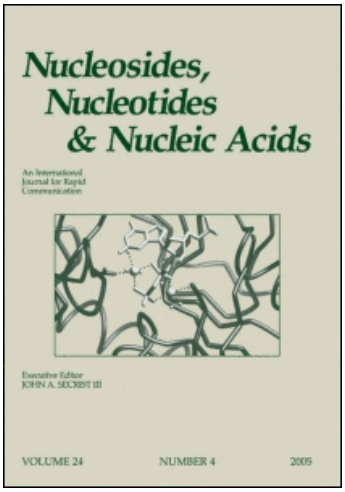

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

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Online publication date: 09 August 2003

To cite this Article Yamaguchi, Toyofumi , Takahashi, Hazuki , Jinmei, Hiroshi , Takayama, Yuko and Saneyoshi, Mineo(2003) 'Inhibition of Vertebrate Telomerases by the Triphosphate Derivatives of Some Biologically Active Nucleosides', Nucleosides, Nucleotides and Nucleic Acids, 22: 5, 1575 — 1577
To link to this Article: DOI: 10.1081/NCN-120023037
URL: http://dx.doi.org/10.1081/NCN-120023037

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS
Vol. 22, Nos. 5–8, pp. 1575–1577, 2003

Inhibition of Vertebrate Telomerases by the Triphosphate Derivatives of Some Biologically Active Nucleosides

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ABSTRACT

In order to clarify the effect of the base moiety of nucleotide analogs on telomerase inhibition, triphosphate derivatives of biologically active nucleosides, 3′-azido-3′-deoxythymidine (AZT), 2′-deoxy-2′-fluoroarafuranosylthymine (FaraT), acycloguanosine (ACG) and their guanine or thymine counterparts (AZdG, FaraG and ACT, respectively) were investigated. In all of the present cases, guanine derivatives showed more potent inhibition than their thymine counterparts.

INTRODUCTION

Telomerase, which catalyses telomere DNA elongation in eukaryotic cells through addition of the G-rich repeat,[1] especially 5′-TTAGGG-3′ in vertebrates, using an RNA template in the enzyme molecule, is classified as one of the reverse

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DOI: 10.1081/NCN-120023037
1525-7770 (Print); 1532-2335 (Online)

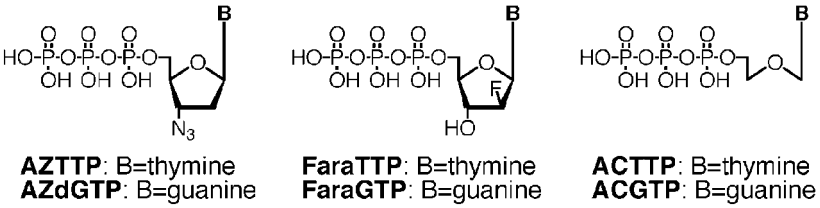

Figure 1. Nucleotide analogs examined in this study.

transcriptases. To clarify the susceptibility of telomerase to sugar-modified nucleotide analogs, we investigated the inhibitory effect of 3′-azido-3′-deoxythymidine 5′-triphosphate (AZTTP), 2′-deoxy-2′-fluoroaraTTP (FaraTTP), acycloguanosine triphosphate (ACGTP)–triphosphate derivatives of typical biologically active nucleosides–and their guanine or thymine counterparts (AZdGTP, FaraGTP and ACTTP, respectively) (Fig. 1).

MATERIALS AND METHODS

Nucleotide Analogs–AZdGTP and FaraGTP were obtained from the corresponding nucleosides[2,3] by chemical phosphorylation.

Telomerase Activity Measurement–To study the properties of telomerase and for inhibition studies with some compounds, the stretch PCR assay was performed as described for human HeLa cell extracts.[4]

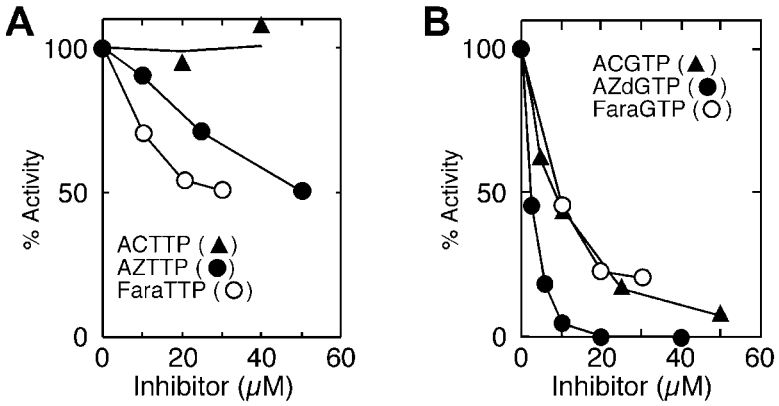

Figure 2. After an extension reaction with telomerase (HeLa cell S100 extract) in the presence of various concentrations of the compound, the DNA products were purified by ethanol precipitation and then amplified by the stretch PCR method. The PCR products were separated by polyacrylamide gel electrophoresis and detected by staining with SYBR green I. The amounts of PCR products, constituting a DNA ladder, were estimated using a fluorescence image analyzer. Telomerase activity was measured in the presence of the indicated concentrations of nucleotide analogs, 10 μM dTTP (Fig. 2A) or 10 μM dGTP (Fig. 2B) and the other two dNTPs each at 200 μM. Activity without an inhibitor was taken as 100%, and the activities remaining are shown.

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RESULTS AND DISCUSSION

As shown in Fig. 2A and 2B, the thymine analogs AZTTP and FaraTTP produced moderate or weak inhibitory effects. On the other hand, the guanine analogs ACGTP, AZdGTP and FaraGTP exhibited potent inhibitory effects. As a preliminary experiment, we analyzed the primer extension products that were synthesized by cherry salmon testis telomerase in the presence of nucleotide analogs. AZdGTP was effectively incorporated at the 3′-terminus of the primer strand and chain termination was elicited. Telomerase incorporates as many as three dGMP residues during an extension reaction of six residues. As expected, dGTP analogs appear to show promise as telomerase inhibitors. Further study of telomerase inhibitors is now under way in our laboratory.

ACKNOWLEDGMENTS

We thank Dr. Hiroshi Shiragami, Ajinomoto Company Inc., for kindly providing acycloguanosine. This work was supported in part by a Grant-in-Aid for Scientific Research on Bioscience/Biotechnology Areas from the Ministry of Education, Culture, Sports, Science and Technology, and a grant from the Promotion and Mutual Corporation for Private Schools in Japan.

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