Bioorganic & Medicinal Chemistry Letters 21 (2011) 6161–6165

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

A new *N*-methyl thymine derivative comprising a dihydroxyisobutenyl unit as ligand for thymidine kinase of herpes simplex virus type 1 (HSV-1 TK)

Svjetlana Krištafor [a], Ivana Novaković [a], Tatjana Gazivoda Kraljević [a], Sandra Kraljević Pavelić [b], Pero Lučin [c], Yvonne Westermaier [d], Lucile Pernot [d], Leonardo Scapozza [d], Simon M. Ametamey [e], Silvana Raić-Malić [a,*]

[a] Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, HR-10000 Zagreb, Croatia
[b] Department of Biotechnology, University of Rijeka, HR-51000 Rijeka, Croatia
[c] School of Medicine, University of Rijeka, HR-51000 Rijeka, Croatia
[d] Pharmaceutical Biochemistry, School of Pharmaceutical Sciences, University of Geneva, CH-1211 Geneva 4, Switzerland
[e] Center for Radiopharmaceutical Sciences, ETH Zurich (Swiss Federal Institute of Technology), CH-8093 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 5 July 2011
Revised 27 July 2011
Accepted 29 July 2011
Available online 6 August 2011

Keywords:
6-(1,3-Dihydroxyisobutenyl)thymine
Herpes simplex virus type 1 thymidine kinase (HSV-1 TK)
Molecular docking
Positron emission tomography (PET)

ABSTRACT

Molecular modeling and phosphorylation assay in vitro were employed to select a novel unsaturated 1,3-dihydroxyisobutenyl thymine derivative **6** as ligand for HSV-1 TK which may be of interest as lead for the development of an positron emission tomography (PET) imaging agent. Compound **6** was successfully prepared using modified approaches. A significant improvement over the syntheses involving pathways A and B (1% and 3% overall yield, respectively), was observed using synthetic route C (14% overall yield).

A large number of thymidine analogs and acyclic guanosine derivatives show antiviral activities against herpes simplex virus type 1 (HSV-1) and 2 (HSV-2).[1] The antiviral activity of these compounds is due to their selective and efficient in vivo activation through monophosphorylation by the viral enzyme.[2,3] The monophosphates are converted to diphosphates, and then to the corresponding triphosphates by cellular enzymes. The triphosphates prevent viral replication by inhibition of the viral DNA polymerase.[4] The molecular basis of the herpes viral thymidine kinase (TK)-based therapy is its difference in substrate specificity as compared to the human cellular isoenzyme. Therefore, HSV-1 TK in combination with a nucleoside analog as fraudulent substrate can be used as a suicide enzyme in the gene therapy of cancer.[5–7] Furthermore, these compounds labeled with positron-emitting radioisotopes can be used as in situ reporter probes to allow non-invasive imaging of HSV-1 TK gene expression using positron emission tomography (PET).[8–11] However, most of the PET reporter probes have several shortcomings, such as unfavorable cytotoxicity and pharmacokinetics. As found earlier, the pronounced biological activities exhibited by C-6 substituted pyrimidine derivatives provide a good rationale for further exploring the chemistry and biological activities of these compounds.[12–16] Thus, we have synthesized nucleoside mimetics in which the acyclic sugar moiety is attached at the C-6 rather than at the *N*-1 position. While some C-6 fluoroalkylated pyrimidines revealed pronounced cytostatic activities,[17–19] thymines with 6-(2,3-dihydroxypropyl)[20] and 6-(1,3-dihydroxyisobutyl)[21] side-chain have been developed as tracer molecules for monitoring HSV-1 TK expression by means of PET. Although the *N*-methylated thymine with 1,3-dihydroxyisobutyl unit at C-6, *N*-Me-[^{18}F]FHBT, was not superior to purine analog [^{18}F]FHBG, *N*-Me-[^{18}F]FHBT clearly showed the feasibility of its use as a PET probe to monitor HSV-1 TK gene expression in vivo.[22] Moreover, it was demonstrated that the introduction of a double bond in some acyclic nucleoside analogs[23] gave a slight rigidity allowing the nonnatural substrates to improve the interactions with the enzyme with respect to the natural ones. This structural modification changed the flexibility of the chain and stabilized compounds in the best phosphorylation enabling conformation.

Thus, in continuation of our previous work, we selected unsaturated *N*-methyl thymine derivative with dihydroxyisobutenyl side chain at C-6 (**6**) as a target compound. In order to evaluate whether compound **6** would be a substrate for HSV-1 TK, this compound was docked into the active site of this enzyme complexed

* Corresponding author. Tel.: +385 1 4597 213; fax: +385 1 4597 224.
E-mail address: sraic@fkit.hr (S. Raić-Malić).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.07.115

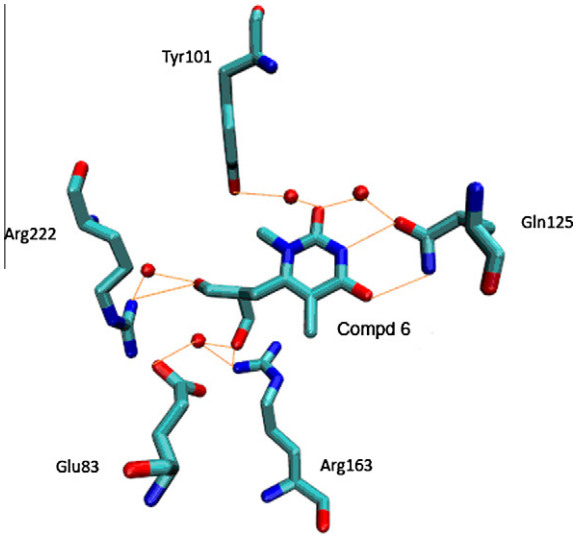


Figure 1. Representative binding mode for **6** obtained by molecular docking.

with 5′-deoxythymidine (dT) using the docking program Gold 4.0.[24] The interactions of **6** with residues of the binding site are depicted in Figure 1. Compound **6** forms H-bonds to several binding site residues either directly or via water molecule.

The extensive H-bonding network between HSV-1 TK and **6** includes the same residues as for dT, except that no H–bond is formed to the LID domain residue Glu225. It is interesting to note that Tyr101 is involved in an H-bond with thymine instead of the sugar moiety. Importantly, the hydroxyl group of compound **6** points towards the catalytic center of the enzyme formed by Glu83 and Arg163. Due to these interactions, it was predicted that this compound would undergo phosphorylation since its hydroxyl functionality mimics the 5′-hydroxyl group of the natural substrate thymidine.

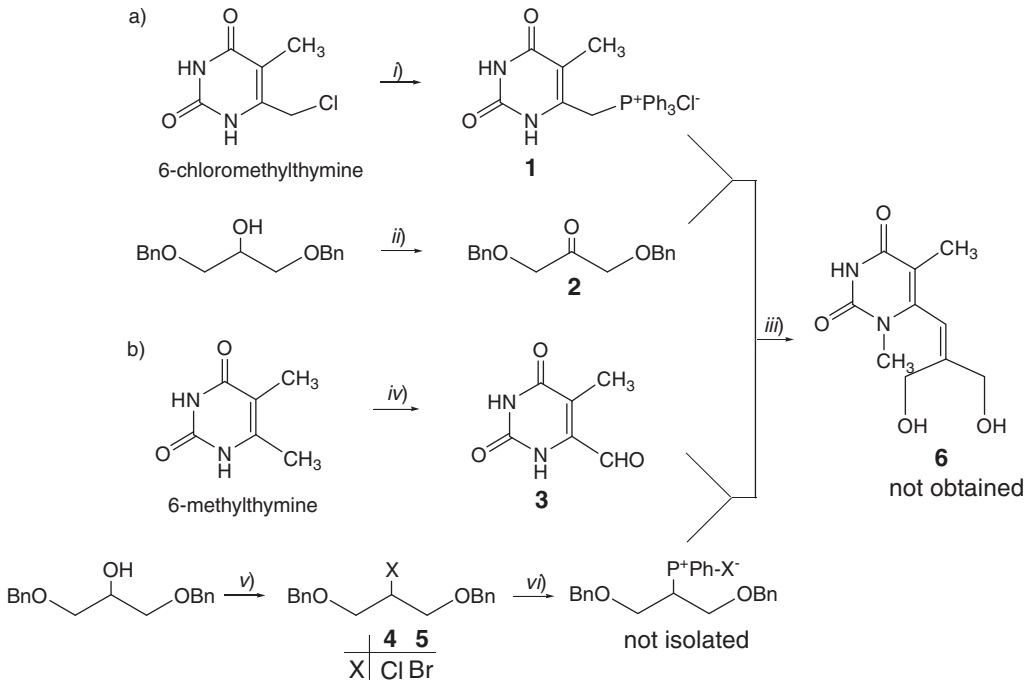
Table 1
An overview of the yields in the pathways A, B, and C for preparation of *N*-methylated thymine derivative **6**

Reactions	Substrate	Product	Pathway	Yield (%)
Debenzylation	**7**	**8**	A	57
	12	**13**	B	54
	12	**14**	C	68
Acetylation	**8**	**9**	A	52
	13	**15**	B	52
	14	**16**	C	78/93[a]
Fluorination	**9**	**10**	A	26
	15	**11**	B	29
	16	**17**+**18**	C	24+27
Deacetylation and dehydrohalogenation	**11**	**6**	A, B	56
	17+**18**	**6**	C	64
N-methylation	**10**	**11**	A	27
	7	**12**	B, C	66
Overall yield of **6**			A	1
			B	3
			C	14

[a] The acetylation of **14** using Ac_2O in the presence of 4-dimethylaminopyridine (DMAP) and pyridine in CH_2Cl_2 at lower temperature afforded **16** with an excellent yield (93%), while acetylation of **14** using Ac_2O in pyridine at rt gave same product in lower yield (78%) (Supplementary data).

Different synthetic routes to the target compound **6** with the double bond as a rigid linker between hydroxymethyl groups and a base residue were evaluated (Table 1). For the synthesis of the compound **6**, we assumed that the Wittig reaction using the corresponding triphenylphosphonium ylide and 1,3-dibenzyloxy-2-propanone (**2**), or thymine-6-carboxaldehyde (**3**)[25] would give **6**. However, the coupling of the phosphonium salt of 6-chloromethyl thymine (**1**) to ketone **2** did not afford the unsaturated C-6 acyclic thymine (Scheme 1).

Moreover, attempts to apply another approach using thymine-6-carboxaldehyde (**3**) and phosphonium salts of related alkyl



Scheme 1. Reagents and conditions: (i) PPh_3, DMF, 70 °C, 12 h; (ii) (1) NCS, CH_2Cl_2, $(CH_3)_2S$, rt, −25 °C, 0.5 h; (2) Et_3N, rt, 12 h; (iii) KO-*t*-Bu, DMF, −30 °C, 0.5 h, rt, 24 h, 70 °C, 2 h; (iv) SeO_2, AcOH, reflux, 4 h, rt, 24 h; (v) CCl_4 (or CBr_4), PPh_3, CH_2Cl_2, rt, 3 h; (vi) PPh_3, DMF, rt, 2 h, 70 °C, 1 h.