
No.	Structure	R^1	R^2	R^3		
4a		$-(CH_2)_5-$		H–	21	–
4b		$-(CH_2)_4-$		H–	14	–
4c		H–	H–	$CH_2{=}CH_2CH_2O-$	10	~300[c]
4d		Ph–	H–	H–	9	–
4e		H–	H–	PhO–	1	750
5a		$(HO)_2(O)P-$	Ph–	–	47	–
5b		$(HO)_2(O)P-$	$BrCH_2-$	–	26	–
5c		$BrCH_2$	$(HO)_2(O)P-$	–	20	–
5d		Ph	$(HO)_2(O)P-$	–	4	–
6a		$(HO)_2(O)PC(O)-$	–	–	2	45
6b		$(HO)_2(O)PCH_2-$	–	–	0	250
7		$(HO)_2(O)PCH_2-$	–	–	17	–
8a		$(HO)_2(O)PCH_2C(O)-$	–	–	59	–
8b		$(HO)_2(O)PCH_2-$	–	–	29	–
8c		$(HO)_2(O)PC(O)-$	–	–	3	350
8d		$(HO)_2(O)PC(S)-$	–	–	9	190
9a		$(HO)_2(O)PC(O)-$	H–	HO–	4	220
9b		$(HO)_2(O)PCH_2-$	HO–	H–	1	45
9c		$(HO)_2(O)PC(O)-$	HO–	H–	0	11
9d		$(HO)_2(O)PC(S)-$	HO–	H–	0	15

[a] TP assay conditions: 20 mM bis-Tris–HCl, pH 6.7, 1 mM EDTA, 2 mM DTT, 100 μM [^{3}H-methyl]thymidine, 200 μM PO_4^{3-}, 25.5 pU enzyme, 10 μM inhibitor; K_m = 83.2 ± 8.69 μM.
[b] Inhibitor concentration 0–1 μM.
[c] Estimated value (see Fig. 3).