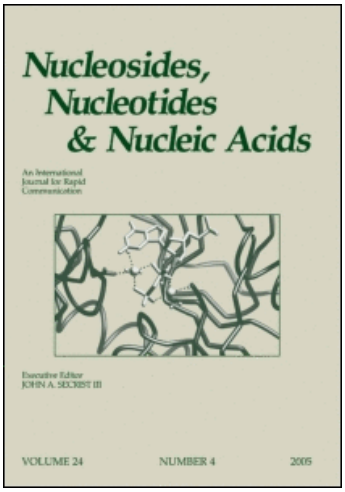

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Sandrine Pairot[a]; Audrey Farese-Di Giorgio[a]; Christophe Di Giorgio[a]; Nadia Patino[a]; Roger Condom[a]; Roger Guedj[a]
[a] Laboratoire de Chimie Bio-Organique, CNRS ESA 6001, Nice Cedex, France

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CONCEPTION, PREPARATION AND BIOLOGICAL EVALUATION OF ONE INHIBITOR OF THE HIV-1 TAT-TAR COMPLEX

Sandrine Pairot, Audrey Farese-Di Giorgio, Christophe Di Giorgio, Nadia Patino, Roger Condom*, Roger Guedj

Laboratoire de Chimie Bio-Organique, CNRS ESA 6001, Université de Nice-Sophia Antipolis F-06108 Nice Cedex 2, France

ABSTRACT : A new compound Z-AG5-E potentially inhibitor of the HIV-1 Tat-TAR complex was prepared. This compound is constituted by a dinucleotide analog (PNA dimer) bound, through a linker, to an arginine residue. Z-AG5-E inhibits viral development in cell culture with a micromolar IC50 and without cellular toxicity until 200μM concentration. Circular dichroism studies have shown that Z-AG5-E binds a synthetic TAR-RNA.

INTRODUCTION

The therapies used at the moment against HIV, try to limit viral replication with drugs inhibiting one or several steps of the replication cycle. HIV contains sequences coding for regulation proteins among which Tat protein. In fact, transcription activation gene expression by Tat protein involves complex formation with mRNA target sequence named TAR ("Trans-Activation-Responsive-element") that is located downstream of the transccription start site in the viral Long-Terminal-Repeat (LTR). TAR is a nascent RNA transcript that has a stable stem loop structure, formed by base-pair interactions between nucleotide +1 to +59. It contains a six-nucleotide loop (residues 30-35) and a three-nucleotide bulge (residues 20-23) that are both necessary for Tat function [1,2].

RESULTS AND DISCUSSION

Tat is able to bind to TAR RNA to form a one to one complex. The basic region of Tat is directly involved in RNA binding through, among others, an arginine residue. Upon

complexation, only the region around the bulge appears to be involved. The fixation of Tat protein induces a conformational change of TAR. The stacked arrangement of the three bulge nucleotides is disrupted. The residues C24 and U25 of the bulge as well as the residues C30 and U31 of the loop are free from interactions. The fixation site of Tat is found to be spacially close both to the bulge and to the six-nucleotide loop region [3,4].

We described here, the synthesis of one molecule that could inhibit the formation of the Tat-TAR complex. The general structure of the molecule is : $(PNA)_2$-Linker-Arginine.

Thus compound is constituted by a dinucleotide analog (A-G-PNA dimer) complementary to the residues C and U of the loop or the bulge. The dimer is bound through a linker to an arginine, which should "drive" the molecule towards the fixation site.

We have chosen Polyamide Nucleic Acids (PNAs) as structural nucleotide analogs because of their high affinity for DNA or RNA. They are more stable towards nucleases and more lipophilic than their natural homologs. We have previously described the synthesis of the PNA dimer [5]. The preparation of Z-AG5-E results from first the condensation of linker-arginine moiety with the PNA dimer, and then from the remove of all the protecting groups.

The linker-arginine moiety was prepared in three steps starting from commercially available N-ε-Z-ε-aminocaproic acid and H-Arg(Pmc)-OH.

A circular dichroism study shows that Z-AG5-E interacts with a synthetic TAR RNA (G17-C45).

Moreover, Z-AG5-E shows a micromolar activity as well on PBMC / IIIB as on CEM-SS / LAI infected cells, without cellular toxicity until 200μM.

CONCLUSION

The compound Z-AG5-E constituted by a PNA dimer and an arginine residue has been shown to be active on HIV-1 infected cells. This molecule could target TAR RNA. Z-AG5-E constitutes a "lead compound" of a new class of inhibitors and structure / activity studies are in progress to improve the anti HIV activity and to confirm the target.

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