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Synthesis and biological evaluation of novel β-carboline derivatives as Tat–TAR interaction inhibitors

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Abstract—Four new β-carboline derivatives were synthesized bearing guanidinium group or amino group-terminated side chain targeting the TAR element. Compounds **5** and **6** with terminal guanidinium group showed inhibitory activities on Tat–TAR interaction as well as to HIV-1 in MT4 cells. Furthermore, capillary electrophoresis assay implied that compound **6** could not only bind to TAR but also hinder the Tat–TAR interaction.

HIV-1 gene expression is regulated through a complex interplay of specific *cis*-acting elements within its long terminal repeat (LTR) with host cell proteins or factors as well as with its own accessory proteins. One of these interactions is that HIV-1 regulatory protein Tat stimulates transcriptional elongation by binding the *trans*-activation response region (TAR), a 59-nucleotide stem-loop found at the 5′ untranslated end of all newly transcribed HIV mRNAs.[1] In the absence of Tat, transcription does occur but yields only short polyadenylated transcripts.[2] This interaction between TAR RNA and Tat is essential for virus replication. Therefore, blocking the Tat–TAR complex formation seems to be a promising target for inhibiting virus multiplication.

Intensive research over the last decade on the transactivation mechanism involving Tat–TAR interaction has yielded significant biological and virological insights. TAR RNA forms a stable hairpin structure with six apical nucleotides (CUGGGA) and two stem regions, upper and lower, separated by a bulge made of three unpaired nucleotides (UCU).[3] Tat binds to this region of the three-base bulge and recognizes both the identity of adjacent Watson–Crick base pairs and the positions of surrounding phosphate groups.[4] HIV-1 Tat is a protein with 86 amino acid residues, which includes a basic region (amino acid residues 48–59), termed arginine-rich motif (ARM), is responsible for Tat–TAR specific interaction.[5] Studies have shown that arginine 52 is largely responsible for the binding specificity and thus for the conformational change in TAR RNA in such a way that guanidinium group interacts with the three-base bulge region of TAR RNA.[6] Furthermore, the observation that a single arginine residue (or arginine amide) binds specifically to TAR and induces a change in RNA conformation that largely mimics the conformation of a portion of Tat–TAR complex suggests a strategy that introducing additional functionalities around the arginine guanidinium group could enhance the affinity and the specificity of drug–TAR interaction.[7] And also many experiments provided evidences that the high-affinity binding of small molecules to the RNA targets is governed by the electrostatic interaction due to amino group, guanidinium group, or arginine residue.[8] Our previous studies have indicated that β-carboline derivatives could interact with nucleic acids for their fused aromatic heterocycle ring structures, and that the optimized linker length and some electronic substituents on the β-carboline ring are important in the binding of nucleic acids.[9]

Based on the above idea, four new β-carboline derivatives were designed bearing guanidinium group or amino group-terminated side chain targeting the TAR element. In order to probe whether the four compounds could block the Tat-mediated transactivation, a transient co-transfection bioassay was performed by using a reporter plasmid construct expressing the chloramphenicol acetyltransferase (CAT) under the control of

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the HIV-1 LTR and a Tat expression vector. Further, their anti-HIV-1 activity in MT4 cells was evaluated. In addition, we used capillary electrophoresis (CE) to study their binding specificity of TAR RNA in inhibiting the Tat–TAR interaction.

The route used for the preparation of the compounds **3**–**6** was carried out as outlined in Scheme 1. β-Carboline derivatives **1** and **2** were synthesized according to the procedure described in the literature.[10,11] Compounds **3** and **4** were obtained by β-carboline methyl ester reacted with 1,3-propanediamine. Finally, coupling of β-carboline carboxamide derivatives with S-methylisothiourea yielded compounds **5** and **6**. Molecules **3**–**6** were characterized using MS, ^{1}H NMR, and elemental analysis.[12]

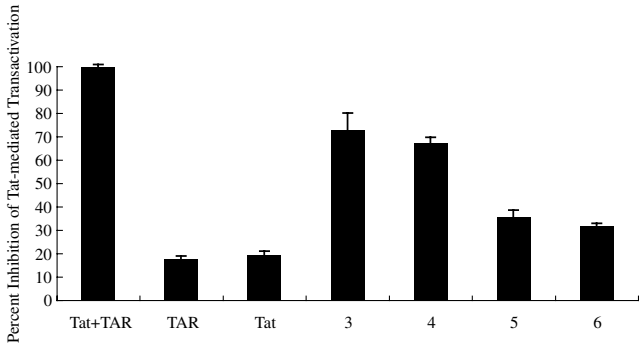
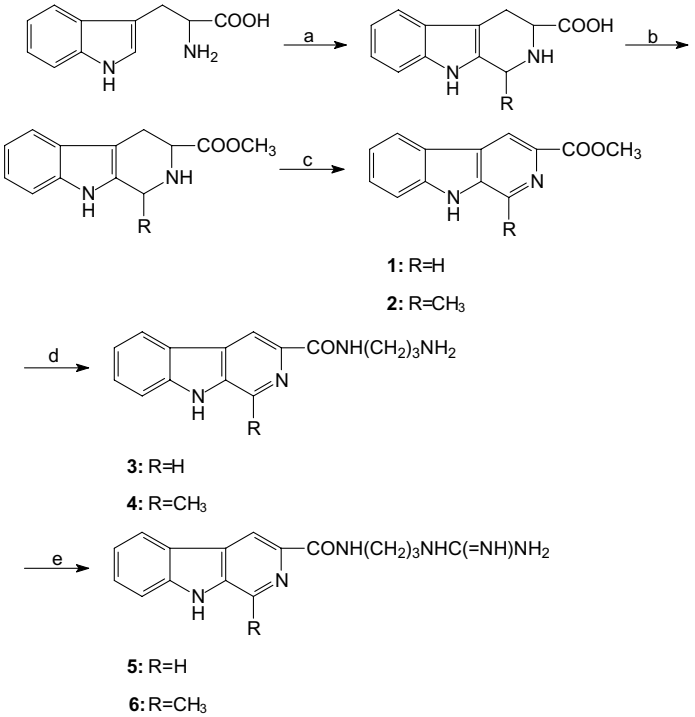


Figure 1. Effects of compounds **3**–**6** on Tat-mediated transactivation in 293T cells.

Activation of transcriptional elongation occurred following the recruitment of Tat to the transcription machinery via a specific interaction with TAR RNA. To evaluate the ability of these compounds to block Tat–TAR interaction, we used a reporter gene-based assay.[13] 293T cells were co-transfected with the reporter plasmids, pLTRCAT, which express CAT under the control of the HIV-1 LTR and a Tat expression vector pSVCMVTAT, along with the individual compound of 30 μM. None of the tested compounds at concentrations up to 30 μM showed any significant cytotoxicity (data not shown). Inhibitory effect of the tested compounds to transactivation was measured by quantitatively determination of CAT expression 48 h after transfection by colorimetric enzyme immunoassay. As seen from Figure 1, a substantial increase in the CAT activity upon co-transfection with pSVCMVTAT and pLTRCAT indicated a significant stimulation of the basal level of transcription of the HIV-1 LTR. The percent inhibition of Tat-mediated transactivation of the HIV-1 LTR varied with the individual compound. Compounds bearing guanidinium group-terminated side chain (compounds **5** and **6**) were somewhat more inhibitory than those with terminal amino group (compounds **3** and **4**), thus the terminal guanidinium group did play an important role in Tat–TAR interaction. Furthermore, the substituted methyl group at C-1 position on the β-carboline ring of the compounds (**4** and **6**) resulted in the appearance of some more inhibitory activities. It is likely that the increased molecular polarity induced by the methyl group might give these compounds more binding affinities to TAR RNA, especially in the complicated eukaryotic system.



Scheme 1. Reagents and conditions: (a) HCHO or CH_3CHO, H^+; (b) $SOCl_2$, CH_3OH; (c) S, xylene, dioxane; 59% for **1** and 71% for **2**; (d) 1,3-propanediamine, $CHCl_3$, MeOH, reflux, 8 h, 44.4% for **3** and 40.9% for **4**; (e) S-methylisothiourea, 2 N NaOH, 4 °C to rt, reflux, 1 h, 51.6% for **5** and 64.8% for **6**.

Since the four compounds showed inhibitory activities on Tat–TAR interaction, they were expected to inhibit virus replication. Thus the anti-HIV activity together with the cytotoxic data of the four compounds was evaluated in MT4 cells infected with the HIV-1_{IIIB} strain.[14] Both compounds **5** and **6** showed inhibitory activity to HIV-1_{IIIB} cytopathic effects (CPE) of cell fusion ($EC_{50} \geqslant 50\,\mu M$) and without apparent cellular toxicity ($IC_{50} \geqslant 100$–$1000\,\mu M$), however, compounds **3** and **4** were neither cytotoxic nor inhibitory to HIV-1 (Table 1).

CE has become a powerful analytical technique in biochemical studies, it has been used in the analysis of RNA–protein interactions, here it has been used to analyze the binding of compound **6** with HIV-1 TAR RNA and its inhibition of the Tat–TAR interaction.[15] As shown in Figure 2, the well-shaped peak of compound **6** with $t_m = 7.2$ min was clearly visible under the experimental condition used, so was Tat protein with $t_m = 8.5$ min. The migration time of free TAR RNA and its complex form with compound **6** or Tat protein was in the range of 28–30 min. Free TAR RNA migrated faster than its complex forms because of the smaller charge to mass ratio of the complexes. The binding of compound **6** with TAR RNA was observed as electrophoresis mobility shift of TAR's peak and the decrease of the height of free compound's peak in the presence of TAR. Then the inhibition of compound **6** on the binding of Tat–TAR complex was probed. Figure 2g showed the height of Tat's peak was higher than that of Figure 2f, which implied that compound **6** could not only bind to TAR but also hinder the Tat–TAR interaction.

All the experiments reported here showed that the newly designed compounds **5** and **6** bearing guanidinium group-terminated side chain could block the Tat–TAR interaction and were active against HIV-1. These studies provide a new idea for the design of new Tat–TAR inhibitors. In addition, CE assay is an effective and sensitive method with which to evaluate RNA–peptide or RNA–drug interaction.

Table 1. Anti-HIV-1 activity and cytotoxicity data of compounds **3**–**6** determined in MT4 cells

Compound	EC_{50}[a] (μM)	CC_{50}[b] (μM)
3	>1000	>1000
4	>100	>100
5	⩾50	>1000
6	⩾50	>100

[a] EC_{50}: concentration required to protect cells against the cytopathogenicity of HIV by 50%.
[b] CC_{50}: concentration required to inhibit uninfected cells proliferation by 50%.

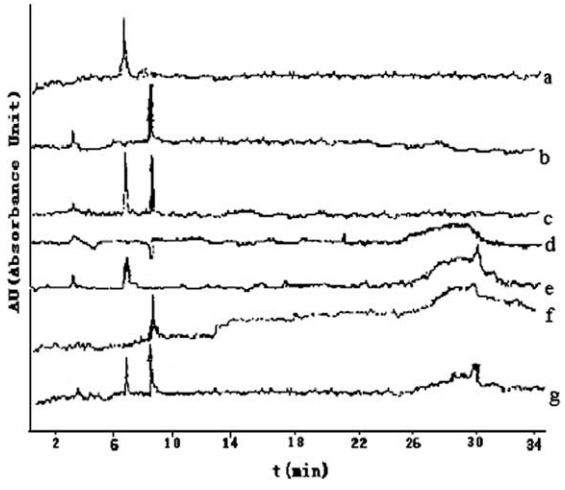


Figure 2. CE electropherograms of the studies on the binding of compound **6** with TAR as well as inhibition of compound **6** on the Tat–TAR interaction. (a) Compound **6** alone (100 μM); (b) Tat alone (100 μM); (c) compound **6** + Tat (100 μM, each); (d) TAR alone (100 μM); (e) compound **6** + TAR (100 μM, each); (f) Tat + TAR (100 μM, each); (g) compound **6** + Tat + TAR (100 μM, each).

Acknowledgements

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References and notes

1. Rana, T. M.; Jeang, K. *Arch. Biochem. Biophys.* **1999**, *365*, 175.
2. Turpin, J. A. *Expert Rev. Anti-infect.* **2003**, *1*, 97.
3. (a) Aboul-ela, F.; Karn, J.; Varani, G. *J. Mol. Biol.* **1995**, *253*, 313; (b) Aboul-ela, F.; Varani, G. *J. Mol. Struct.-Theochem.* **1998**, *423*, 29.
4. Churcher, M.; Lamont, C.; Hamy, F.; Dingwall, C.; Green, S. M.; Lowe, A. D.; Butler, P. J. G.; Gait, M. J.; Karn, J. *J. Mol. Biol.* **1993**, *230*, 90.
5. Verhoef, K.; Koper, M.; Berkhout, B. *Virology* **1997**, *237*, 228.
6. (a) Watson, K.; Edwards, R. J. *Biochem. Pharmacol.* **1999**, *58*, 1521; (b) Calnan, B. J.; Biancalana, S.; Hudson, D.; Frankel, A. D. *Gene* **1991**, *5*, 201.
7. Nifosi, R.; Reyes, C. M.; Kollman, P. A. *Nucl. Acids Res.* **2000**, *28*, 4944.
8. (a) Peytou, V.; Condom, R.; Patino, N.; Guedj, R.; Aubertin, A. M.; Gelus, N.; Bailly, C.; Terreux, R.; Cabrol-Bass, D. *J. Med. Chem.* **1999**, *42*, 4042; (b) Mei, H. Y.; Cui, M.; Heldsinger, A.; Lemrow, S. M.; Loo, J. A.; Sannes-Lowery, K. A.; Sharmeen, L.; Czarnik, A. W. *Biochemistry* **1998**, *37*, 14204.
9. Xiao, S.; Lin, W.; Wang, C.; Yang, M. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 437.
10. Brossi, A.; Focella, A.; Teitel, S. *J. Med. Chem.* **1973**, *16*, 418.
11. Lippke, K. P.; Schunack, W. G.; Wenning, W.; Müller, W. E. *J. Med. Chem.* **1983**, *26*, 499.
12. Melting points were determined on a XA-4 instrument and are uncorrected. All ^{1}H NMR spectra were taken on a Varian Unity 300 NMR spectrometer. Chemical shifts (δ)

for ^{1}H spectra were expressed in ppm relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were measured on a VG-ZAB-HS spectrometer or an ABI QSTAR spectrometer. Elemental analyses were determined on an Elementar Vario EL apparatus.

^{1}H NMR, MS, elemental analysis, mp for representative compounds: **3:** ^{1}H NMR (DMSO-d_6): δ 13.08 (s, 1H, NH), 8.89 (d, $J = 7.2$ Hz, 1H, 1-H), 8.80 (t, $J = 6.0$ Hz, 1H, 4-H), 8.39 (d, $J = 7.8$ Hz, 1H, 8-H), 7.64 (m, 1H, 5-H), 7.57 (d, $J = 9.0$ Hz, 1H, 6-H), 7.29 (t, $J = 7.2$ Hz, 1H, 7-H), 3.38 (m, 2H, CH_2), 2.60 (m, 2H, CH_2), 1.63 (m, 2H, CH_2); MS (EI) (*m*/*z*): 269($M + H^+$); elemental analysis calcd (%) for $C_{15}H_{16}N_4O$: C 67.15, H 6.01, N 20.81; Found C 67.39, H 6.46, N 20.34; mp: 218–222 °C. **4:** ^{1}H NMR (acetone-d_6): δ 12.03 (s, 1H, NH), 8.77 (s, 1H, 4-H), 8.35 (d, $J = 7.8$ Hz, 1H, 8-H), 7.67 (d, $J = 7.8$ Hz, 1H, 5-H), 7.59 (t, $J = 7.8$ Hz, 1H, 6-H), 7.30 (t, $J = 7.8$ Hz, 1H, 7-H), 3.56 (m, 2H, CH_2), 3.33 (m, 2H, CH_2), 2.83 (s, 3H, CH_3), 1.90 (m, 2H, CH_2); MS (EI) (*m*/*z*): 283($M + H)^+$; elemental analysis calcd (%) for $C_{16}H_{18}N_4O$: C 68.09, H 6.38, N 19.86; Found C 67.85, H 6.40, N 20.01; mp: 175–177 °C. **5:** ^{1}H NMR (DMSO-d_6): δ 13.14 (s, 1H, NH), 8.92 (s, 1H, 1-H), 8.81 (s, 1H, 4-H), 8.37 (d, $J = 8.1$ Hz, 1H, 8-H), 7.64 (d, $J = 8.1$ Hz, 1H, 5-H), 7.58 (d, $J = 6.6$ Hz, 6-H), 7.27 (t, $J = 7.8$ Hz, 1H, 7-H), 3.41 (m, 2H, CH_2), 3.11 (m, 2H, CH_2), 1.75 (m, 2H, CH_2); TOF-MS: 311 ($M + H^+$); elemental analysis calcd (%) for $C_{16}H_{18}N_6OCl$: C 50.24, H 5.22, N 21.93; Found C 50.51, H 5.24, N 22.30; mp: 199–201 °C. **6:** ^{1}H NMR (DMSO-d_6): δ 12.03 (s, 1H, NH), 8.66 (s, 1H, 4-H), 8.28 (d, $J = 7.2$ Hz, 1H, 8-H), 7.64 (d, $J = 8.4$ Hz, 1H, 5-H), 7.53 (d, $J = 7.2$ Hz, 1H, 6-H), 7.23 (t, $J = 8.1$ Hz, 1H, 7-H), 3.36 (m, 2H, CH_2), 3.12 (m, 2H, CH_2), 2.82 (s, 3H, CH_3), 1.69 (m, 2H, CH_2); TOF-MS: 325 ($M + H^+$); elemental analysis calcd (%) for $C_{17}H_{20}N_6OCl$: C 51.39, H 5.04, N 21.16; Found C 51.21, H 5.30, N 21.27; mp: 270–272 °C.

13. Transient transfection and CAT assays: 293T cells were grown as monolayer in Dulbecco's modified Eagle's medium (DMEM) (Gibco BRL) supplemented with 10% (v/v) fetal calf serum, penicillin (100 U mL^{-1}), and streptomycin (100 U mL^{-1}) at 37 °C in 5% CO_2 containing humidified air. The cells were seeded at a six-well plate 24 h prior to transfection, which was performed by standard calcium phosphate co-precipitation techniques with optimum amounts of the plasmids pLTRCAT and pSVCMVTAT. Twenty four hours later, the culture medium was removed and the cells were washed twice with phosphate-buffered saline (PBS). Then the transfected cells were added to fresh medium together with diluted compounds of final concentration 30 μM, respectively, and incubated for another 24 h. Forty eight hours post-transfection, the cells were harvested and analyzed for CAT activity using a commercial CAT ELISA kit (Roche Molecular Biochemicals) in accordance with the manufacturer's protocol. All data were reported as a percentage of CAT activity (±SD). Results shown are representative of three independent experiments.
14. (a) Balzarini, J.; Pannecouque, C.; Clercq, E. D.; Pavlov, A. Y.; Printsevskaya, S. S.; Miroshnikova, O. V.; Reznikova, M. I.; Preobrazhenskaya, M. N. *J. Med. Chem.* **2003**, *46*, 2755; (b) Ayisi, N. K.; Nyadedzor, C. *Antivir. Res.* **2003**, *58*, 25.
15. Capillary electrophoresis assay: CE experiments were carried out on a Beckman P/ACE 2100 capillary electrophoresis system using a Beckman 57 cm × 75 μm I.D. bare fused-silica capillary, 50 cm to the UV detector. Phosphate buffer (50 mM, pH 8.0) was used as running buffer. Reverse polarity, a constant voltage of 11 kV, a temperature of 25 ± 0.1 °C and pressure injection for 10 s at 20 psi (1 psi = 6894.76 Pa) were used. Absorption of samples was detected at 210 nm. Prior to use, the capillary was pretreated with 0.2 M NaOH for 60 min, water for 30 min, and finally with the running buffer until the baseline become smooth. To decrease the adsorption of the samples on the capillary wall through electrostatic interaction and to insure the reproducibility of migration time, the capillary was washed between runs with 0.2 NaOH, water, and running buffer each for 4 min orderly. Solutions were filtered through a 0.22 μm PTFE membrane before use.