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Design, synthesis and bioactivities of TAR RNA targeting β-carboline derivatives based on Tat–TAR interaction

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Abstract

A series of new β-carboline derivatives **3**–**14** bearing guanidinium group or amino group-terminated side chain targeting the TAR RNA were designed and synthesized. Molecular modeling studies indicated that the minimal interaction energy was obtained for compound **11**, which contained the optimal linker of three methylene groups and the terminal guanidinium group interacted with the three-base bulge of TAR element by hydrogen bonds, which were the main contributor to the stability of drug–TAR RNA complex. To evaluate the ability of compounds **3**–**14** to block Tat–TAR interaction, we established a rapid, sensitive quantitative bioassay based on transient cotransfection of a Tat expression vector and a long terminal repeat region–chloramphenicol acetyltransferase (LTR–CAT) reporter construct in eukaryotic cells, monitoring the influence of the compounds on CAT expression levels with ELISA. Compounds **11** and **12** were the most active compounds of all in inhibiting Tat–TAR interaction bearing the terminal guanidinium group, and the optimal linker of the three methylene groups. Both compounds also exhibited anti-HIV-1 activity in MT4 cells, and their LD_{50} values of intraperitoneal acute toxicity for mice were 320.0 and 104.3 mg/kg, respectively. Furthermore, the results of capillary electrophoresis (CE) suggest that it is through targeting TAR RNA that this series of compounds block the Tat–TAR interaction.

Keywords: HIV; Tat–TAR interaction; β-Carboline; Anti-HIV activity

1. Introduction

In the past decade, remarkable clinical success in the field of target-directed AIDS treatment has been achieved with drugs against the reverse transcriptase and the protease [1]. However, the rapid development of viral resistance against existing drugs has accelerated the search for alternative targets. Recently, increasing evidence has shown that the viral trans-activator of transcription Tat protein binding to the trans-activation response region (TAR) RNA is an essential step in the human immunodeficiency virus type 1 (HIV-1) replication cycle [1,2]. Therefore, the blockage of Tat–TAR interaction is a potential route for AIDS chemotherapy. TAR RNA is a 59-base stem-loop structure located in the long terminal repeat region (LTR) at the 5′ end of all viral transcripts [3,4]. It contains a six-base loop and a three-base bulge (U23–C24–U25), which separate two helical stem regions [5,6]. Sequence and secondary structure of the apical portion of the HIV-1 TAR RNA containing the Tat binding site and apical loop is shown in Fig. 1. Deletion analyses of TAR RNA and other studies have established that Tat binds to TAR RNA at the three-base bulge and interacts with two base pairs above and

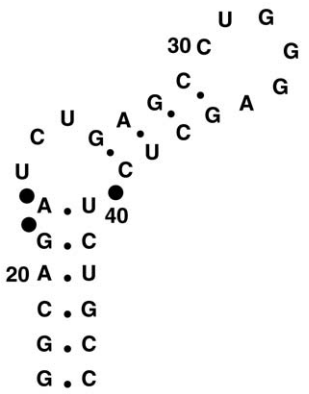

Fig. 1. Sequence and secondary structure of the HIV-1 TAR RNA containing the Tat binding site and apical loop.

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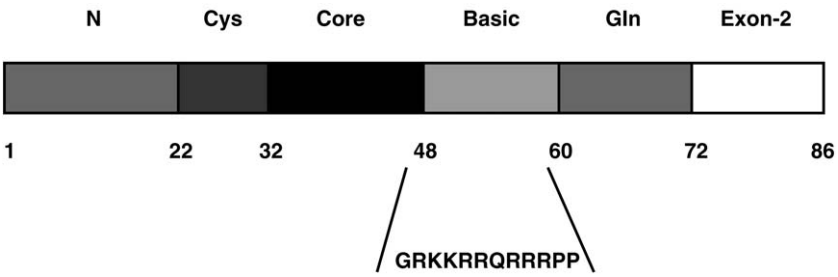

Fig. 2. Functional regions of the Tat protein and sequence of the basic region (48–59), which is one of the crucial sites of TAR recognition.

below the bulge in the major groove of TAR RNA [7], and appears to involve a two-step mechanism [3]. First, in intermolecular recognition involves the binding of arginine residues within the basic region of Tat to the TAR RNA bulge, which induces a conformational change in TAR RNA. Second, recognition by the protein of functional groups presented in the major groove of the TAR bulge. This mechanism has significant implication for the discovery of novel anti-HIV compounds directed at inhibiting the essential Tat–TAR interaction. The unique structure of TAR RNA in this three-base bulge region seems to be an attractive target for designing specific TAR-binding compounds as HIV-1 Tat–TAR inhibitors [8]. Numerous studies have shown that a short region of basic amino acids in Tat (amino acid residues 48–59), termed arginine-rich motif (ARM), is responsible for Tat–TAR specific interaction (Fig. 2) [9,10], and the arginine residue in the basic region is critically important in the binding of Tat and TAR in such a way that guanidinium group interacts with the three-base bulge region of TAR [11,12]. The existing structural information should allow the possibility of rational drug discovery. And also many experiments have provided evidences that the high-affinity binding of small molecules to their RNA targets are governed by the electrostatic interaction due to amino group, guanidinium group or arginine residue [13,14]. It has been proved that acridine derivatives bearing both amino side chain and planar aromatic ring are the most potent Tat–TAR low molecular weight inhibitors known to date by inhibiting the trans-activation of Tat protein and therefore represent a significant lead for the development of new active heterocycle drugs [15]. Our previous studies have indicated that β-carboline derivatives could interact with nucleic acids for its fused planar heterocycle ring structure, the optimized linker length and also some electronic substituents were important in the binding of nucleic acids [16,17]. Therefore, with molecular modeling insights into the interaction between HIV-1 TAR RNA and small molecules, we designed 12 β-carboline derivatives (compounds **3**–**14**) bearing guanidinium group or amino group-terminated side chain targeting the TAR element. To evaluate the ability of compounds **3**–**14** to block Tat–TAR interaction, we established a rapid, sensitive quantitative bioassay based on transient cotransfection of a Tat expression vector and a LTR–CAT reporter construct in eukaryotic cells, monitoring the influence of the compounds on chloramphenicol acetyltransferase (CAT) expression levels with the colorimetric enzyme immunoassay (ELISA). Based on the bioactivity determination in transient cotransfection assay for the new compounds, their anti-HIV-1 activity in vitro and intraperitoneal acute toxicity on mice were also evaluated. In addition, we used capillary electrophoresis (CE) to study the binding specificity of this series of compounds with TAR RNA in inhibiting the Tat–TAR interaction, as CE has become a powerful analytical means in biochemical studies, because of its high efficiency, easy of automation, short analysis time, and low sample consumption in the study of protein–RNA or drug–RNA interaction [18,19].

2. Chemistry

The synthesis of the β-carboline derivatives was outlined in Scheme 1, which was carried out in three steps. First, modified β-carboline compounds **1** and **2** were synthesized using previously described procedures [20,21]. We introduced a carboxyl ester function at position 3 of β-carboline ring for use in anchoring an amino-terminated or guanidine-terminated chain via an amide linkage. Second, we synthesized six molecules (**3**–**8**) containing an amino-terminated side chain by **1** or **2** reacted with 1,2-diaminoethane, 1,3-propanediamine and 1,6-diaminohexane respectively. Third, these compounds reacted with S-methylisothiourea to yield the compounds **9**–**14** bearing guanidine-terminated side chain. New compounds were characterized by MS, ^{1}H and ^{13}C NMR spectroscopy.

3. Molecular modeling

The calculating interaction energies of the 12 compounds with TAR RNA were presented in Table 1. Result of TAR RNA docking of compound **11** as an example was shown in Fig. 3.

4. Biological studies

4.1. Transient cotransfection assay

The results of the transient cotransfection assay for evaluating activities of compounds on Tat–TAR interaction were shown in Fig. 4.