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Guanine α-carboxy nucleoside phosphonate (G-α-CNP) shows a different inhibitory kinetic profile against the DNA polymerases of human immunodeficiency virus (HIV) and herpes viruses

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Dedicated to my formal mentor at NIH, Dr. David G. Johns who passed away in December 2016.

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(1*R*,4*S*)-(−)-*cis*-4-acetoxy-2-cyclopenten-1-ol (PubChem CID: 9855498)
(1S,4R)-(+)-*cis*-4-acetoxy-2-cyclopenten-1-ol (PubChem CID: 10154103)
Tris (PubChem CID: 6503)
EDTA (PubChem CID: 6049)
NaCl (PubChem CID: 5234)
Formamide (PubChem CID: 713)
Bromophenol blue (PubChem CID: 8272)
Polyacrylamide (PubChem CID: 6579)
Sodium cacodylate (PubChem CID: 2724247)
$MgCl_2$ (PubChem CID: 5360315)
Dithiothreitol (PubChem CID: 446094)
Glutathione (PubChem CID: 124886)
KCl (PubChem CID: 4873)
Triton X-100 (PubChem CID: 5590)
Trichloroacetic acid (PubChem CID: 6421)
Sodium pyrophosphate ($Na_4P_2O_7$) (PubChem CID: 24003)
$(NH_4)_2SO_4$ (PubChem CID: 22921)
Ethanol (PubChem CID: 702)
Glycerol (PubChem CID: 753)

ABSTRACT

α-Carboxy nucleoside phosphonates (α-CNPs) are modified nucleotides that represent a novel class of nucleotide-competing reverse transcriptase (RT) inhibitors (NcRTIs). They were designed to act directly against HIV-1 RT without the need for prior activation (phosphorylation). In this respect, they differ from the nucleoside or nucleotide RTIs [N(t)RTIs] that require conversion to their triphosphate forms before being inhibitory to HIV-1 RT. The guanine derivative (G-α-CNP) has now been synthesized and investigated for the first time. The (L)-(+)-enantiomer of G-α-CNP directly and competitively inhibits HIV-1 RT by interacting with the substrate active site of the enzyme. The (D)-(−)-enantiomer proved inactive against HIV-1 RT. In contrast, the (+)- and (−)-enantiomers of G-α-CNP inhibited herpes (i.e. HSV-1, HCMV) DNA polymerases in a non- or uncompetitive manner, strongly indicating interaction of the (L)-(+)- and the (D)-(−)-G-α-CNPs at a location different from the polymerase substrate active site of the herpes enzymes. Such entirely different inhibition profile of viral polymerases is unprecedented for a single antiviral drug molecule. Moreover, within the class of α-CNPs, subtle differences in their sensitivity to mutant HIV-1 RT enzymes were observed depending on the nature of the nucleobase in the α-CNP molecules. The unique properties of the α-CNPs make this class of compounds, including G-α-CNP, direct acting inhibitors of multiple viral DNA polymerases.

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1. Introduction

A broad variety of antiviral drugs are clinically approved (for an overview, see Ref. [1]). The majority of these drugs are nucleoside analogues for which the virus-encoded DNA and RNA polymerases are the prime targets for the inhibition of virus replication. Indeed, a variety of nucleoside analogues efficiently inhibit the herpes DNA polymerases encoded by herpes simplex virus type 1 and type 2 [2], varicella zoster virus (VZV) [2,3] and human cytomegalovirus (HCMV) [2,4], the reverse transcriptase of retroviruses such as HIV-1 and HIV-2 [5], the DNA polymerase of hepatitis B virus [6], as well as the RNA polymerases specified by several RNA viruses such as respiratory syncytial virus [7], influenza viruses [8], and also flaviviruses as exemplified by hepatitis C virus [9,10]. Such inhibitors need to be activated (phosphorylated) by virus-encoded kinases or by cellular nucleoside/nucleotide kinases to their 5′-triphosphate derivatives, or in case of the acyclic nucleoside phosphonates to their diphosphate derivatives before these nucleoside/nucleotide analogues can be recognized by the viral DNA or RNA polymerases [1–10]. The nucleoside analogues are often incorporated in the growing viral DNA or RNA chain, and function as chain terminators [2,5]. Instead, several non-nucleoside derivatives have also been discovered to act against viral polymerases, either at a non-substrate active site of the enzyme [such as the non-nucleoside RT inhibitor (NNRTI) interaction with HIV-1 RT] [11–13] or at the substrate-active site of the RT enzyme [such as the nucleotide-competing RT inhibitor (NcRTI) (i.e. INDOPY)] [14–16]. Such agents differ from a classic nucleoside structure and do not need prior metabolic conversion to exhibit inhibitory activity against their specific polymerase target(s).

Members of the nucleoside analogue class of compounds represent the highest number of clinically approved antiviral drugs [1]. However, due to their obligatory dependence on cellular metabolism and often inefficient intracellular conversion to their antivirally-active phosphorylated metabolites, efforts have been devoted to deliver directly and more efficiently the nucleoside/nucleotide drugs intracellularly as their activated (mono)phosphorylated derivatives [17–20]. Although this approach proved successful in several cases, the compounds usually still need to be converted to their eventual triphosphate equivalent before being inhibitory against the viral polymerase.

Recently, we have reported an entirely different approach to design nucleotide analogues (Fig. 1) that are directly inhibitory to viral DNA polymerases without the need of additional metabolic conversions [21,22]. The prototype of these novel nucleotide analogues consists of a nucleobase, connected to an α-carboxy phosphonate moiety through a cyclic cyclopentyl linker entity. Crystallographic, kinetic and biochemical studies have shown that such compounds could directly bind to the substrate-active site of HIV-1 RT without any prior metabolic conversion enabling base pairing and active site Mg^{2+} ion chelation similar to the natural dNTPs [21,23]. It was shown that one oxygen of the carboxylate and two oxygens of the phosphonate part of the α-CNP backbone are involved in Mg^{2+} ion coordination. These three oxygens mimic the three chelating α-,β- and γ-phosphate oxygen atoms of a dNTP [21,23].

Although the thymine-, uracil-, cytosine- and adenine-α-CNPs are potent inhibitors of HIV RT, they are less inhibitory to herpes DNA polymerases [21]. However, the guanine α-CNP derivative had not been synthesized and evaluated in previous studies. Given the importance of guanine nucleoside analogues as antiherpes (i.e. acyclovir, ganciclovir) or anti-HIV (i.e. abacavir) agents, we now synthesized the guanine α-CNP (G-α-CNP) and investigated its inhibitory activity against HIV-1 RT and herpes DNA polymerases. The G-α-CNP derivative displayed comparable inhibitory activity against HIV-1 RT and the herpes DNA polymerases as the prototype thymine-α-CNP, but showed differences in its inhibitory activity against mutant HIV-1 reverse transcriptases. Its mechanism of action appears to be significantly different for HIV-1 *versus* the herpes DNA polymerases. This novel compound is of interest as a potential dually-active compound with significant concomitant anti-HIV and anti-herpes virus DNA polymerase activity.

2. Materials and methods

2.1. Compound synthesis and characterization data

2.1.1. G-α-CNP [cis-9-{4-[Carboxy(phosphono)methoxy]cyclopentan-1-yl}guanine]

Racemic G-α-CNP was prepared in nine steps starting from 2-cyclopentenone. The materials were purchased from Acros Organic, Fisher Scientific Ireland Ltd. (Dublin, Ireland). The enantiomers (L)-(+)-G-α-CNP and (D)-(−)-G-α-CNP were prepared in seven steps starting from (1*R*,4*S*)-(−)-*cis*-4-acetoxy-2-cyclopenten-1-ol and (1*S*,4*R*)-(+)-*cis*-4-acetoxy-2-cyclopenten-1-ol, respectively. Full details of the synthesis will be described elsewhere. Characterization data: ^{1}H NMR (400 MHz, D_2O): δ 1.08–2.00 (4H, m), 2.00–2.14 (1H, m), 1H 2.40–2.50 (1H, m), 3.93 (0.5H, d, *J* = 18.2), 3.99 (0.5H, d, *J* = 18.3) 4.03–4.10 (1H, m), 4.51–4.62 (1H, m), 8.03 (0.5H, s), 8.04 (0.5H, s); ^{13}C NMR (125 MHz, D_2O): δ 9.4, 30.5, 30.6, 37.8, 38.8, 53.3, 53.5, 80.0, 80.5, 115.8, 138.8, 151.2, 153.4, 158.8, 158.9, 177.6; ^{31}P NMR (162 MHz, D_2O): δ 12.36, 12.48; *m*/*z* (ES−) 372.1 $[M-H]^-$; HRMS (ES+) Exact mass calculated for $C_{12}H_{17}N_5O_7P$ $[M+H]^+$ 374.0866; found 374.0861.

2.2. Reverse transcriptase, nucleic acids, and small molecules

HIV-1 reverse transcriptase was expressed and purified as described previously [33]. Oligodeoxynucleotides were synthesized and purchased from Integrated DNA Technologies, Coralville, USA. The following sequence was used as template: PPT57, CGTT GGGAGTGAATTAGCCCTTCCAGTCCCCCCTTTTCTTTTAAAAAGTGGCT AAGA. Primer sequences used were: PPT-17, GCCACTTTTTAAAA GAAAAGGGGGG; PPT+16, AAAGGGGGGACTGGAAGGGCTAATT. Deoxynucleotides were purchased from Integrated DNA Technologies.

2.3. DNA synthesis

A 3-fold excess of PPT-57 DNA template was heat-annealed to 50 nM 5′-fluorolabeled PPT-17 primer, then incubated with 250 nM of RT in a buffer containing 50 mM Tris-HCl (Sigma, St. Louis, MO), pH 7.8, 50 mM NaCl (Sigma), 0.3 mM EDTA (Sigma), and 0.5 μM of each of dATP, dTTP, dGTP, and dCTP (GE Healthcare,