Explore Novel Drugs To Target HIV RNA for AIDS Therapy

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Introduction

In the three decades since HIV was isolated, the AIDS pandemic remains one of the most devastating diseases in human history. Millions of people are infected every year. Much still needs to be done in the global fight against HIV and AIDS. The development of a highly efficient drug with less toxicity and low cost is in urgent need. This proposal will focus on developing innovative cyclic peptoids to target HIV TAR RNA as antiviral drugs. An efficient and facile synthetic method to generate a large library of compounds and quick structure-activity relationships (SAR) study is proposed here for comparative screening of libraries of synthetic molecules against HIV for their inhibitory effects. By integrating chemical synthesis with the studies on the mechanism of functions of cyclic peptoids in HIV development, our approach will significantly decrease the amount of time and effort required to screen chemical libraries to tandem target HIV RNA. Our studies will have high impact on developing novel and specific methods for the treatment of HIV. Our central goal is to identify novel compounds to facilitate antiviral drug development.

Experimental

NMR data will be collected to interpret the HIV RNA structure complexity upon ligand binding. The RNA constructs applied to our testing include HIV TAR and 7SK RNA. The ligands of interests are focused on cyclic peptides/peptoids. One Bead One Compound (OBOC) libraries of peptoids have been shown to be an excellent source of selective protein ligands. We will further develop new generation of peptoid libraries incorporating more conformational constraints and greater chemodiversity**.** Many cyclic peptide and depsipeptides have been found to display potent biological activities and often have better pharmacokinetic properties than their linear counterparts. This has prompted interest in the synthesis and screening of large libraries of cyclic, peptide-like compounds. To ensure the success of our screening effort to identify peptoid ligands that modulate HIV transactivation, we will further optimize the strategy that incorporates a peptoid library into a cyclic scaffold. Our experiments are performed using *a* Bruker Avance II 600 console with a 5mm TXI cryoprobe and a Magnex 14.1T/54 mm AS magnet in the facility of Advanced Magnetic Resonance Imaging and Spectroscopy, McKnight Brain Institute, University of Florida.

Results and Discussion

Unlabeled 27mer HIV-1 TAR RNA 5'GCAGAUCUGAGCCUGGGAGCUCUCUGC3' (bases in the loop are underlined) was synthesized and was purified by 20% urea-PAGE and desalted on a NAP-25 column. Purified RNA oligomer was resuspended in NMR buffer (50 mM NaCl, 10 mM Phosphate, 0.5 mM EDTA, pH 6, 90:10 H2O/D2O). Spectra were collected on a Bruker Avance II 600 MHz NMR spectrometer at 27 °C. Spectrum of free HIV-1 TAR RNA was collected first; titration of concentrated peptide or peptoid conjugate into the free RNA was monitored by inspecting the imino proton region of RNA. NMR experiments can provide useful structural information on the HIV-1 TAR RNA and the ligands such as peptides and peptoids, especially the structural change upon complex formation. In particular, imino protons from RNA base pairs in the stem produce unique and easily detectable signals that are easy to monitor. NMR spectra were collected in order to distinguish HIV-1 TAR RNA binding between different ligands. NMR spectra of free HIV-1 TAR RNA such as ^{1}H-^{1}H 2D-COSY NMR was shown in Fig. 1.

Conclusions

Our analyses aim to reveal that the novel cyclic peptoids will have substantially improved RNA-binding affinity and specificity relative to the peptide alone. Our work supports the idea that peptide- and peptoid-based binding can be combined to create a new class of high-affinity, high-specificity RNA-binding ligands. This approach provides straightforward optimization to enhance the affinity of known modules and facilitate the discovery of powerful new hybrid ligands with novel functionalities.

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References

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