CYTOSINE CONTAINING NUCLEOSIDE β-HYDROXY-PHOSPHONATE ANALOGS AS POTENTIAL INHIBITORS OF CYTOSOLIC 5'-NUCLEOTIDASE

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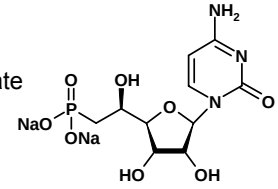
Introduction

The cytosolic 5'-nucleotidase (cN-II) is a promising target in cancer's chemotherapy, as inhibition of this enzyme may reduce resistance phenomenon and thus repotentiate the clinical use of cytotoxic nucleosides.[1] Thus, a first lead was previously identified in our laboratory, the nucleoside β-hydroxyphosphonate of cytosine (UA1776, Figure 1), which inhibits nucleotidase activity of cN-II *in vitro* and could be used as starting point for further optimization.[2] Indeed, molecular modelling studies highlighted the presence of an hydrophobic pocket closed to the nucleobase and involving two phenylalanine residues (Phe 157 and 354, Figure 2). Therefore, we decided to explore the effect of modifications at position C5 of the heterocycle through the introduction of hydrophobic and/or aromatic substituents.

Figure 1. Structure of β-hydroxyphosphonate of cytosine UA1776.



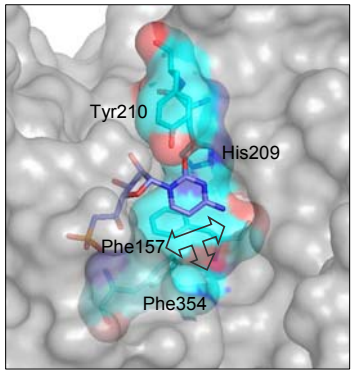
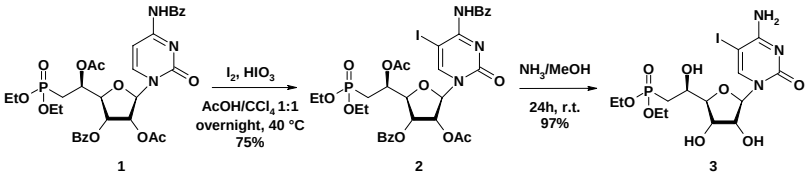


Figure 2. Surface representation of the IMP binding site of cN-II in presence of UA1776 (lead compound for further optimization) and showing a large cavity (triple arrow) available in the vicinity of the C5 position of the nucleobase.

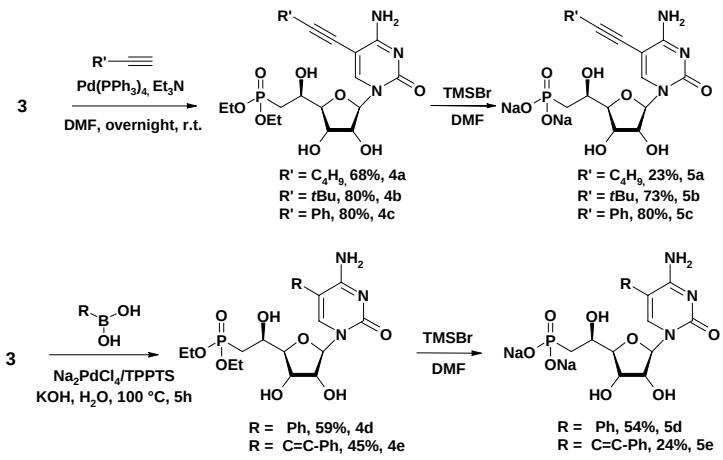
Chemistry

To introduce apolar and aromatic groups, we used the well-know reactions of Sonogashira and Suzuki-Miyaura, and requiring a 5-iodo-nucleoside intermediate **3** (Scheme 1). This last was obtained in two steps, following an adapted procedure, from the protected nucleoside β-hydroxyphosphonate of cytosine **1**.[3]

Then, the required alcynes or arylboronic acids were coupled (Scheme 2), leading to derivatives **4a-e** in modest to good yields. Finally, removal of the phosphonoester protecting groups was performed, giving rise to the targeted phosphonic acids **5a-e**, as sodium salts.



Scheme 1. Synthesis of the 5-iodo-nucleoside intermediate **3**



Scheme 2. Synthesis of C5-modified derivatives **5a-e**

Conclusion

We have synthesized C5-modified nucleoside β-hydroxyphosphonate of cytosine using Suzuki and Sonogashira couplings. The inhibitory activity of these compounds was evaluated towards purified recombinant cN-II (Table). Molecular modelling studies were also performed (Figure 3) and shown interactions between apolar residues of the catalytic site of the protein and aromatic/aliphatic groups. However, this last were close to a clamp of polar residues and generate steric clash.

In vitro inhibition assays

R		Inhibition (%) at 1 mM	K_i (mM)
H		85 ± 5	1.74
-C≡C-nBu	**5a**	40 ± 10	n.d.
-C≡C-tBu	**5b**	46 ± 20	~ 10
-C≡C-Ph	**5c**	n.d.*	8.90
-Ph	**5d**	60 ± 10	2.00
-C=C-Ph	**5e**	n.d.*	1.14

* Precipitate in presence of the chemical reagent used (green malachite)

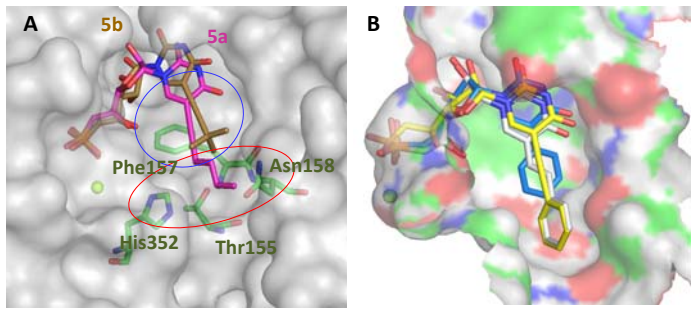


Figure 3. (A) Binding mode obtained with nucleobase-modified derivatives **5a** and **5b** showing the hydrophobic pocket and including Phe157 (blue circle) and polar residues Thr155, Asn158 and His352 belonging to the same cavity (red circle). (B) Binding poses of derivatives **5c** (yellow), **5d** (blue) and **5e** (white) to compare the aromatic substitutions bearing a double or a triple bond.

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References: 1) Sève P. et al. *Lung Cancer*, **2005,** *49*, 363-370. Jordheim L.P. et al. *Curr. Med. Chem.*, **2013**, in press. 2) Gallier F. et al. *PLoS Computational Biology*, **2011**, 7, e1002295. 3) Gallier F. et al. *Eur. J. Org. Chem.*, 2007, 38(7), 925-33.