Discovery of Novel Latency-Associated Nuclear Antigen Inhibitors as Antiviral Agents Against Kaposi's Sarcoma-Associated Herpesvirus

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Abstract

With the aim to develop novel antiviral agents against Kaposi's Sarcoma Herpesvirus (KSHV) we are targeting the latency-associated nuclear antigen (LANA). This protein plays an important role for the viral genome maintenance during latent infection. LANA has the ability to tether the viral genome to the host nucleosomes and, thus, ensures latent persistence of the viral genome in the host cells. By inhibition of the LANA–DNA interaction, we seek to eliminate or reduce the load of the viral DNA in the host. To achieve this goal, we screened our in-house library using a dedicated fluorescence polarization (FP)-based competition assay, which allows for the quantification of the LANA–DNA-interaction inhibition by small organic molecules. We successfully identified three different compound classes capable of disrupting this protein-nucleic acid interaction. We characterized these compounds by IC_{50} dose-response evaluation and confirmed the compound–LANA interaction using surface plasmon resonance (SPR) spectroscopy. Furthermore, two of the three hit scaffolds showed only marginal cytotoxicity in two human cell lines. Finally, we conducted STD-NMR competition experiments with our new hit compounds and a previously described fragment-sized inhibitor. Based on these results, future compound linking approaches could serve as a promising strategy for further optimization studies in order to generate highly potent KSHV inhibitors.

INTRODUCTION

Kaposi's Sarcoma Herpesvirus (KSHV, also known as HHV-8) is a human γ_2 -herpesvirus that was identified as the etiological agent of Kaposi Sarcoma (KS) and two lymphoproliferative disorders, Primary Effusion Lymphoma (PEL) and the plasma cell variant of Multicentric Castleman's Disease (MCD) (1). Most of the disease burden caused by KSHV occurs in immunocompromised individuals, mainly patients suffering from the acquired immunodeficiency syndrome (AIDS) and transplant recipients. In contrast, KSHV-associated disease is infrequent in otherwise healthy individuals; however, KSHV also causes 'classic' KS mainly in elderly men from KSHV-endemic areas and 'endemic' KS in East and Central Africa (2–5). No specific treatments directed against the latent phase of the KSHV life cycle and KSHV-associated diseases are available (6). For the latent persistence and the regulation of KSHV in the human host, the latency-associated nuclear antigen (LANA) plays an important role (7–9). All KSHV-infected tumor cells express LANA, which is hence used as a biomarker for diagnostics by immunohistochemistry (10,11). It functions as an origin-binding protein via tethering the viral genome with its C-terminus and the host nucleosome with its N-terminus (12–14). LANA ensures a stable and latent persistence of the viral genome in the human cells (15). There are three adjacent LANA binding sites (LBS) located in the terminal repeat (TR) region on the viral genome, which are referred to as LBS1, LBS2 and LBS3 (13,16). Previous studies showed that disruption of LANA expression leads to a reduction of viral DNA copies (17). On the basis of these findings we aim to prevent latent KSHV persistence and reduce the viral load of infected cells through inhibition of the interaction between LANA and the viral genome (18). In a previous work, we discovered first inhibitors, which interfere with the LANA–DNA interaction, using a fragment-based drug-discovery approach (12). The functional activity of our LANA–DNA interaction inhibitors was evaluated using a fluorescence

polarization (FP)-based competition assay as a rapid and quantitative method. The most promising fragment-sized inhibitor **I** showed an IC_{50} value of $17 \pm 1 \mu M$ (Figure 1). Additionally, target binding was confirmed via microscale thermophoresis (MST) and saturation transfer difference (STD)-NMR experiments as well as in an electrophoretic mobility shift assay (EMSA) measuring the interaction between the LANA DNA-binding domain (DBD) and DNA oligonucleotides representing LBS1 and LBS2 (12).

Figure 1. Previously discovered LANA–DNA interaction inhibitor **I** using a fragment-based approach.

In this present study, we exploit our FP-based competition assay as a new screening tool to search our in-house HIPS-library for compounds with the ability to disturb the LANA–DNA interaction. In order to exclude assay artifacts, we used surface plasmon resonance (SPR) spectroscopy to confirm target binding and affinity of the screening hits and assessed their applicability by determining their cytotoxic potential. Moreover, with the view to compare the novel hit scaffolds with inhibitor **I**, we performed STD-NMR-based competition experiments. By this means, we were able to demonstrate non-overlapping binding sites for two of our novel scaffolds with inhibitor **I**. Our screening protocol served as an effective strategy for the discovery of LANA–DNA interaction inhibitors and the identification of novel scaffolds for future medicinal chemistry campaigns.

RESULTS and DISCUSSION

General screening protocol and hit evaluation

In order to identify new potential scaffolds, we decided to screen our in-house HIPS-library (670 compounds) comprising various chemical classes of small molecules with diverse biological activities. We used an FP-based competition assay, which we have established and described previously (12). By this assay, we are able to quantitatively assess the functional activity of compounds in terms of LANA–DNA interaction inhibition. We employ a mutant of the C-terminal DBD of LANA, which is multimerization deficient. A fluorescence-labelled double-stranded DNA oligo is used as the probe. Hit identification was done in two steps as shown in Figure 2A. The first screening step was carried out at a single fixed concentration (100 μ M) for preselection. Then, a three-point dose-dependency test was performed for evaluating the activity profiles. This procedure resulted in nine hits that can be clustered into three classes according to their chemical structure (Figure 2B). Subsequently, we determined the IC₅₀ values using our (FP)-based competition assay and confirmed target binding via SPR measurements of the hit compounds. Moreover, we investigated the binding mode of most promising inhibitors using STD-NMR competition experiments with the inhibitor I.

Figure 2. (A) Flowchart illustrating the screening procedure and the following hit evaluation methods. Using FP-based primary screening for identification of LANA–DNA interaction inhibitors and dose-dependent screening as secondary filters. IC₅₀ determination, SPR and STD-NMR experiments were performed for hit characterization and to confirm target binding; (B) Core structures of our screening hit compound classes.

Library screening using FP-based Assay

To apply our FP-based competition assay as a medium-to-high throughput screening method, we modified assay conditions slightly. For the primary screening, we used the same concentrations of LANA DBD mutant and LBS2 probe as described before (12). The library compounds were screened in two independent experiments at a final concentration of 100 μM. Additionally, we used a high control (HC) comprising samples with LANA DBD mutant, LBS2 probe and DMSO in buffer without any compound and low control (LC) containing the same components without LANA DBD mutant in each screening plate.

Compounds, which contained strong chromophores or precipitated under assay conditions were neglected to exclude false positives. Compounds showing an inhibitory effect greater than 50% were defined as hits. According to these criteria, we selected 86 compounds out of 670 (hit rate 12.8%) for further investigations (Figure S1). Next, we tested the 86 primary actives in a concentration-dependent three-point test in two independent FP-based experiments at final concentrations of 100 μ M, 50 μ M and 10 μ M (Figure S2-S4). By this means, we were able to focus on well-behaved compounds that can display a concentration-dependent inhibition of the LANA–DNA interaction avoid being misguided by a strong initial effect in the spot test. The largest of the three classes (Class I) comprises 64 2-ureidothiophene-3-carboxylic acid derivatives (19,20). This scaffold was previously described as a dual antibacterial and antiviral inhibitor for methicillin-resistant *Staphylococcus aureus* (MRSA) and human immunodeficiency virus type 1 (HIV-1) co-infections (21). The antibacterial activity can be attributed to the inhibition of RNA polymerase (RNAP) activity, which is an essential enzyme for bacterial viability. RNAP is responsible for the transcription of double-strand DNA into single-strand RNA. Antiviral potency of these compounds was rooted in their ability to inhibit the HIV-reverse transcriptase (HIV-RT), which reversibly transcribes the single-strand viral RNA to the double-strand DNA (21). Obviously, there is a certain degree of functional relationship between these two targets and our target LANA, as all three possess the ability to interact with DNA and/or RNA (nucleic acids). The second class (Class II) consists of 21 carboxamido benzoic acid derivatives, which were also identified as bacterial RNAP inhibitors (22). Additionally, we identified a unique compound in our LANA-DNA interaction inhibition assay possessing an indole-2-carboxylic acid scaffold (Class III).

Based on these results, we selected representative examples from Class I (compound **1–4**, Table 1) and Class II (compound **5–8**, Table 1), which show suitable concentration-dependent activity and structural diversity in addition to the single member of class III (compound **9**, Table 1) for further investigations.

Hit characterization

Selected hits were further characterized for their relative potency and target affinity. We carried out dose-dependent FP-assay experiments using serial dilutions up to 100 μ M or 125 μ M with and without addition of LANA DBD mutant. Importantly, for none of our hit compounds we observed fluorescence quenching or enhancement. Representative curves show the results of compounds **1–9** (Figure 3A–C, and S6–S14). Curves were fitted to a four-parameter dose–response model using OriginPro 2018 to calculate IC_{50} values. Generally, the observed IC_{50} values are in the low micromolar range (Table 1). Importantly, we carried out SPR experiments to confirm that our hits are real target binders and not interfering with the DNA. We screened the hit compounds at a final concentration of 100 μ M for binding to an immobilized LANA DBD mutant. For all hit compounds, we observed a significant binding response (Figure S16A–I). The bar diagram (Figure 3D) shows the mean of SPR response values in RU normalized to the molecular weights (MW) of the compounds. These results indicate that protein–DNA-interaction inhibition is most probably due to LANA binding rather than DNA or fluorescence-interfering effects.

Figure 3. **(A–C)** Dose-dependent FP-based competition experiments for hit compounds **1–9**. Curves were based on normalized data points (inhibition from 0–100%) from FP values of duplicates $\pm$ standard deviation; **(D)** Mean of SPR response values (RU) of duplicates $\pm$ standard deviation of the screened compounds at 100 μ M injected over an immobilized LANA DBD mutant.

Within class I (**1–4**), compound **1** showed the lowest IC_{50} value of $24 \pm 1 \mu$ M and the lowest MW (Table 1). The IC_{50} values of hits **2–4** are in the same low micromolar range. However, bulkiness and complexity are increased compared to hit **1**. In the SPR study, compounds **1–3** displayed reproducible responses between 9–14 RU and their sensorgrams showed clear association and dissociation phases indicating a typical reversible binding to LANA (Figure S16A–C). Hit **4** showed a relatively high response value (42 RU), which may indicate unspecific binding because of the two carboxylic acid motifs. Based on these results, we

picked out hit **1** showing the lowest MW, least structure complexity, best IC₅₀ value and good SPR response for further characterization. For class II (**5–8**), hit compound **8** showed the highest inhibitory activity (IC₅₀ value: 11 ± 2 μM). Moreover, all hits displayed prominent SPR responses and binding curves (Figure S16E–H). Interestingly, sensorgram of compound **6** revealed a slow dissociation interaction with LANA among this set compounds (**5–8**) and a potential high affinity. Accordingly, we choose compound **6** for further evaluation as it represents class II preferably, having the lowest MW, low IC₅₀ value (30 ± 2 μM) and favorable binding kinetics. Furthermore, compound **6** showed the highest solubility in our assay conditions. In addition, compound **9** (class III) showed also high activity (IC₅₀ value: 11 ± 1 μM) as well as binding response and was selected for further investigations.

Table 1: Characterization of hit compounds **1–9**.

Comp d	Structure	MW [g/ mol]	IC ₅₀ [μM] (FP assay)	Response [RU] at 100 μM (SPR)	K _D [μM] (SPR)
1		407.27	24 ± 1	9.5 ± 0.7	131.0 ± 9.0
2		437.29	33 ± 1	14.4 ± 0.6	n.d. ^a
3		500.37	26 ± 2	8.6 ± 0.1	n.d.
4		516.57	31 ± 2	42.3 ± 0.8	n.d.
5		396.24	33 ± 5	22.1 ± 1.2	n.d.
6		385.34	30 ± 2	12.2 ± 0.6	9.9 ± 0.4

7		412.24	38 ± 1	10.1 ± 0.5	n.d.
8		399.46	11 ± 2	21.2 ± 1.1	n.d.
9		504.74	11 ± 1	9.1 ± 0.7	9.3 ± 0.4

^a not determined.

Next, we evaluated compounds **1**, **6** and **9** for affinity to LANA and determined binding kinetics using SPR. Compounds **6** and **9** showed high affinity to LANA (K_D values: 9–10 μ M) and about 13-fold stronger than compound **1** (K_D value: 131 μ M) (Table 1 and Figure 4). The on-rate of compounds **6** and **9** was one order of magnitude faster than compound **1**, while all compounds displayed a comparable off-rate (see Figure S17–S19 and Table S2 for detailed results including Langmuir isotherms and kinetic parameters). The dissociation rate constant (k_{off}) and the ligand–protein residence time ($1/k_{off}$) are known to play a major role for potency, efficacy and duration of effect (23). Interestingly, we found that the inhibitory activities (IC_{50} values) obtained from the FP assay show a better correlation with the off-rates of the compounds rather than the binding affinities (K_D values). Such a correlation is known to be target-specific and was observed previously for other protein targets (23,24). These results indicate that the dissociation rate and consequently the duration of binding seem to be the driving force for the activity on LANA.

Figure 4: SPR Sensorgrams (black) of the hit compounds **1** (A), **6** (B) and **9** (C) at concentrations of 1.6–100.0 μ M using immobilized LANA. Global fitting of the association and dissociation curves (red).

Saturation Transfer Difference (STD) NMR Competition Experiments

As the new hits were able to compete with the DNA in the FP-based competition experiments, they seem to bind to the DNA binding site of LANA. Having confirmed compound–LANA binding by the SPR study, we were interested in studying the LANA–ligand interactions and getting insights into the mode of binding. To this end, we performed ligand-observed STD-NMR studies using compounds **1**, **6** and **9** in a competition experiment with our previously discovered inhibitor **I** (Figure 1). STD-NMR competition experiments can provide important information on the putative binding of the hit compounds to the target in the absence of any structural data of the protein (25). In our previous work, we observed a defined binding orientation for inhibitor **I** to LANA where the pyridine moiety of inhibitor **I** interacts strongly with the LANA surface (12). A competition experiment, should allow for evaluating whether the new hit compounds bind at the same binding site as inhibitor **I** or if they bind simultaneously at different binding regions. For the STD studies, we used a fixed concentration (250 μ M) of compound **1**, **6** or **9** and different concentrations (125 μ M, 250 μ M and 500 μ M) of inhibitor **I**. The observed STD spectra for hit **6** in competition to inhibitor

I is shown in Figure 5. Spectrum 6 (red) shows the off resonance spectrum of inhibitor **I**, spectrum 5 (blue) the off resonance spectrum of compound **6**. The next spectrum (4) is the off resonance spectrum of inhibitor **I** mixed with **6**. The spectra 1–3 show the STD signals with fixed concentration of 250 μ M of compound **6** and increasing concentrations of inhibitor **I**. The observed STD effects indicate that both compounds can bind simultaneously to the LANA surface as signals for compound **6** have identical peak intensities regardless any concentration of inhibitor **I**. The STD signals for inhibitor **I** increase with increasing concentration of inhibitor **I** as expected. For the STD competition experiments with compound **1** and Inhibitor **I** we observed similar results (see Figure S26). Unfortunately, we were not able to observe STD-NMR data for compound **9**.

Figure 5. STD-NMR competition experiments with inhibitor **I** and hit **6** in complex with LANA DBD mutant. The respective protons of each compound are highlighted with a corresponding color, Inhibitor **I** in red and Hit **6** in blue; Spectrum 6 (red): off resonance spectrum of

Inhibitor **1** with final concentration of 250 μ M; Spectrum 5 (blue): off resonance spectrum of hit **6** with final concentration of 250 μ M; Spectrum 4: off resonance spectrum of Inhibitor **1** mixed with hit **6**, each with a final concentration of 250 μ M; Spectrum 3: STD spectrum of Inhibitor **1** mixed with hit **6**, inhibitor **1** with a final concentration of 500 μ M and hit **6** with a final concentration of 250 μ M; Spectrum 2: STD spectrum of Inhibitor **1** mixed with hit **6**, each with a final concentration of 250 μ M; Spectrum 1: STD spectrum of Inhibitor **1** mixed with hit **6**, inhibitor **1** with a final concentration of 125 μ M and hit **6** with a final concentration of 250 μ M.

It is quite possible that compounds **1** and **6** bind in different binding modes and on different binding sites of LANA considering the huge DNA binding interface of LANA. Another explanation could be that the hit compounds have an allosteric effect on LANA, which could prevent the LANA–DNA interaction. Importantly, with the STD-NMR competition experiments we demonstrated that different and non-overlapping binding sites for our novel scaffolds do exist compared to inhibitor **1**.

Cytotoxicity

We complemented our hit characterization by testing the compounds for cytotoxicity. Hit **1** and **6** showed only a marginal cytotoxicity on Hek293 and HepG2 cells, whereas compound **9** seems to be highly toxic. Cytotoxicity assessment is very important in prospect of testing cell-based activities of antiviral compounds, which renders Hit **6** the most promising starting point for subsequent optimization efforts as it showed the lowest cytotoxic potential.

Table 2: Cytotoxicity data of compounds **1**, **6** and **9**.

Hit	Relative viability after 48 h [%] @100 μ M	
	HepG2	Hek293
1	114 $\pm$ 42	43 $\pm$ 3
6	147 $\pm$ 12	85 $\pm$ 3
9	20 $\pm$ 9	7 $\pm$ 6

However, also scaffolds found in classes I (**1**) and III (**9**) might provide valuable structural motifs to be exploited in compound merging studies.

Conclusion

We have identified three chemical scaffolds as novel LANA–DNA interaction inhibitors by screening our in-house library using a dedicated FP-based competition assay. These new LANA inhibitors were found to inhibit the LANA–DNA interaction with IC₅₀ values in the low micromolar range providing a very good starting point for medicinal chemistry optimization. Additionally, we applied SPR to confirm target binding and exclude DNA interaction in the FP-assay. All hit compounds displayed a significant binding to LANA, and the obtained affinity (K_D) results were comparable to the inhibitory activity (IC₅₀ values). Furthermore, we performed STD-NMR competition experiments with previously discovered inhibitor **1**. We found that compounds **1** and **6** are binding at a different binding site on the LANA surface than inhibitor **1**. Cytotoxicity was also evaluated for one representative compound from each class, whereby compounds **1** and **6** only show a marginal cytotoxicity and in contrast compound **9** was highly toxic. Importantly, our study demonstrated that our FP-based competition assay can be used as a fast and simple method to identify new LANA–DNA interaction inhibitors, which can open up avenues for further studies. Moreover, the results

obtained in this study can serve as a starting point for further development of LANA–DNA interaction inhibitors with greater potency and selectivity as future therapeutic agents against latent KSHV infections.

METHODS

Expression of His-tagged oligomerization-deficient LANA DBD (aa1008–1146) mutant

The expression of His-tagged oligomerization-deficient LANA DBD (aa1008–1146) mutant was done as described previously (12,13).

FP-based Library Screening

The in-house HIPS small molecule library contained 670 compounds dissolved in DMSO to 10 mM stock solutions. The fluorescence polarization assay was performed as described previously (12) with slight modifications. All experiments were performed in two independent experiments and each sample was tested in duplicates. The primary spot-test screening was performed with a final concentration of 100 μ M of each compound. The three point secondary screening was carried out with final compound concentrations of 10 μ M, 50 μ M and 100 μ M. LANA DBD mutant was used with a final concentration of 200 nM and fluorescent labelled LBS2 oligomer was used with a final concentration of 10 nM (12). IC₅₀ determination of the final screening hits was performed as described previously (12). Curves were fit to a four-parameter dose-response model using OriginPro 2018 to calculate IC₅₀ values. For data reliability, high control (HC) comprising 24 samples with LANA DBD mutant, LBS2 probe and DMSO (5% [v/v] final) in buffer without any compound and low control (LC) comprising 24 samples containing the same components without LANA DBD mutant were distributed over each screening plate.

Binding studies using surface plasmon resonance (SPR)

The SPR experiments were performed using a Reichert SR7500DC surface plasmon resonance spectrometer (Reichert Technologies, Depew, NY, USA), and medium density carboxymethyl dextran hydrogel CMD500M sensor chips (XanTec Bioanalytics, Düsseldorf, Germany). Double distilled (dd) water was used as the running buffer for immobilization. Phosphate-buffered saline (PBS) buffer (10 mM Na₂HPO₄, 1.8 mM KH₂PO₄, 137 mM NaCl, 2.7 mM KCl, 0.05% [v/v] tween 20, pH 7.4) containing 5% [v/v] DMSO was used as the running buffer for binding study. All running buffers were filtered and degassed prior to use. The LANA 1008-1147 (17.653 kDa) was immobilized in one of the two flow cells by amine coupling procedure. The other flow cell was left blank to serve as a reference. The system was initially primed with borate buffer 100 mM (pH 9.0), then the carboxymethyl dextran matrix was activated by a 1:1 mixture of *N*-ethyl-*N'*-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) 100 mM and *N*-hydroxysuccinimide (NHS) 100 mM at a flow rate of 10 $\mu\text{L min}^{-1}$ for 7 min. The LANA protein (aa1008-1147) was diluted to a final concentration of 50 $\mu\text{g mL}^{-1}$ in 10 mM sodium acetate buffer (pH 4.0) and was injected at a flow rate of 10 $\mu\text{L min}^{-1}$ for 7 min. Non-reacted surface was quenched by 1 M ethanolamine hydrochloride (pH 8.5) at a flow rate of 25 $\mu\text{L min}^{-1}$ for 3 min. A series of 7 buffer injections was run initially on both reference and active surfaces to equilibrate the system resulting in a stable immobilization level of approximately 5000 μ refractive index unit (μRIU). Binding experiments were performed at 20 °C. Compounds dissolved in DMSO were diluted with PBS buffer (final DMSO concentration of 5% [v/v]) and were injected (in duplicate) at a flow rate of 30 $\mu\text{L min}^{-1}$. Single-cycle kinetics were applied for K_D determination. The association time was set to 60 s, and the dissociation phase was recorded for 120 s. Ethylene glycol 80% in the running buffer or 10 mM glycine hydrochloride (pH 2.0) was used for regeneration of the

surface. Differences in the bulk refractive index due to DMSO were corrected by a calibration curve (nine concentrations: 3–7% [v/v] DMSO in PBS buffer). Data processing and analysis were performed by Scrubber software (Version 2.0c, 2008, BioLogic Software). Sensorgrams were calculated by sequential subtractions of the corresponding curves obtained from the reference flow cell and the running buffer (blank). SPR responses are expressed in resonance units (RU). Concentration-dependent SPR experiments were performed at final compound concentrations of 100 μ M, 50 μ M, 25 μ M, 12.5 μ M, 6.25 μ M and 3.125 μ M. The K_D values were calculated by global fitting of the kinetic curves.

Saturation-Transfer Difference (STD) NMR.

The STD experiments were recorded at 298 K on a Bruker Fourier spectrometer (500 MHz). The samples contained 5 μ M (final concentration) C-terminal His-tagged oligomerization-deficient LANA DBD (aa1008–1146) mutant and different compound concentrations. The control spectra were recorded under the same conditions containing the free compound to test for artifacts. Conditions for compound **1**: The STD buffer for experiments using compound **1** and inhibitor **I** consisted of 5 mM HEPES, 125 mM NaCl, pH 8.5 in D₂O containing 10% [v/v] DMSO-d₆. The experiments were recorded with a carrier set at -1 ppm for the on-resonance and -40 ppm for the off-resonance irradiation. Selective protein saturation was carried out at 1 s by using a train of 50 ms Gauss-shaped pulses, each separated by a 1 ms delay. For all experiments, a constant concentration of 250 μ M of **1** was used combined with different concentrations of inhibitor **I** (125 μ M, 250 μ M and 500 μ M). Conditions for compound **6**: The STD buffer for experiments using compound **6** and inhibitor **I** consisted of 20 mM tris-Cl, 150 mM NaCl, pH 7.4 in D₂O containing 10% [v/v] DMSO-d₆. The

experiments were recorded with a carrier set at -2 ppm for the on-resonance and -40 ppm for the off-resonance irradiation. Selective protein saturation was carried out at 1.5 s by using a train of 50 ms Gauss-shaped pulses, each separated by a 1 ms delay. For all experiments, a constant concentration of 250 μ M of **6** was used combined with different concentrations of inhibitor **I** (125 μ M, 250 μ M and 500 μ M).

Cytotoxicity Assay

HepG2 and Hek193 cells (2×10^5 cells per well) were seeded in 24-well flat bottom plates. The procedure for culturing the cells, incubation times and OD measurements were performed as described previously (26) with slight modifications. 24 h after seeding the cells, the compounds were added (final DMSO concentration of 1%) and incubated for 24 h. The living cell mass was determined after 24 h. Each compound was tested in two independent experiments.

ASSOCIATED CONTENT

Supporting Information Available: This material is available free of charge via the Internet.

[Data of primary and secondary FP-based library screening, concentration-dependent FP experiments, SPR response curves, information on \$K_D\$ determination via SPR, STD-NMR spectra, and cytotoxicity assay data.](#)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

AIDS, acquired immune deficiency syndrome; DMSO, dimethylsulfoxide; DNA, deoxyribonucleic acid; DBD, DNA binding domain; EDC, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride; FP, fluorescence polarization; HHV-8, human herpesvirus 8; HIPS, Helmholtz Institute for Pharmaceutical Research Saarland; KS, Kaposi Sarcoma; KSHV, Kaposi's sarcoma-associated herpesvirus; LANA, latency-associated nuclear antigen; LBS, LANA binding site; MST, microscale thermophoresis; NHS, *N*-hydroxysuccinimide; PBS, phosphate-buffered saline; STD NMR, saturation transfer difference nuclear magnetic resonance; SPR, surface plasmon resonance; TR, terminal repeat.

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