European Journal of Medicinal Chemistry 46 (2011) 1165–1171

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Structure–activity relationship of 2,4,5-trioxoimidazolidines as inhibitors of thymidine phosphorylase

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ARTICLE INFO

Article history:
Received 3 August 2010
Received in revised form
14 October 2010
Accepted 22 January 2011
Available online 31 January 2011

Keywords:
Thymidine phosphorylase
Trioxoimidazolidine
Parabanic acid
Hydantoin
Anti-angiogenic
Molecular modelling

ABSTRACT

Novel non-nucleobase-derived inhibitors of the angiogenic enzyme, thymidine phosphorylase, have been identified using molecular modelling, synthesis and biological evaluation. These inhibitors are 2,4,5-trioxoimidazolidines bearing *N*-(substituted)phenylalkyl groups, together with, in most cases, *N*′-$(CH_2)_n$-carboxylic acid, ester or amide side chains. The best compound from this series is 3-(2,4,5-trioxo-3-phenylethyl-imidazolodin-1-yl)propionamide, with an IC_{50} of 40 μM against *Escherichia coli* TP. Molecular modelling suggests that this ligand, when complexed with closed-cleft human TP, would have the phenylalkyl group in the active site region normally occupied by a thymine-containing structure.

1. Introduction

Thymidine phosphorylase (TP, dThPase, E.C. 2.4.2.4), also known as the platelet-derived endothelial cell growth factor (PD-ECGF), catalyses the phosphorolysis of thymidine (**1**) to 2-deoxyribose-1-phosphate (**2**) and thymine (**3**) (Fig. 1) [1]. TP is highly expressed in many solid human tumours, facilitating tumour growth by promoting angiogenesis, metastasis and suppressing apoptosis [2–5].

Furthermore, intracellular hydrolysis of **2** generates 2-deoxy-D-ribose [6] which promotes angiogenesis, the chemotactic activity of endothelial cells and also confers resistance to hypoxia-induced apoptosis in some cancer cell lines [2,7]. Thus, TP is considered an attractive therapeutic target for inhibition of tumour angiogenesis and concomitant tumour growth and metastasis [5].

Almost all literature TP inhibitors are based on uracil or very closely related ring systems. One of the most potent inhibitors, 5-chloro-6-[1-(2-iminopyrrolidinyl)methyl]uracil hydrochloride (**4**, TPI, Fig. 2), caused a reduction in the rate of tumour growth when administered to mice carrying tumours that over-expressed human TP [8]. Prodrugs of inhibitors of TP have also been synthesised in which 2-nitroimidazolyluracil prodrugs (**5A**) require bioactivation under tumour conditions to form the active species, 2′-amino-imidazolyluracils (**5B**) (Fig. 2) [9–11], one of which demonstrated activity similar to TPI (apparent IC_{50} 0.023 μM against human TP) [10,11]. These zwitterionic tight-binding inhibitors are proposed to mimic parts of the oxacarbenium ion-like transition state formed during thymidine phosphoroylsis, see Fig. 1 [12]. Despite the potency levels of these inhibitors, their highly ionic nature and poor pharmacokinetic profiles remain as substantial limitations.

Recently, novel scaffolds for TP inhibitors have been targeted with the aid of 3D-homology models of human pyrimidine nucleoside phosphorylase and *Escherichia coli* TP [12,13]. We have previously employed structure-based virtual screening of the National Cancer Institute (NCI) database against the open conformation of the homology model of human TP [9] based on a crystal structure of the *E. coli* enzyme [13]. This study identified hydantoin **7** (Fig. 3) as a TP inhibitor lead with micromolar activity against human and *E.coli* TP [13], comparable to that of the known, non-uracil-based TP inhibitor, 7-deazaxanthine (**6**). This novel scaffold lacks the undesirable ionic sites of the existing tight-binding nucleobase-derived inhibitors and therefore may possess improved pharmacokinetic and cell-penetrating properties.

Based on our knowledge of this hydantoin (2,4-dioxoimidazolidine) lead, we here employ molecular modelling, synthesis and biological assays to identify further active non-nucleoside lead scaffolds as a basis for development of novel TP inhibitors.

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doi:10.1016/j.ejmech.2011.01.035

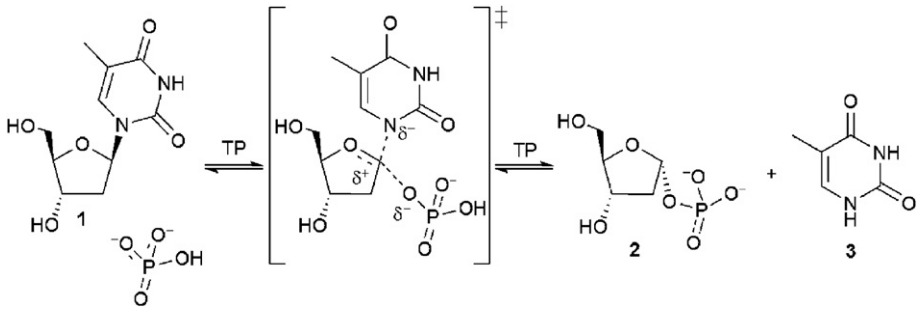

Fig. 1. Phosphorolysis of thymidine (**1**) to 2-deoxyribose-1-phosphate (**2**) and thymine (**3**).

2. Results and discussion

In an initial modelling step, the NCI and Available Chemicals Directory (ACD) databases were filtered to identify all imidazolidine-containing compounds [14]. The identified compounds were then screened *in silico* against an X-ray structure of closed-cleft human TP [15], in which phosphate was modelled into the active site; these compounds were ranked according to calculated docking scores [14]. Interestingly, the top-ranked NCI ligand from this screen was the hydantoin, 4-hexyl-1-methyl-2,5-dioxo-4-imidazolidinecarbaldehyde semicarbazone (**8**). This ligand is very similar to the previously identified hydantoin derivative **7** [13], the only difference being the presence of an extra methylene group in the aliphatic chain. The top-ranked ACD ligand from the screen was [3-(2-methylbenzyl)-2,4,5-trioxo-imidazolidin-1-yl]ethanoic acid (**9**). Its complex with TP was predicted to be more stable (by 36.4 kJ/mol) than that of compound **8** [14].

2.1. Chemistry

Based on this predicted preference of imidazolidines for the active site of TP, the chemical space surrounding the highest scoring compound, **9**, was explored: a series of 3-arylalkyl-2,4,5-trioxoimidazolidine-1-ethanoic acids (parabanic acid derivatives), including **9**, and their corresponding esters and amides were prepared (Scheme 1) and tested for their TP inhibitory activities (Table 1). We note that some of these compounds have previously been investigated for their therapeutic potential for reduction of diabetic complications such as neuropathy, nephropathy, retinopathy, keratopathy, angiopathy and cataracts by selectively inhibiting the enzyme aldose reductase [16–18].

The general synthetic route for the acids and their esters is shown in Scheme 1 and is based on chemistry developed by Ishii and coworkers [19]. The synthesis starts from benzylamine derivatives **10**–**13**, which are reacted with urea in the presence of acid to yield *N*-(benzyl)urea derivatives **14**–**17**. Treatment of the *N*-(benzyl)urea derivatives **14**–**17** with oxalyl chloride gave the 1-(benzyl)-imidazolidine-2,4,5-triones **18**–**21** in good yields. The ethyl [3-benzyl-2,4,5-trioxo-imidazolidin-1-yl]ethanoates **22**–**25** were then prepared by reaction of **18**–**21** with ethyl bromoethanoate in the presence of potassium hydroxide. Acid-catalysed hydrolysis of esters **22**–**25** gave the 3-benzyl-2,4,5-trioxo-imidazolidin-1-yl-ethanoic acids **9** and **26**–**28** in good yields. The novel amide analogues **29**–**32** were obtained by reaction of the imidazolidine-2,4,5-trione derivatives **18**–**21** with 3-bromopropionamide (Scheme 1). Amides **29**–**32** were fully characterized by ^{1}H and ^{13}C NMR spectroscopy, elemental analysis and mass spectrometry.

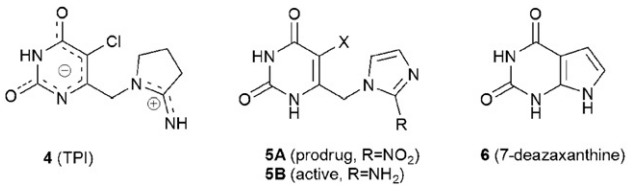

Fig. 2. Examples of known TP inhibitors.

2.2. Structure–activity relationship

Compound **9** and **18**–**32** were tested for their TP inhibitory activity using *E. coli* TP. A spectrophotometric assay was used to measure the decrease in absorbance (at 265 nm) of the natural substrate thymidine upon the addition of the compounds [10,20]. The experimental IC_{50} values are presented alongside their calculated docking scores in Table 1, together with the data for the inhibitor TPI (**4**) as a positive control.

The data presented in Table 1 for the 1-arylalkyl-2,4,5-trioxoimidazolidines (**18**–**21**) indicate that there is little difference in TP inhibition activity between these compounds. This indicates that the nature of substituent, X, on the phenyl ring has a rather weak influence on the overall binding affinity of the ligands. Correspondingly, these groups do not appear to interact directly with the protein in the predicted binding poses. This is also evident from their similar calculated energy scores. Indeed, compounds **18**–**21** all bind in very similar conformations with good interactions formed between the imidazolidine-2,4,5-trione ring and Ser217 and Arg202 residues, with the phenyl ring located in the centre of the active site.

Ligands **22**–**32** examine the effect of the presence of side chains opposite the lipophilic aryl substituent (Table 1). The ester groups in ligands **22**–**25** constitute hydrogen bond acceptors and the ethyl groups have the ability to make lipophilic interactions. The presence of the ester functionality generally decreased TP inhibition when compared with other analogues in Table 1. Replacing the ester functional group with the carboxylic acid group led to a marked improvement in the calculated GoldScore value, comparable to the value obtained for TPI (Table 1). The most stable binding conformation predicted for compound **9**, which was the top-ranked ligand in the original screen of the ACD database and displayed the highest calculated score (−72.2 kJ/mol) of all the

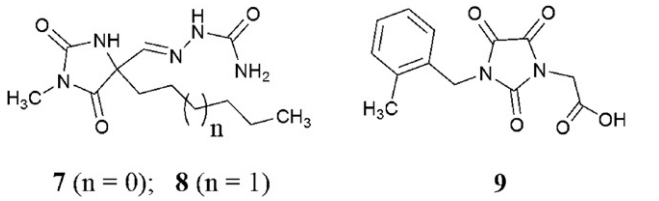

Fig. 3. Hydantoins **7** and **8** and trioxoimidazolidine **9** were TP inhibitors identified by *in silico* screening of NCI and ACD databases.