研究报告

# Molecular Mechanism of Macrocyclic Polyamines with Anti-HIV-1 Activity to Recognize RNA and Its Effect on Apoptosis*

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**Abstract** The molecular recognition of macrocyclic polyamines (MP-1, MP-2 and MP-3) to RNA, and its effects on apoptosis of cos-7 cells were studied in order to explore their mechanism of anti-HIV-1 activity. Cleavage of RNA was observed by agarose electrophoresis; and apoptosis was determined by flow cytometry assay. Computer modeling was used to investigate the theoretical possibility of compounds binding to TAR RNA. Results showed that: (1) Compounds MP-1, MP-2 and MP-3 could not only cleave the polyA·polyU and TAR RNA, but also inhibit the interaction of Tat-RNA. (2) Compounds could affect the percentage of hypodiploid cell. It is proposed that compounds MP-1, MP-2 and MP-3 could recognize the polyA·polyU and TAR RNA molecules and cleave them, and affect the interaction of Tat-RNA. The compounds may affect the apoptosis of cos-7 cells.

**Key words** HIV-1, macrocyclic polyamines, apoptosis, docking

Replication of the human immunodeficiency virus 1 (HIV-1) is dependent upon the expression of the trans-activator protein, Tat (Trans-activator of transcription), an 86 amino acid, basic, cysteine-rich, nuclear protein encoded by the tat gene of the virus[1~4]. Tat could bind specially to a RNA stem-loop structure named TAR (trans-activation-responsive region), which is located in the HIV long terminal repeat, and RNA binding is essential for Tat-dependent transcriptional activation. The overall charge density of the Tat peptides is important for binding; it provides a favorable electrostatic environment for its interaction with TAR RNA in the major groove[5~7]. Furthermore, arginine as the free amino acid could also bind specially to TAR RNA through the interaction of guanidium group with $U^{23}$ in the bulge, $A^{27}$·$U^{38}$ in the upper helix and with guanosine-26 ($G^{26}$) that is next to $A^{22}$·$U^{40}$ just below the bulge. Thus, we prospected that the base A and U played an important role in RNA molecular recognition by regulatory protein.

Macrocyclic polyamines coordinating with metal ions are rich in positive charge, and interact with phosphate in aqueous solution and have the characteristics of recognizing the TAR RNA[8~11]. They are known to be a group of compounds providing prospective agents against HIV-1[8]. After coordinating with metal ions, their delayed toxicity was overcome. But their antiviral mechanism was not clear. In this work, we investigated the molecular mechanism of the compounds to recognize the RNA and their effect on apoptosis.

## 1 Materials and Methods

### 1.1 Reagents

polyA·polyU was purchased from Sigma, and agarose and ethidium bromide (EB) from Sigma. TAR RNA with 27 nucleotides and a 16-amino acid peptide used as Tat model were a kind gift from Mr. H. Mazarguil. The test compounds (macrocyclic polyamines) were provided by our laboratory. PE buffer contained 7.5 mmol/L $NaH_2PO_4$, 1.0 mmol/L EDTA, pH 7.0 and 0.5 × TBE contained 45 mmol/L Tris, 45 mmol/L boric acid and 1.0 mmol/L EDTA, pH 8.3.

Cell Line: Cos-7 cells were provided by Professor Ma Da-Long (Department of Immunology, Peking University).

### 1.2 Electrophoretic analysis

A: Mobility shift assay was performed by agarose electrophoresis. Mixture of polyA·polyU and compounds were kept at room temperature for 10 min in the presence or absence of Tat and 1% agarose was conducted in 0.5 × TBE for 1 h at 200 V. polyA·polyU was stained with EB, and then was detected by UV-visible method.

B: Mixture of TAR RNA and compounds were kept at room temperature for 10 min and 1% agarose was conducted in 0.5 × TBE for 1 h at 200 V. TAR RNA was stained with EB, and then was detected by UV-visible method.

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### 1.3 Cell culture

An attached monolayer of Cos-7 cells was continuously incubated at 37 ℃ in a humidified atmosphere containing 5% $CO_2$ in RPMI-1640 supplemented with 10% heat-inactivated fetal calf serum.

### 1.4 Flow cytometry analysis

Cos-7 cells with $1 \times 10^5$/ml were transferred into new culture medium. After incubating 24 h, compounds were added into the culture dishes (their final concentration about 5.0 μmol/L and incubated for 48 h at 37 ℃. Cell monolayer were digested, washed three times with cold PBS, fixed by 70% ethanol for 30 min at 4 ℃. Then the cells were diluted into $1 \times 10^6$/ml suspension, digested with RNase and stained by PI. Percentage of hypodiploid cell was measured by FACScan.

### 1.5 Molecular modeling

All modeling studies were conducted with Biosym Technologies Modules INSIGHT II, BIOPOLYMER, DISCOVER, DISCOVER-3, DOCKING (Version 95.0), run on a Silicon Graphics Indigo workstation. The structure was then subjected to an energy minimization procedure using the Discover. The model was further refined by molecular dynamics stimulated annealing. The docking procedure used involved minimizing electrostatic and Van der waals' interaction energies of conformational rigid structure. Systematic searching for orientations of the compounds, which offered the best register and resulted in the lowest interaction energy, performed docking of the compounds into the 27-residue TAR fragment.

## 2 Results

### 2.1 Cleavage of compounds on polyA·polyU

Mixture of polyA·polyU and compounds kept at room temperature for 10 min was analyzed by 1% agarose electrophoresis. From Figure 1 we observed that compounds MP-1, MP-2 and MP-3 could cleaved polyA·polyU strongly (6.0 μmol/L) and made RNA bands disappeared, especially the compound MP-2.

### 2.2 The inhibition of polyA·polyU binding to Tat peptide

Mobility shift assay was performed by agarose electrophoresis. Mixture of polyA·polyU and compounds were kept at room temperature, Tat was added into the system after 10 min later and 1% agarose was conducted (Figure 1). The results demonstrated that the compounds MP-1, MP-2 and MP-3 could inhibit the binding of Tat to polyA·polyU resulted from cleaving polyA·polyU, and made the free RNA bands vanished.

### 2.3 Interaction between TAR RNA and compounds

Mixture of TAR RNA and compounds were kept at room temperature for 10 min and 1% agarose was conducted (Figure 2). Electrophoresis pattern indicated that compounds MP-1, MP-2 and MP-3 could recognize TAR RNA molecules and made them cleaved (4.5 μmol/L), especially the compounds MP-2.

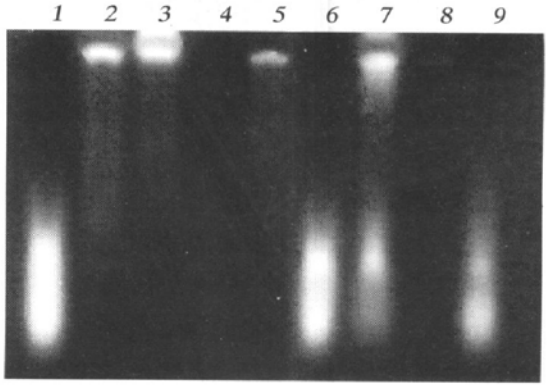


**Fig. 1 The agarose electrophoresis map of polyA·polyU cleaved by compounds in the presence(lane *3* ~ *5*) and absence of Tat(*7* ~ *9*)**

*1* and *6*: polyA·polyU only; *2*: polyA·polyU + Tat.

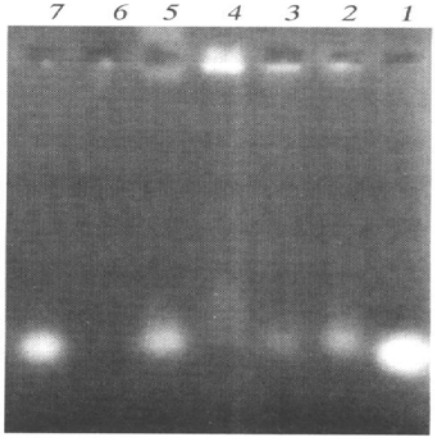


**Fig. 2 The agarose electrophoresis pattern of TAR RNA recognized by compounds(6 μmol/L)**

*1*: TAR RNA only; *5* ~ *7*: compounds MP-3, MP-2 and MP-1, respectively (lane *2* ~ *4*: other macrocyclic polyamines).

### 2.4 The effects on cell apoptosis

Flow cytometry analysis was conducted after incubating cos-7 cells with compounds for 48 h (Figure 3). Results showed that compounds MP-1 and MP-3 (5.0 μmol/L) could decrease the percentage of hypodiploid cell while MP-2 could increase the percentage of hypodiploid cell.

### 2.5 Computer docking calculation

From Figure 4 we observed that the compound Mp-1 (as an example, others not showed) existed in the upper three-nucleotide bulge in the major groove of TAR RNA. The total energy by docking showed that complex of MP-1 with TAR RNA had lower interaction energy (Table 1).

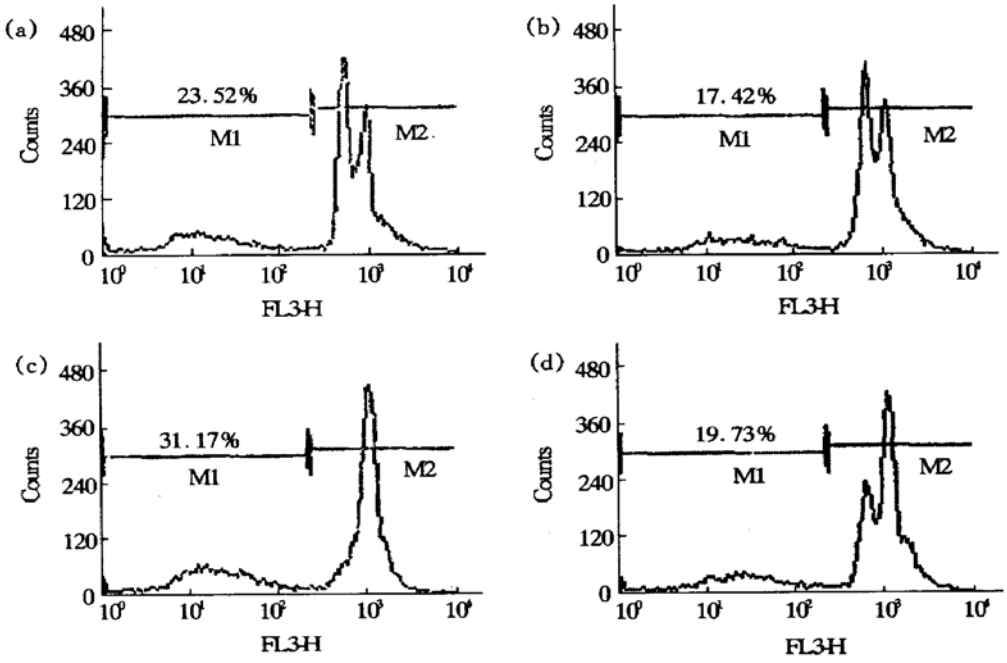


**Fig. 3 Apoptosis effect of cos-7 cells induced by different compounds**
(a) control; (b) MP-1; (c) MP-2; (d) MP-3.

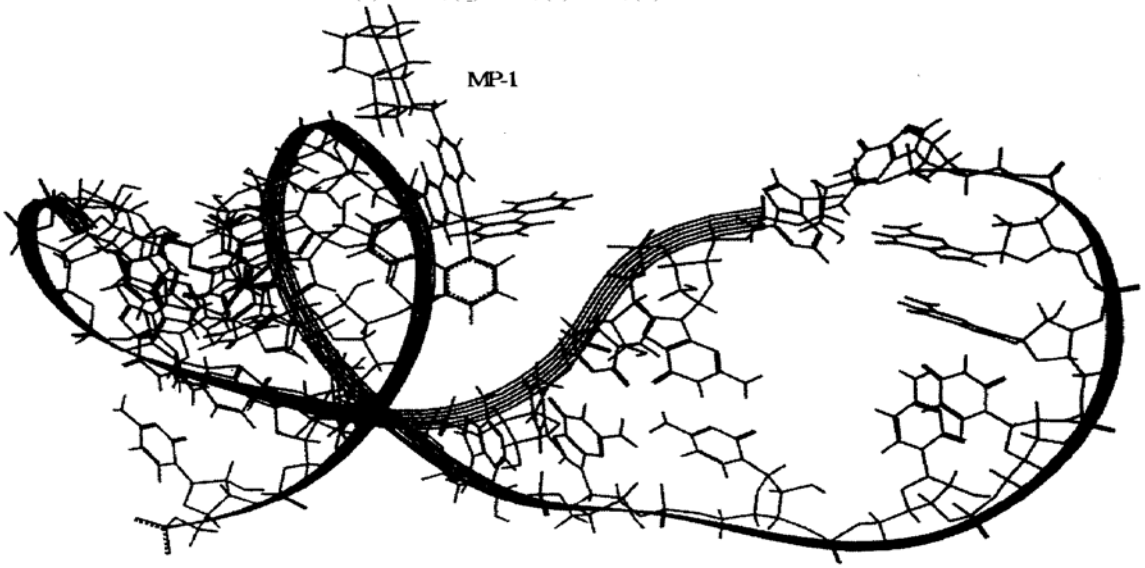


**Fig. 4 Molecular modeling of the interaction between compound MP-1 and TAR RNA upper stem/loop**

**Table 1 The total energy of interaction between TAR RNA and compounds**

| Compound | Elet/kJ·mol$^{-1}$ | Vdw/kJ·mol$^{-1}$ | Total/kJ·mol$^{-1}$ |
|---|---|---|---|
| MP-1 | -4.56 | -54.7 | -59.3 |
| MP-2 | -0.752 | -38.6 | -40.6 |
| MP-3 | -4.93 | -35.7 | -38.7 |

Elet: electrostatic, Vdw: Van der waals, Total: Vdw+Elet.

## 3 Discussion

Complicated replication cycles of HIV-1 provide several possible methods of developing antiviral drugs. However, the genome RNA of HIV-1 is easy to mutant resulting to generate drug resistance. This brings great difficulties in combating against diseases. TAR RNA sequence is a RNA stem/loop structure located between residues +1 to +59 of long terminal region (LTR) and forms part of the 5′ untranslated region of all the mRNAs encoded by HIV-1[1]. This area is high conserved so as to make itself a specific target with obvious advantages in drug resistance.

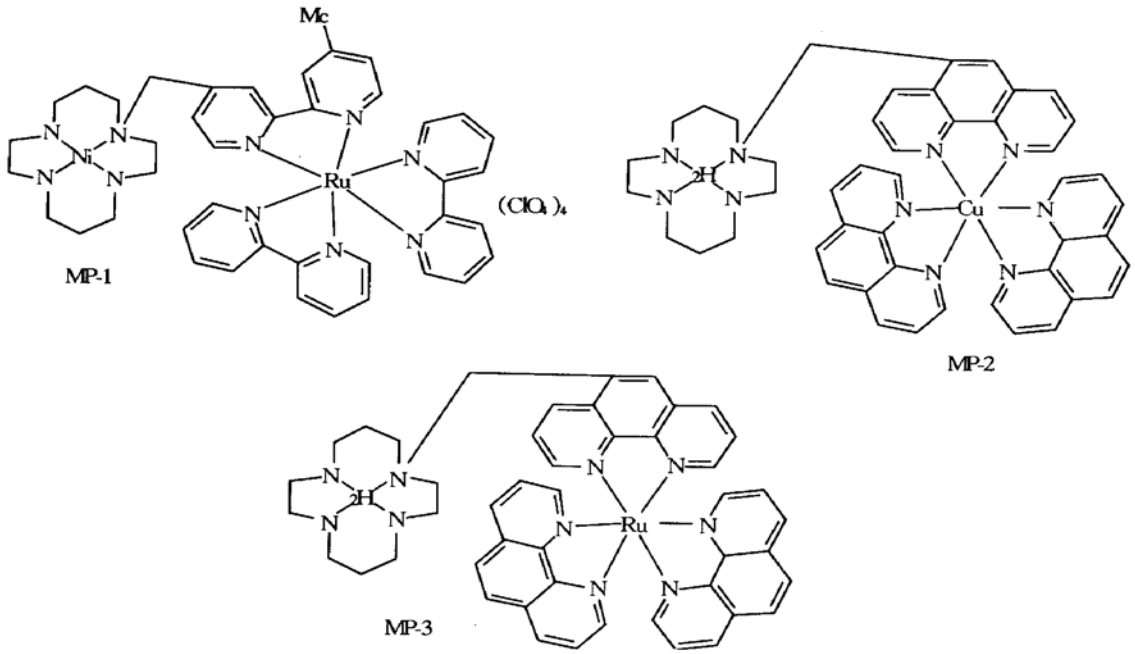


**Fig. 5 The Structure of compounds**

The complex of macrocyclic polyamine with metal ion ($Zn^{2+}$-cyclen) has a unique propensity to bind with deprotonated imides such as thymine (T) and uracil (U)[12]. It selectively recognizes them among DNA and RNA bases by reversibly forming a stable complex in physiological pH aqueous solution, and these complexes, moreover, seemed to disrupt the A-U hydrogen bonds in polyA · polyU as demonstrates by lowering the RNA duplex melting temperature. In addition, this kind of compounds has anti-HIV-1 activity and the metal complex of phenanthroline with chemical nuclease showed cleaved activity on nucleic acids. In this work, we studied the molecular recognition of macrocyclic polyamines (MP-1, MP-2 and MP-3, See Figure 5) to RNA, and the effects on inducing apoptosis of cos-7 cells in order to explore their mechanism of anti-HIV-1 activity. First of all, the activity of compounds was investigated by using polyA · polyU as the test system. The results indicated that compounds MP-1, MP-2 and MP-3 made polyA · polyU be small fragments which could not bind enough EB molecules and resulted in the fluorescence bands disappeared in electrophoresis pattern. Although the structures of MP-2 and MP-3 are very similar, the cleavage activities showed obviously different behavior on polyA · polyU. The activity of MP-2 was much higher than that of MP-3. The only difference between them is that MP-2 contains copper ion while Mp-3 does not. Thus, we prospected that this difference was related to copper ion; Moreover, reported study[13] demonstrated that the chemical nuclease activity possessed by phenanthroline is resulted from reactive oxygen species such as free radical · OH, which was generated through Harner-Weiss reaction. Therefore, we prospected that the strong cleavage showed by MP-2 on polyA · polyU may be related to reactive oxygen species.

To ascertain whether the cleavage of polyA · polyU by MP-1, MP-2 and Mp-3 could affect the binding of polyA · polyU with Tat peptide, the inhibition effects were observed by 1% agarose gel after mixing the three compounds with polyA · polyU respectively at room temperature for 10 min followed by adding Tat peptide. The electrophoretic analysis displayed that the compounds MP-1, MP-2 and MP-3 could inhibit the binding of polyA · polyU with Tat because of their cleaved activities on polyA · polyU.

In addition, published studies reported that the compounds had the anti-HIV-1 activities[8,14], and in our experiments these compounds could cleave the polyA · polyU and inhibit the binding of Tat to polyA · polyU. To further explore the mechanism of their anti-HIV activities, gel shift assay was performed by agarose electrophoresis having TAR RNA as a target. The results showed that the MP-1, Mp-2 and Mp-3 could definitely cleave the TAR RNA, especially MP-2, which has a stronger effect with TAR RNA molecules. This was consistent with the above observation in the test system of polyA · polyU that very shallow bands were seen in the agarose gel shadowed by 254 nm in the presence of compounds with lower concentration, and also there was a band with small shifting rate near the origin. However, when compounds with higher concentration, all the fluorescence bands vanished. From

this phenomenon, we proposed that the compounds could make the RNA molecules cross-linked first and then cleaved, in which the band with small shift rate maybe the outcome that many RNA molecules cross-linked together before cleaved.

To understand the mechanism of the anti-HIV activities of the compounds, the flow cytometric analysis was used to investigate the apoptosis in cos-7 cells induced by compounds. The results indicated that compounds MP-1 and MP-3 (5.0 μmol/L) could decrease the percentage of hypodiploid cells demonstrating that they could inhibit apoptosis in cos-7 cells. It is known that when infected in HIV-1, normal cells are induced to excessive death, which result to the down-regulation of the function of immunological system. Thus we proposed that the activities of anti-HIV of compounds MP-1 and MP-3 maybe also related to their inhibition of apoptosis. But compound MP-2 could increase the percentage of hypodiploid cells. This observation led to an intriguing question what the mechanism was, which needs to be investigated further. However, from above, it was known that compound MP-2 had stronger cleaved activity on RNA, thus compound MP-2 may have similar action to nuclease that can cleave DNA in apoptosis cells and result to the decrease of the percentage of hypodiploid cells. The computer modeling pictures showed that the compound Mp-1 (as an example, others not showed) existed in the upper three-nucleotide bulge in the major groove of TAR RNA. The compounds were rich positive charges and could better match phosphate backbones that were rich negative charges. The total energy by docking showed that complex of MP-1 with TAR RNA had lower interaction energy (Table 1). Although compound MP-2 showed stronger cleavage activity on RNA than other two compound MP-1 and MP-3, the total conformational energy of complex between MP-2 and TAR RNA was not the lowest. It was proposed that compound MP-2 and MP-3 had larger steric bulk than MP-1, and this structural characteristic made itself not to approach to the narrow and deep groove of RNA easily; Also the conformational energy was only a simple and direct parameter in principle, and it was not represent the solution reaction completely.

From the above, it can be seen that macrocyclic polyamines (MP-1, MP-2 and MP-3) showed RNA recognition ability not only on the molecular level but also on the cell level. Yet the advanced studies need to be performed to investigate whether they could affect the interaction between TAR and Tat on expression level and also what the exact mechanism is that MP-2 increased the percentage of hypodiploid cells.

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# 抗 HIV-1 活性的大环多胺类化合物与 RNA 的识别作用及其对细胞凋亡的影响*

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**摘要** 研究了具有抗 HIV-1 活性的大环多胺类化合物与 RNA 的识别作用，以及其对 cos-7 细胞凋亡的影响，以进一步探讨其抗 HIV-1 的作用机理. 实验采用琼脂糖凝胶电泳方法，观察化合物与 RNA 的识别作用；通过流式细胞计数法探讨其对 cos-7 细胞凋亡的影响；运用计算机分子模型，从理论上 Docking 计算化合物与 TAR RNA 结合的可能性. 结果表明，大环多胺类化合物 MP-1、MP-2 和 MP-3 不仅具有断裂 RNA 的作用，并可抑制 Tat-RNA 的相互作用，还可影响 cos-7 细胞亚二倍体的含量；理论化学计算数据与实验结果基本一致. 这一结果提示化合物的抗 HIV-1 活性可能通过作用于病毒基因组 RNA 而发挥作用，是多靶作用的结果.

**关键词** HIV-1，大环多胺，凋亡，Docking

**学科分类号** Q524

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