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Structure–activity relationships of β-hydroxyphosphonate nucleoside analogues as cytosolic 5′-nucleotidase II potential inhibitors: Synthesis, *in vitro* evaluation and molecular modeling studies

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ABSTRACT

The cytosolic 5′-nucleotidase II (cN-II) has been proposed as an attractive molecular target for the development of novel drugs circumventing resistance to cytotoxic nucleoside analogues currently used for treating leukemia and other malignant hemopathies. In the present work, synthesis of β-hydroxyphosphonate nucleoside analogues incorporating modifications either on the sugar residue or the nucleobase, and their *in vitro* evaluation towards the purified enzyme were carried out in order to determine their potency towards the inhibition of cN-II. In addition to the biochemical investigations, molecular modeling studies revealed important structural features for binding affinities towards the target enzyme.

1. Introduction

Enzymes are attractive biological targets for small-molecule drug discovery, especially those involved in the nucleic acid biosynthesis. Polymerases are key enzymes in these processes and they have been extensively studied as targets for the treatment of viral infections and cancers [1]. Thus, nucleos(t)ide analogues are widely used as therapeutic agents because they mimic physiological metabolites and interfere with key steps in viral particle production and/or in cancer cell proliferation, respectively [2]. To obtain these effects, the phosphorylated forms (nucleotides) must compete with endogenous nucleotides. While much effort has been carried out for the identification of novel analogues that are intrinsically virotoxic or cytotoxic, very little is known regarding the relationship between the size of the pools of endogenous nucleos(t)ides and the therapeutic effect of these drugs. It is expected that their biological effect is directly related to the ability of unnatural nucleotides to compete with the physiological ones, thereby emphasizing the importance of mechanisms regulating nucleotide pools [3,4]. Although the individual steps involved in nucleoside transport and metabolism have been dissected and the majority of the related proteins identified, the overall regulation of nucleos(t)ide pools in mammalian cells remains unclear. Thus, intracellular monophosphorylation of nucleoside analogues is balanced by a family of enzymes called 5′-nucleotidases (5′-NTs: EC 3.1.3.5), which catalyze the hydrolysis of deoxyribo- and ribonucleoside 5′-monophosphates into the corresponding nucleosides and inorganic phosphate [3,5,6]. Among the eight members of this family, our interest for the cytosolic 5′-nucleotidase II (cN-II, EC 3.1.3.5) arose from the fact that its expression level is of crucial

Abbreviations: AML, acute myeloid leukemia; cN-II, cytosolic 5′-nucleotidase II; DMAP, 4-(dimethylamino)pyridine; DMF, *N*,*N*-dimethylformamide; DMSO, dimethylsulfoxide; IMP, inosine 5′-monophosphate; 5-NTs, 5′-nucleotidases; RNA, ribonucleic acid; TMSBr, trimethylsilyl bromide; TMSCl, trimethylsilyl chloride; TPPS, 4,4′,4″,4‴-(porphine-5,10,15,20-tetrayl)tetrakis benzenesulfonic acid; Ts, *p*-toluenesulfonyl.

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interest for patients treated with nucleoside analogue-based chemotherapy [7,8]. Indeed, a high level of expression of cN-II mRNA in blasts is predictive of worse outcome in patients receiving cytarabine-based regimens (a well-known nucleoside analogue used to treat acute myeloid leukemia, AML) [9,10]. In addition, the inhibition of cN-II expression by short hairpin RNA was associated with the induction of apoptosis in human astrocytoma cells, suggesting that cN-II could be a therapeutic target in brain tumors [11]. Finally, recent reports showed that hyperactive mutated cN-II in relapsed children with acute lymphoblastic leukemia, and treated with the antimetabolite 6-mercaptopurine, was associated with a worse survival [12,13].

In light of the various structural, functional and regulatory properties of this enzyme, we envisaged cN-II as an attractive target for developing different types of inhibitors that could interfere with protein function or regulation [14,15]. It must be highlighted that cN-II is called high-K_m nucleotidase indicating that K_m value for its favorite substrate, IMP (inosine 5′-monophosphate) is in the millimolar range. Therefore, potential competitive inhibitors are expected to exhibit K_i values in the same range. In addition, cN-II acts as a tetramer (dimer of dimer) and possesses two regulatory or effector sites. The effector site 1 is located closed to the IMP binding site at the interface between two monomers and regulates the enzyme activity (for instance, in presence of ATP, the k_{cat} is increased and K_m is lowered for IMP [16]. Several attempts to target this site have been performed to find other type of inhibitors (such as non-competitive ones) but yet not successful. Our previous studies led to the selection of a first series of 5′-monophosphate nucleoside analogues, such as β-(S)-hydroxyphosphonate derivatives (compounds **2** and **4**, Fig. 1), able to interfere with the hydrolysis of IMP by purified recombinant cN-II [15]. Thus, these two derivatives were considered as a starting point for further optimization and we elaborated several computer-based strategies to improve their efficiency. First, we investigated the effect of the following sugar modifications: (i) inversion of the C2′ and C3′-configurations (Fig. 1, compounds **5**–**16**) or of the C5′ stereochemistry *i.e.* the (*R*)-isomer of the β-hydroxyphosphonate (Fig. 1, compounds **17**–**18**), (ii) opening of the sugar ring (Fig. 1, compounds **19**–**22**).

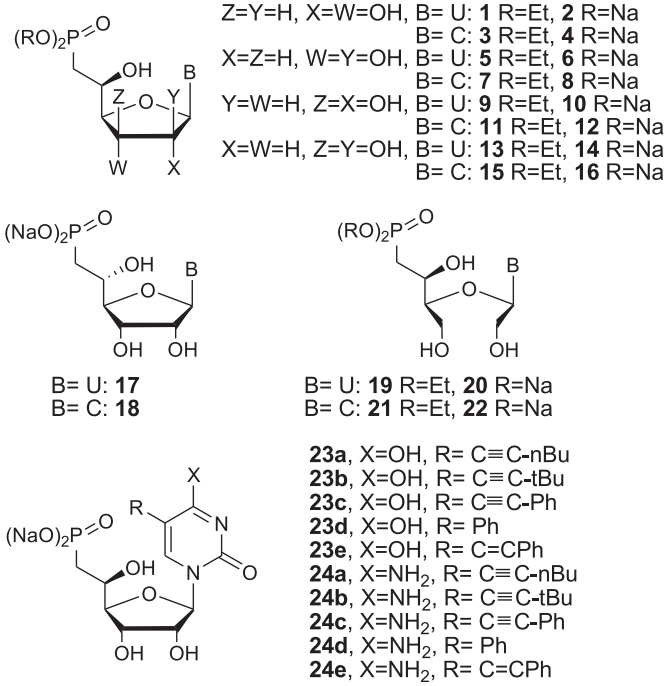

Fig. 1. Structure of the studied compounds.

As we previously reported the crucial role of a hydrophobic clamp around the nucleobase for substrate recognition, that is promoted by three protein residues Phe157, His209 and Tyr210 (Fig. 2) [15], we performed further modeling studies of compounds **2** and **4** within the active site of the enzyme. It revealed that a large hydrophobic pocket was available near the nucleobase and therefore, we envisaged a second series of derivatives including non-natural pyrimidines. The volume of this cavity has been estimated to be ~1200 Å^3 (Fig. 2) allowing various kind of modifications at the C5 position of uracil and cytosine. Thus, we decided to append lipophilic or aromatic substituents on the nucleobase (Fig. 1, compounds **23a**–**e** and **24a**–**e**), that should allow additional Π–Π and/or hydrophobic interactions between the ligand and the protein residues (especially Phe157 and farther Phe354, see Fig. 2).

We have synthesized and evaluated a series of thirty-two β-hydroxyphosphonate derivatives as potential cN-II ligands to determine their activity against cN-II and possible mechanisms of inhibition. In addition, molecular modeling and docking studies were performed to identify the interaction profile of these compounds, as well as key structural parameters responsible for their properties.

2. Results and discussion

2.1. Chemistry

Synthesis of altrofuranoside derivatives **5**–**8** and acyclophosphonates **19**–**22** was initiated from the previously obtained 1-[6-deoxy-6-diethylphosphono-β-D-allofuranosyl] uracil **1** (Scheme 1) [17]. Inversion of the C2′ configuration was envisaged through a well-known two-steps sequence [18] involving the formation of the 2,2′-anhydro intermediate **29** and subsequent opening of the nucleobase-sugar ring. Thus, diethylphosphoester analogue **5** was reacted with trimethylsilyl bromide affording the corresponding phosphonic acid (as sodium salt after percolation on a Dowex ion-exchange resin) **6** in low yield, due to purification losses. From intermediate **5**, the

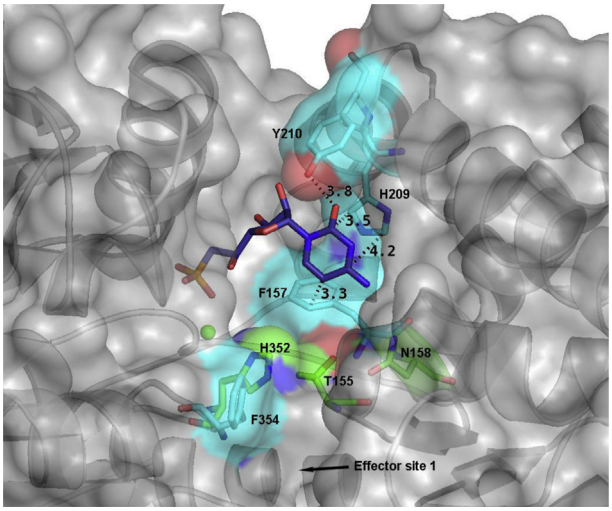

Fig. 2. Surface representation of the IMP binding site (PDB: 2XCW) in presence of the cytosine derivative **4** obtained by molecular docking (starting point for optimization) and showing a large cavity (delineated by the three residues F157, H209 and Y210) surrounding the nucleobase. The magnesium ion is depicted as a green sphere and secondary elements of cN-II are shown in gray. The contacts between derivative **4** and the hydrophobic clamp are indicated in Å. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)