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Original article

Inhibition of human thymidine phosphorylase by conformationally constrained pyrimidine nucleoside phosphonic acids and their "open-structure" isosteres

Ivana Košiová [1], Ondřej Šimák [1], Natalya Panova, Miloš Buděšínský, Magdalena Petrová, Dominik Rejman, Radek Liboska, Ondřej Páv, Ivan Rosenberg*

Institute of Organic Chemistry and Biochemistry, Academy of Sciences v. v. i., Flemingovo 2, 166 10 Prague 6, Czech Republic

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ABSTRACT

A series of conformationally constrained uridine-based nucleoside phosphonic acids containing annealed 1,3-dioxolane and 1,4-dioxane rings and their "open-structure" isosteres were synthesized and evaluated as potential multisubstrate-like inhibitors of the human recombinant thymidine phosphorylase (TP, EC 2.4.2.4) and TP obtained from peripheral blood mononuclear cells (PBMC). From a large set of tested nucleoside phosphonic acids, several potent compounds were identified that exhibited K_i values in the range of 0.048–1 μM. The inhibition potency of the studied compounds strongly depended on the degree of conformational flexibility of the phosphonate moiety, the stereochemical arrangement of the sugar-phosphonate component, and the substituent at position 5 of the pyrimidine nucleobase.

1. Introduction

Thymidine phosphorylase (TP), the enzyme identical to platelet-derived endothelial cell growth factor [1–5], catalyzes the reversible phosphorolysis of thymidine to thymine and 2-deoxy-α-D-ribofuranosylphosphate (Fig. 1).

In contrast to non-neoplastic tissues, the elevated levels of TP have been found in colorectal, ovarian, pancreatic, and breast tumors [6,7], and in other hyperproliferative disease states such as rheumatoid arthritis [8] and psoriasis [9]. The inhibition of TP may result in the reduction of tumor growth and metastasis [10–17]. To date, a number of TP inhibitors of human TP based on the analogues of uracil and thymine nucleobases and nucleosides have been reported [18–32]. The most potent and therapeutically promising inhibitor of human TP is 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]uracil hydrochloride (**1**, TPI [13], Taiho Pharmaceutical Company) with a K_i of 17 nM [33] which, in a combination with the anticancer drug trifluorothymidine, is currently in clinical trials (Fig. 2) [34,35].

In addition to these mono-substrate-like inhibitors, there is a class of compounds exists in which both nucleoside and phosphoryl moieties are combined in one molecule that can bind simultaneously to both nucleobase and phosphate binding sites, respectively. Balzarini et al. [36,37] and Votruba et al. [38] reported the inhibition of *Escherichia coli* TP with structurally diverse acyclic nucleoside phosphonic acids (Fig. 2, structures **2**–**4**), and Lequeux et al. [39] recently published a thorough structural study on the inhibition of the enzyme by various α,α-difluoromethyl-moiety-containing 1-thyminylalkylphosphonic acids **4a**. Comparative study on the inhibition of *E. coli* and human TPs with 3-pyrimidinylalkylphosphonic acids was recently reported by Pomeisl et al. [40]. Irrespective of the structural and genetic similarities among thymidine phosphorylases from various sources including *E. coli* and human recombinant TPs, the sensitivity of the two enzymes toward inhibitors based on nucleoside phosphonic acids appears to differ. Recently, we described the potent bi-substrate-like inhibitors **5** and **6a**–**b** of TP isolated from T-cell lymphomas of the Sprague–Dawley rat strain; the inhibitors were based on pyrrolidine nucleoside phosphonic acids [41], with IC_{50} values in the range of 11–45 nM at a thymidine concentration of 100 μM. Interestingly, these compounds did not inhibit healthy rat liver, *E. coli*, or human TPs.

* Corresponding author. Tel.: +420 220 183 381.
E-mail address: rosenberg@uochb.cas.cz (I. Rosenberg).
[1] These authors contributed equally to this paper.

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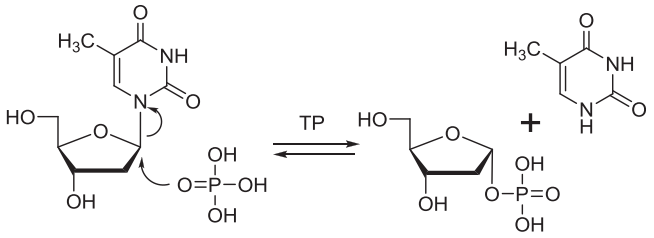

Fig. 1. TP-catalyzed thymidine cleavage.

In contrast to guanine-based pyrrolidine nucleoside phosphonic acids, recently reported as potent bi-substrate-like inhibitors [42] of human purine nucleoside phosphorylase, the similar thymine-based compounds did not inhibit the human TP.

The first nucleoside phosphonic acid inhibiting human TP in the submicromolar range, 5-methyl-2′,3′-*O*-(2-phosphono-1,1-ethylidene)uridine (**50**, Fig. 1), a conformationally constrained bi-substrate-like inhibitor with a K_i value of 236 nM, was reported by Li [43]. Recently described [44] carba analogues of **50**, i.e., nucleoside phosphonic acids **7a** and **7b**, inhibited the human TP in the high micromolar range.

In this paper, we describe the synthesis of two new series of pyrimidine nucleoside phosphonic acids, conformationally constrained and flexible ("open-structure"), respectively, and describe their ability to inhibit human TP. The target compounds were designed to offer a variety of structural types believed to cover a range of potential candidates that could provide significant data on the structure–activity relationship.

2. Results and discussion

2.1. Chemistry

2.1.1. Nucleosid-2′,3′-di-O-ylmethanephosphonic acids (Scheme 1)

Conformationally constrained pyrimidine nucleotide analogs **12a**,**b**–**14a**,**b**, the isopolar phosphonate analogs of pyrimidine nucleoside 2′(3′)-phosphates, were synthesized from the appropriate nucleosides **8** and **9** by several step syntheses (Scheme 1) [45]. These compounds were obtained as epimeric mixtures that provided individual epimers after separation by RP HPLC. 5′-Chlorouridine phosphonates **13a** and **13b** were prepared from the protected 5-chlorouridine **9** which was easily obtained by chlorination of the uridine derivative **8** with *N*-chlorosuccinimide (NCS) according to the described procedure [46]. Protected nucleosides **8** and **9** were transformed into the 2′,3′-*O*-orthoester derivatives **10** and **11**, respectively, by the reaction with trimethyl orthoformate in the presence of anhydrous HCl in DMF. Subsequent reaction of **10** and **11** with two equivalents of dimethyl chloro phosphite gave, after removal of the silyl protecting group with TBAF and the following treatment with bromotrimethylsilane in a CH_3CN–2,6-lutidine mixture (to remove the phosphoester groups), a mixture of **13a** (*endo*) and **13b** (*exo*) epimers (25:75). The epimers were separated by RP HPLC. In contrast to the synthesis of **13a**,**b**, the 5-iodo derivatives **14a** and **14b** were prepared by a direct iodination of pure epimers **12a** and **12b** with iodine in diluted nitric acid [47]. Compounds **12c** and **12d** were synthesized according to the method described in the literature [45].

2.1.2. Nucleosid-2′(3′)-O-ylmethanephosphonic acids (Scheme 2)

The regioisomeric 3′- (**23a**–**26a**) and 2′-*O*-methanephosphonic acids (**23b** and **24b**), which are conformationally flexible congeners related to the constrained phosphonates **12** and **13**, were prepared from the corresponding protected nucleosides **15a**–**18a**, **15b** and **16b** in three steps (Scheme 2). The alkylation of these nucleosides with methyl tosyloxymethanephosphonate [48] provided phosphonate methyl esters **19a**–**22a**, **19b** and **20b**, respectively. Detritylation of these compounds using 80% acetic acid followed by removal of the ester groups by bromotrimethylsilane treatment afforded the final 3′- and 2′-*O*-methanephosphonic acids **23a**–**26a** and **23b**, **24b**, respectively. The synthesis of **23a** was already reported [49].

2.1.3. 2-(Nucleosid-3′(2′)-O-yl)-ethanephosphonic acids (Schemes 3 and 4)

For the synthesis of nucleoside 3′- and 2′-*O*-(hydroxyethane) phosphonic acids (**31a** and **31b**, respectively), and 3′- and 2′-*O*-ethanephosphonic acids (**33a** and **33b**, respectively) which are one methylene group longer congeners of compounds **23a** and **23b** with an additional methylene group, we used the following synthetic strategy. The *O*-allylation of 5′-*O*-dimethoxytrityl nucleosides **15a** and **15b** with allyl bromide [50] afforded the allyl derivatives **27a** and **27b**. Treatment of these compounds with osmium tetroxide in the presence of *N*-methylmorpholine-*N*-oxide provided, after silica gel chromatography, pure *O*-(2,3-dihydroxypropyl) derivatives **28a** and

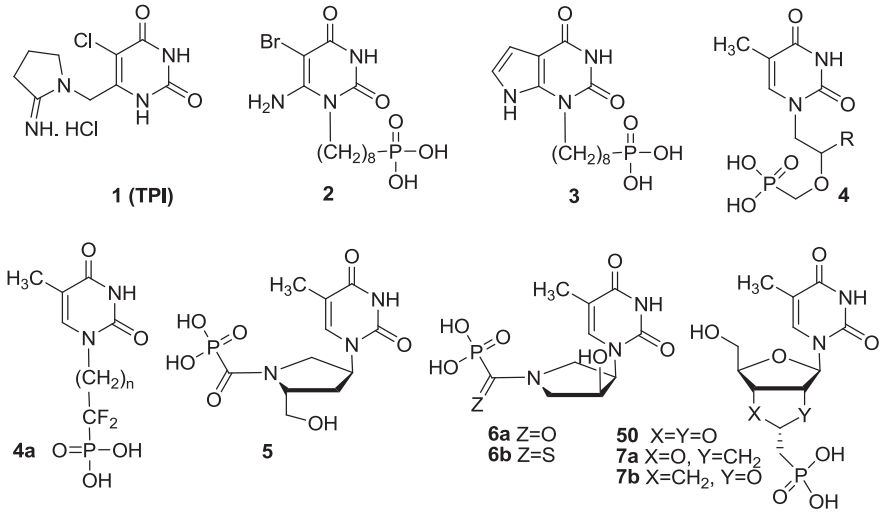

Fig. 2. TPI and bi-substrate like nucleoside phosphonic acid-based TP inhibitors.