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Synthesis and assay of isoquinoline derivatives as HIV-1 Tat–TAR interaction inhibitors

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Abstract—Four new isoquinoline derivatives bearing guanidinium group or amino group-terminated side chain were synthesized to target the HIV-1 TAR element. Their abilities to bind TAR RNA and inhibit Tat–TAR RNA interaction were determined by CE analysis, a Tat-dependent HIV-1 LTR-driven CAT assay and SIV-induced syncytium evaluation.

The early phase of the HIV-1 replication is controlled by the interaction of trans-activator of transcription (Tat) protein with the trans-activation responsive region (TAR) RNA.[1,2] A three-nucleotide bulge (U23, C24, and U25) of HIV-1 TAR is essential for high affinity and specific binding of Tat protein.[3] The Arginine residue at position 52 of Tat is the only sequence-specific contact mediating the complex formation between Tat and TAR, and its guanidine group interacts with the residue U23 of the trinucleotide bulge.[4–6] On the basis of the structural information from HIV-1 Tat–TAR interaction as well as the design principle put forward previously,[7] we defined requirements for potential low-molecular weight inhibitor of TAR recognition by the Tat protein. A series of isoquinoline derivatives with fused aromatic moiety bearing guanidinium group or amino group-terminated side chain were designed and synthesized under the molecular modeling direction. Their bioactivities were evaluated by CE, a Tat-dependent HIV-1 LTR-driven CAT assay and SIV-induced syncytium evaluation.

The route used for the preparation of *N*-(2-aminoethyl)-isoquinoline-3-carboxamide, *N*-(3-aminopropyl)-isoquinoline-3-carboxamide, *N*-(2-guanidinoethyl)-isoquinoline-3-carboxamide bisulfite, and *N*-(3-guanidinopropyl)-isoquinoline-3-carboxamide bisulfite (I_1, I_2, IG_1, and IG_2) was carried out as outlined in Scheme 1. Isoquinoline derivatives **3** and **4** were synthesized according to the procedure described previously.[8,9] Compounds I_1 and I_2 were obtained by isoquinoline methyl ester reacted with ethylenediamine and 1,3-propanediamine, respectively. Finally, coupling of isoquinoline carboxamide derivatives with *S*-methylisothiourea yielded compounds IG_1 and IG_2. Compounds I_1, I_2, IG_1 and IG_2 were characterized by MS and ^{1}H NMR.[10]

Capillary electrophoresis (CE) was used in the analysis of RNA–protein interactions, which provided a quick, sensitive, and precise method to study the binding of Tat–TAR RNA and drug-TAR RNA.[11–14] Herein, we used CE to study the four compounds' binding specificity of TAR RNA and to determine their binding constants.[15] The result of IG_2–TAR interaction is provided here as an example.

TAR, Tat, and IG_2 migrated at about 10 min, 6 min, and 3 min, respectively. As shown in Table 1, the binding of compound IG_2 with TAR RNA was observed with the decrease of the area of free compound's peak in the presence of TAR. Then the inhibition of compound IG_2 on the binding of Tat–TAR complex was probed. Figure 1h showed the height and area of Tat's peak was greater than those of Figure 1c, which implied that compound IG_2 could not only bind to TAR but also hinder the Tat–TAR interaction. The CE analysis of other compounds provided similar results as well.

We assume for simplicity that TAR RNA has a single site for Tat, then define a simplified reaction model for the TAR–Tat system:

Keywords: Tat–TAR interaction; Isoquinoline; CE.
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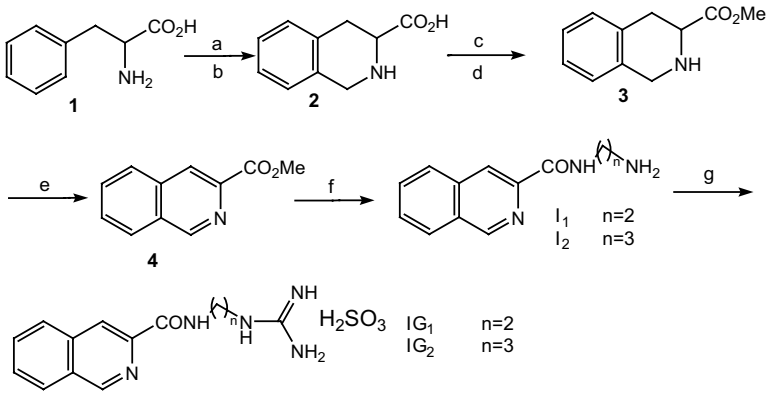

Scheme 1. Reagents and conditions: (a) HCHO, HCl, reflux, 3 h; (b) 10% NH_3, pH 7; (c) $SOCl_2$, MeOH, 3 h; (d) saturated $NaHCO_3$ solution, pH 8; (e) S, xylene, reflux, 48 h; (f) $NH_2(CH_2)_nNH_2$, $CHCl_3$; (g) EtOH, 35 °C, 4 h.

Table 1. CE analysis of IG_2–TAR interaction

Peaks	Migration time (min)	Peak area
1	9.750	1496436.501
2	6.017	174852.453
3	6.022	30264.600
4	8.583	117549.797
5	2.498	53183.648
6	2.352	30466.404
7	7.898	112402.95
8	2.223	23299.400
9	9.300	164893.842
10	2.982	22429.500
11	12.777	36775.083
12	2.453	30264.600
13	6.422	575355.408
14	10.026	100301.579

$$R + P = RP \quad K_p = [PR]/[R][P] = \gamma_P/[P], \qquad (1)$$

where [PR], [R], and [P] is the concentration of TAR–Tat complex, nonbonding TAR, and nonbonding Tat in the mixture.

By the law of mass action, the molar concentration of all TAR–Tat complexes ([PR]) is equal to the concentration of bound Tat peptide and the net concentration of bound Tat peptide is equal to the total Tat peptide concentration (C_P) less the concentration of free Tat ([P]) remaining at equilibrium. The TAR binding ratio γ_P is calculated as Eq. 2.

$$\gamma_P = [PR]/[R] = (C_P - [P])/[R]. \qquad (2)$$

The concentration of free TAR RNA ([R]) in the equilibrium mixture was set equal to the total concentration of TAR (C_R) added to the mixture minus the amount of bound RNA in the complex (equal to [PR]). To estimate the binding constant of TAR–Tat complex using our CE method, we developed Eq. 3.

$$\gamma_P = (C_P - [P])/(C_R - C_P + [P]). \qquad (3)$$

In our method, linear equations by which concentration of free Tat or the compounds in the mixture could be calculated were obtained.[16] Thus, the ratio γ_P and the binding constant K were calculated and summarized in Table 2.

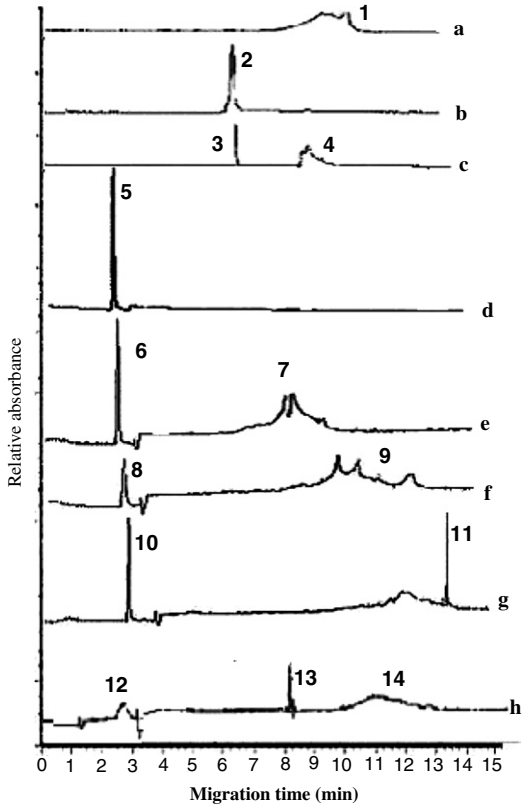

Figure 1. CE elctrophorograms of IG_2–TAR interaction. (a) TAR alone (300 μM); (b) Tat alone (300 μM); (c) Tat + TAR (300 μM, each) incubated for 30 min; (d) IG_2 alone (300 μM); (e), (f) and (g) IG_2 + TAR (300 μM, each), incubated for 15 min, 30 min and 45 min, respectively.

As seen in Table 2, compounds bearing guanidinium group-terminated side chain had stronger affinities than those with terminal amino group, thus the guanidinium group played an important role in the interaction with TAR. Furthermore, the length of the side chain also affected their affinities, but the effect did not appear so obviously as that of guanidinium group.

Table 2. Binding constants of the compounds and Tat with TAR

Compounds	Linear equation	R^2	Peak area	Concentration ($\times 10^{-4}$ M)	K
Tat	A = 22184c + 3759.9	0.9881	30264.600	1.195	1.26×10^4
I_1	A = 37074c − 1645.1	0.9824	28515.699	0.814	3.30×10^4
I_2	A = 18417c + 3577.5	0.9977	23958.539	1.107	1.54×10^4
IG_1	A = 48670c − 2215.4	0.9822	15154.543	0.357	2.07×10^5
IG_2	A = 78175c − 7385.9	0.9783	23299.400	0.393	1.69×10^5

Table 3. Computer simulation of the interaction between the title compounds and TAR RNA

Compounds	E_1[a] (kcal/mol)	E_2[b] (kcal/mol)	H-bond number
I_1	93.8721	−1.70263	2
I_2	79.2974	−4.37024	*N*
IG_1	15.2278	−6.40564	*N*
IG_2	−4.20675	−47.2764	2

[a] Optical conformation energy.
[b] Minimization binding energy. All the compounds, TAR RNA and their complexes were minimized by using DISCOVERwith a steepest descents algorithm in an AMBER FRC potential forcefield on SGI (Silicon Graphics Inc.) IRIX6.5 workstation, respectively.

In order to reveal the mechanism of their TAR RNA-binding properties, molecular modeling experiments were also performed. The docking calculation of the four small molecules, I_1, I_2, IG_1, and IG_2, with target TAR RNA sequences was carried out using the Biosym modeling package (Biosym Technologies) in Insight II. The theoretical calculating energies are presented in Table 3.

The modeling results implied that IG_2 was a good match to the trinucleotide bulge (U23-C24-U25) of TAR RNA and two hydrogen bonds formed between the terminal guanidinium group of the side chain and the phosphate of C24 at the intercalation site. IG_2 exhibited the lowest optical conformation energy and minimization binding energy among the four compounds, which indicated that the complex IG_2–TAR was more stable than the other analogues. Those data agree well with our CE results. Using the program, DISCOVER, water molecular and metal cation have been excluded in the calculation, by which our experimental data of the binding between the compounds and TAR RNA are consistent with the estimation.

In order to probe whether the title compounds could block the Tat-mediated transactivation in human cells, the activities of compounds I_1, I_2, IG_1, and IG_2 were examined by using Tat dependent HIV-1 LTR-driven CAT gene expression colorimetric enzyme assays.[14,17] As shown in Figure 2, the decreased CAT activities in the presence of the title compounds reflect their competition with Tat for TAR RNA binding and the inhibition of Tat function in vivo.

Moreover, the inhibitions of SIV-induced syncytium in CEM174 cells with the compounds were tested to confirm their antiviral effects,[18] and the data were summarized in Table 4. From the two tests above, it is indicated that compounds containing guanidine group were more potent in inhibiting Tat–TAR RNA interaction and showed stronger inhibition of the syncytium

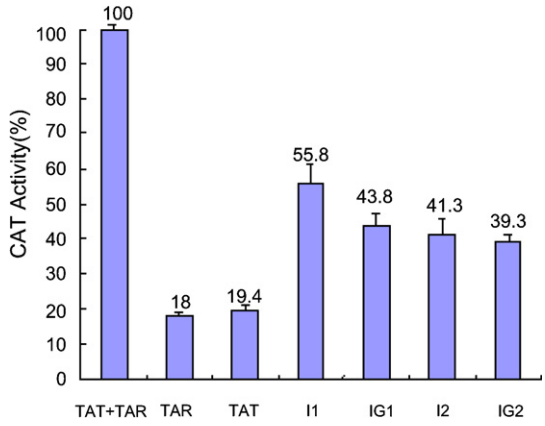

Figure 2. Effect of compounds I_1, IG_1, I_2, and IG_2 on Tat-mediated transactivation in 293T cell.

Table 4. Inhibition effect of compounds on SIV induced syncytium

Compounds	EC_{50} (μM)	Inhibition %
I_1	12.9	44.2
IG_1	6.86	56.2
I_2	8.74	58.7
IG_2	3.92	60.7

formation as well. Also, we found that a side chain with a suitable length linking isoquinoline and guanidine group played an important part in the inhibition.

All the experiments reported here show that the newly designed compound IG_2 bearing guanidinium group-terminated side chain could block the Tat–TAR interaction and have the anti-HIV potency. These studies provide a new idea for the design of HIV-1 Tat–TAR inhibitors.

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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. Aboul-ela, F.; Karn, J.; Varani, G. *Nucleic Acid Res.* **1996**, *24*, 3974.
4. Calnan, B. J.; Tidor, B.; Biancalana, S.; Hudson, D.; Frankel, A. D. *Science* **1991**, *252*, 1167.
5. Karn, J.; Graeble, M. A. *Trends Genet.* **1992**, *8*, 365.
6. Tassew, N.; Thompson, M. *Anal. Chem.* **2002**, *74*, 5313.
7. Chow, C. S.; Bogdan, F. *Chem. Rev.* **1997**, *97*, 1489.
8. Kato, H.; Miyazawa, K.; Etchu, E. Japan. 7307, 115 (C1. C07d, A61k)
9. Cain, M.; Weber, R. W.; Guzman, F. J. *Med. Chem.* **1982**, *25*, 1081.
10. Melting points were determined on a XA-4 instrument and are uncorrected. All ^{1}H NMR spectra were on a Varian Unity 300 NMR spectrometer. Chemical shifts (δ) for ^{1}H spectra were expressed in parts per million relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were measured on a VG-ZAB-HS spectrometer or an ABI QSTAR spectrometer. ^{1}H NMR, MS, mp for representative compounds: I_1: ^{1}H NMR (DMSO-d_6) δ 9.39 (s, 1H, 1-H), 8.56 (s, 1H, 4-H), 8.26 (m, J = 7.8 Hz, 1H, 8-H), 8.19 (m, J = 7.8 Hz, 1H, 5-H), 7.88 (m, J = 7.2 Hz, 1H, 6-H), 7.81 (m, J = 7.2 Hz, 1H, 7-H), 3.43 (m, 2H, $CONHCH_2$), 2.74 (m, 2H, $NHCH_2$), 1.23 (m, 2H, CH_2); MS (EI) (*m*/*z*): 216 (M+H); mp: jelly. I_2: ^{1}H NMR (DMSO-d_6) δ9.38 (s, 1H, 1-H), 8.56 (s, 1H, 4-H), 8.25 (m, J = 7.8 Hz, 1H, 8-H), 8.20 (m, J = 7.8 Hz, 1H, 5-H), 7.88 (m, J = 7.2 Hz, 1H, 6-H), 7.81 (m, J = 7.2 Hz, 1H, 7-H), 3.46–1.71 (m, 6H, $CH_2 \times 3$); MS (EI) (*m*/*z*): 229 ; mp: jelly. IG_1: (FABMS) (*m*/*z*): 258.0 (M+H); mp: 136–137 °C. IG_2: (FABMS) (*m*/*z*): 271.9 (M+H); mp: 136–137 °C.
11. Zhang, B.; Xue, H.; Lin, B. C. *Chromatographia* **1998**, *48*, 268.
12. Mucha, P.; Szyk, A.; Rekowski, P.; Barciszewski, J. *J. Chromatogr. A* **2002**, *968*, 211.
13. Zhao, H.; Dai, D.; Li, J.; Chen, Y.; Jiang, L. *BBRC* **2003**, *312*, 351.
14. Yu, X.; Lin, W.; Lin, J.; Yang, M. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3127.
15. Capillary electrophoresis assay: CE experiments were carried out on a Beckman P/ACE 2100 capillary electrophoresis system using a 50 cm × 50 μm ID bare fused-silica capillary (Beckman). Phosphate buffer (50 mM, pH 8.0) was used as running buffer. Electrophoresis was started at 15 kV and 20 ± 0.1 °C. Samples were injected at 10 kV for 20 s and detected at 214 nm. Prior to use, the capillary was pre-treated successively with 0.1 M NaOH for 60 min, water for 30 min, and finally with running buffer until the baseline becomes smooth. Between runs the capillary was washed sequentially with 0.1 M NaOH, water, and running buffer for 4 min each. Solutions were filtered through a 0.22 μm PTFE membrane prior to use. To ensure proper folding of the TAR RNA structure, the RNA solutions were annealed by heating for 3 min at 95 °C and cooled slowly. TAR-compound or TAR–Tat peptide complex was incubated for 30 min at 4 °C (binding buffer: 10 mM Tris–HCl, 70 mM NaCl, 0.2 mM EDTA, and 5% glycerol, pH 7.4) before CE analysis.
16. The samples were prepared with a wide range of Tat (or the compounds) diluted by binding buffer (10 mM Tris–HCl, 70 mM NaCl, 0.2 mM EDTA, and 5% glycerol, pH 7.4) and CE procedure was as described in Ref. 15. All experiments were in triplicate. Relative standard deviation (RSD) was calculated from a series of three experiments carried out with the same sample in 1 day. Linear equations of the concentration and the peak area were calculated. With the peak areas of free Tat or the free compounds were obtained as described in Ref. 15, their concentration in the mixture could be calculated.
17. 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 ($100\ U \times mL^{-1}$), and streptomycin ($100\ U \times mL^{-1}$) 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. After 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.
18. Inhibition of SIV-induced syncytium in CEM174 cell cultures was measured in a 96-well microplate containing 2×10^5 CEM cells/mL infected with 100 $TCID_{50}$ of SIV per well and containing appropriate dilutions of the tested compounds. After 5 days of incubation at 37 °C in 5% CO_2 containing humidified air, CEM giant(syncytium) cell formation was examined microscopically(COIC). The EC_{50} was defined as the compound concentration required to protect cells against the cytopathogenicity of SIV by 50%.