SUPPORTING INFORMATION

Cyclic peptides with a distinct arginine-fork motif recognize the HIV trans-activation response RNA in vitro and in cells

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Running title: Targeting HIV TAR with a focused library of cyclic peptides
| Name     | Parent TBP | Sequence                  | linker           | $K_D$ (μM) |
|----------|------------|---------------------------|------------------|------------|
| TB-CP-6.3a | 6.3        | (C)PRTRRPRGQ(C)          | methylene        | 3.1 ± 1.4  |
| TB-CP-6.3f-m | 6.3     | (C)PRTRRPRGQ(C)          | 1,3-xylene       | 2.0 ± 0.5  |
| TB-CP-6.3Ra | 6.7(Q48R, T50R) | (C)PRRRRPRGQ(C)        | methylene        | 1.0 ± 0.1  |
| TB-CP-6.3 Pf-m | 6.7(Q48R, T50R) | (C)PRRRRPRGQ(C)        | 1,3-xylene       | 0.8 ± 0.1  |
| TB-CP-6.7a  | 6.7        | (C)PRQRTPRGQ(C)          | methylene        | 23 ± 1     |
| TB-LP-6.9   | 6.9        | (C)PRRRTPRGQ(C)          | linear           | 17.6 ± 7.2 |
| TB-CP-6.9a  | 6.9        | (C)PRRRTPRGQ(C)          | methylene        | 5.2 ± 0.2  |
| TB-CP-6.9a-LD | 6.9   | $(i)(C)PRRRTPRGQ(C)^o$    | methylene        | 24.2 ± 3.4 |
| TB-CP-6.9a-DD | 6.9     | $(i)(C)PRRRTPRGQ(C)^o$    | methylene        | 16.4 ± 4.4 |
| TB-CP-6.9b  | 6.9        | (C)PRRRTPRGQ(C)          | ethyl            | 6.8 ± 0.3  |
| TB-CP-6.9c  | 6.9        | (C)PRRRTPRGQ(C)          | propyl           | 12.1 ± 2.0 |
| TB-CP-6.9d  | 6.9        | (C)PRRRTPRGQ(C)          | acetone          | 4.0 ± 0.7  |
| TB-CP-6.9f-m | 6.9     | (C)PRRRTPRGQ(C)          | 1,3-xylene       | 1.7 ± 0.4  |
| TB-CP-6.9i  | 6.9        | (C)PRRRTPRGQ(C)          | dibromo 1,3-     | 3.1 ± 1.3  |
|            |            |                           | xylene           |            |
| TB-CP-6.9h  | 6.9        | (C)PRRRTPRGQ(C)          | 1,3 dimethyl     | 9.8 ± 2.5  |
|            |            |                           | pyridine         |            |
| TB-CP-6.9l  | 6.9        | (C)PRRRTPRGQ(C)          | 1,4-dimethyl     | 5.0 ± 1.5  |
|            |            |                           | naphthalene      |            |
| TB-CP-6.9m  | 6.9        | (C)PRRRTPRGQ(C)          | 2,3-dimethyl     | 3.7 ± 0.7  |
|            |            |                           | naphthalene      |            |
| TB-CP-6.9SS | 6.9        | (C)PRRRTPRGQ(C)          | disulfide        | 7.5 ± 0.3  |
Supporting Figures

**Figure S1: Workflow for this investigation.** Outline of the steps used to identify, produce and analyze the cyclic peptides of this study with the goal of producing a new class of antivirals that target HIV-1.
Figure S2: Representative ITC thermograms and isotherm fitting analysis of cyclic peptides analyzed in this investigation. ITC was performed at 25 °C with the peptide sample in the syringe and HIV-1 TAR RNA in the cell. The sequence composition for each cyclic peptide is specified in Table S1. All linker cysteines exhibit L stereochemistry unless noted otherwise. (a) Titration of disulfide bonded TB-CP6.9SS into TAR. (b) Titration of methylene-linked TB-CP6.9a into TAR. (c) Titration of methylene-linked TB-CP6.9a-LD into TAR. (d) Titration of methylene-linked TB-CP6.9a-DD into TAR. (e) Titration of ethyl-linked TB-CP6.9b into TAR. (f) Titration of propyl-linked TB-CP6.9b into TAR. (g) Titration of acetone-linked TB-CP6.9d into TAR. (h) Titration of meta-xylene-linked TB-CP6.9f-m into TAR. (i) Titration of pyridine-linked TB-CP6.9h into TAR. (j) Titration of dibromo-meta-xylene-linked TB-CP6.9i into TAR. (k) Titration of 1,4-naphthalene-linked TB-CP6.9l into TAR. (l) Titration of 2,3-naphthalene-linked TB-CP6.9m into TAR. (m) Titration of methylene-linked TB-CP6.3a into TAR. (n) Titration of meta-xylene-linked TB-CP6.3f-m into TAR. (o) Titration of methylene-linked TB-CP6.Ra into TAR. (p) Titration of meta-xylene-linked TB-CP6.Rf-m into TAR. (q) Titration of 1,4-naphthalene-linked-linked TB-CP6.Rl into TAR. (r) Titration of 2,3-naphthalene-linked-linked TB-CP6.Rm into TAR. For each representative experiment, the apparent equilibrium dissociation constant ($K_D$), the stoichiometry of binding (n) and the unitless quality-control indicator (c) — which describes the shape of the profile (I) — are given for rigor and transparency. Average values for duplicate experiments and other thermodynamic parameters are listed in Table 1 of the main text.
Figure S3: Off-target interaction analysis of representative cyclic peptides and RNA motifs that contain TAR-like bulged internal loops or hairpin loops. ITC was performed at 25 °C with the peptide sample in the syringe and RNA in the cell. The sequence composition for each cyclic peptide is specified in Table S1. (a) Secondary structure diagrams depicting RNA samples used in this investigation to assess off-target binding by representative cyclic peptides. (b) Titration of methylene-linked cyclic peptide TB-CP-6.9α into the human U1 snRNA hpII. No appreciable heats of binding were detected. Previous analysis of the U1A protein titrated into the identical hpII RNA revealed a $K_D$ of 145 ±19 under comparable experimental conditions (2). (c) Titration of methylene-linked cyclic peptide TB-CP-6.9α into the bovine immunodeficiency virus (BIV) TAR; no appreciable heats of binding were detected. The structure of BIV TAR in complex with BIV Tat reveals a comparable internal loop structure compared to HIV TAR but major groove features are different (3). (d) Control titration of the Tat ARM peptide into human 7SK RNA SL1. (e) Titration of methylene-linked cyclic peptide TB-CP-6.9α into the human 7SK SL1 RNA. No appreciable heats of binding were detected. (f) Titration of methylene-linked cyclic peptide TB-CP-6. Ra into the human 7SK SL1 RNA. Despite the detection of heat resulting from titration, no fit could be made. (g) Titration of xylene-linked cyclic peptide TB-CP-6.Rf-m into the human 7SK SL1 RNA. Despite the detection of heat resulting from titration, no fit could be made. ITC titrations were repeated two or more times.
Figure S4: Single-round viral infectivity assays conducted on representative cyclic peptides of this investigation. Analysis of luciferase activity (relative luminescence units or RLU) resulted from infection of TZM-bl cells using pseudotyped HIV-1. The control sample was untreated with peptide and set to 100% infectivity. The cyclic peptides tested included: methylene-linked TB-CP-6.Ra (violet); meta-xylene-linked TB-CP-6.Rf-m (green); meta-xylene-linked TB-CP-6.3f-m (red); dibromo-meta-xylene-linked TB-CP-6.9i (sky blue); meta-xylene-linked TB-CP-6.9f-m (brown); 1,4-methyl-naphthalene-linked TB-CP-6.Rl (orange); 2,3-methyl-naphthalene-linked TB-CP-6.Rm (dark blue); linear peptide TB-LP-6.9 (gold), which was synthesized and characterized previously (3). A positive control was treated with the small molecule temacrazine (mint green), which blocks viral transcription in the nanomolar range (4).
Figure S5: Representative HPLC chromatograms and mass spectrometry analysis of TBP-derived β2-β3 loop cyclic peptides synthesized for this investigation. (a) (left) HPLC profile of cyclic peptide TB-CP-6.3a using a C18 column and a gradient of 20-75% buffer B (0.1% TFA acetonitrile) eluted over 30 min. (right) Mass spectrometry of the pure peptide. (b) Analysis of cyclic peptide TB-CP-6.3f-m as described in panel a. (c) Analysis of cyclic peptide TB-CP-6.Ra as described in panel a. (d) Analysis of cyclic peptide TB-CP-6.Rf-m as described in panel a. (e) Analysis of cyclic peptide TB-CP-6.9a as described in panel a. (f) Analysis of cyclic peptide TB-CP-6.9a-LD as described in panel a. (g) Analysis of cyclic peptide TB-CP-6.9a-DD as described in panel a. (h) Analysis of cyclic peptide TB-CP-6.9b as described in panel a. (i) Analysis of cyclic peptide TB-CP-6.9c as described in panel A. (j) Analysis of cyclic peptide TB-CP-6.9d as described in panel a. (k) Analysis of cyclic peptide TB-CP-6.9f-m as described in panel a. (l) Analysis of linear peptide TB-CP-6.9h as described in panel a. (m) Analysis of linear peptide TB-CP-6.9i as described in panel a. (n) Analysis of cyclic peptide TB-CP-6.9l as described in panel a. (o) Analysis of linear peptide TB-CP-6.9m as described in panel a. (p) Analysis of cyclic peptide TB-CP-6.9SS as described in panel a. (q) Analysis of cyclic peptide TB-CP-6.9Rf-m R47K as described in panel a.
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