The Discovery of Novel Bioactive Small Molecules Targeting the Priming Complex of HIV-1

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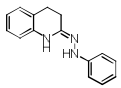
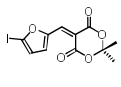
TRANA Discovery

Poster # 50

Abstract

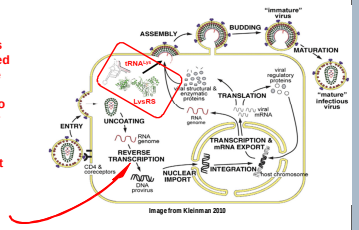
The next generation of antiviral therapeutics will likely target viral functions not yet exploited, such as the HIV viral priming complex. This complex is essential for activation of the reverse transcriptase enzyme and represents a therapeutic target that involves a host factor. HIV selects only human $tRNA^{Lys}_3$ for this primer. Following extensive transformation of the native conformation, HIV modifies the anticodon stem loop (ASL) into a platform for creating this primer complex. In addition to the 18 base pair duplex formed between the viral genome and the 5' end of human $tRNA^{Lys}_3$ at the viral primer binding site, there is a second region of interaction. This region has been shown to be essential for viral replication in both deletion and antisense characterization studies which validate this interaction as a target for therapeutic intervention. To discover small molecule therapeutics, a screening assay design was created using synthetic oligonucleotides, as a mimic of the priming complex and integrating this into an AlphaScreen™ discovery detection platform . After screening a diverse set of 300,000 small molecules, two unique scaffolds were identified. Biochemical and structural docking experiments by NMR have confirmed specific interactions between two of the bioactive hits and the RNA complex. These experiments provided atomic level models of the RNA/RNA/small molecule complexes. Using this NMR information 18 analogs of these scaffolds were selected and tested for inhibition of viral replication in a PBMC assay. Out of the 18 derivatives tested several had good activity in the inhibition of viral replication in a PBMC assay without concomitant toxicity. The results of the bioassay and the SAR will be presented. The two scaffolds are depicted below:

Trana 2010154 

Trana 2010156 

Background

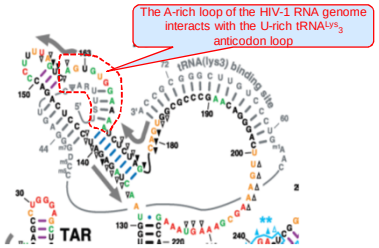
Small molecules would be focused on inhibiting the step where the $tRNA^{Lys}_3$ binds to the HIV-1 primer binding site, in order to ultimately inhibit reverse transcription from occurring.



The reverse transcription primer complex as a target of therapeutic intervention

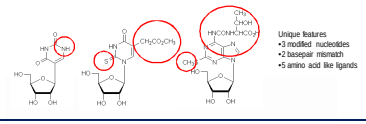
The replication cycle of HIV is well characterized. There are multiple points for therapeutic intervention. Inhibition of viral transcription is a well validated mechanism to therapeutically control HIV. In addition to targeting reverse transcriptase it is possible to inhibit viral replication by inhibiting the formation of the reverse transcription primer complex.

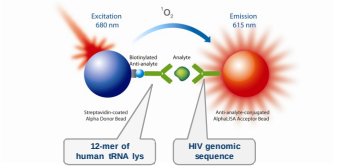
Methods



The target complex

The RT primer complex is formed between the 5' untranslated region of the HIV genome and a host tRNA (human lysine3). The selection of human tRNA lysine 3 as primer is universally conserved and formation of the priming complex is essential for viral replication. In addition to the 18 nucleotide primer binding site (PBS) additional interactions occur between the anticodon region of the tRNA and the viral genome.

A 

B 

HTS screening to identify small molecules that inhibit the formation of the priming complex

To identify small molecules that inhibit the primer binding complex a screen that integrated synthetic oligonucleotides containing the modified nucleotides (see above) contained in the tRNA primers and the 12-mer HIV viral target sequence was integrated into an AlphaScreen® Bead platform. The second region of interaction in the complex is stabilized by 3 post-transcriptional modified nucleotides. Panel A shows the chemical structures of the modified nucleotides. Alphascreen Beads utilize luminescent proximity (Panel B) to report complex formation in the screening assay.

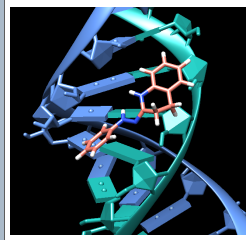
Follow-up screening was performed using the PBMC Assay

PBMC Assays: Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll hypaque gradient centrifugation from whole blood and activated with PHA. Following a seven day acute infection with the clinical clade B strain HT/92/599 in the presence of compound, supernatant reverse transcriptase (RT) activity was measured to quantify virus replication. Cell viability was measured in parallel using XTT dye reduction.

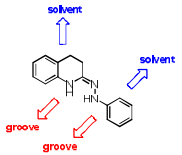
Previous NMR Studies

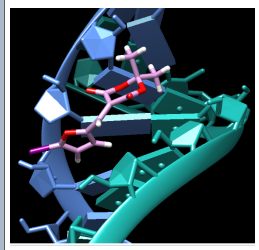
Atomic level models of the RNA/RNA/small molecule complexes were obtained from 2D NOESY NMR studies of the bioactive leads and the RNA complex. Docking of the lead compounds into the RNA complex is shown below.

Reported in Richard Guenther—2013 ICAR poster # 72

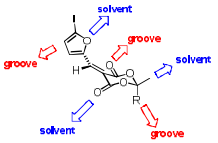


TR 154



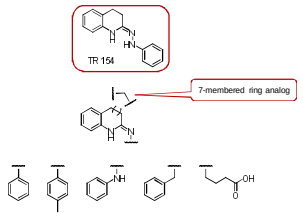


TR 156

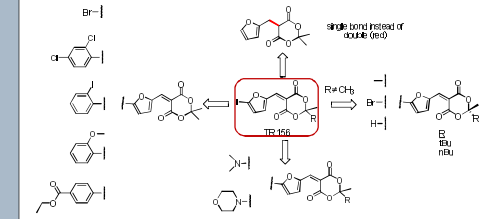


Results

Selected TR 154 Analogs for follow-up testing



***Selected* TR 156 Analogs for follow-up testing**



According to our docking model all of these compounds should bind to the desired groove. Screening of these compounds yielded several bioactive hits in both series, a selection of which is shown below.

PBMC Assay Results

ID #	Structure	EC_{50} µM	TC_{50}	TI
TR 789		89.5	175.0	1.96
TR 452		3.3	114.0	34.24
TR 646		49.5	197.9	4.0
TR 154		51.1	120.2	2.4
TR 662		302.7	>377	>1.25
TR 676		221.9	218	0.98
TR 156		11.7	18.8	1.6

Conclusions and future work

- Results from a proprietary high throughput screen of >300,000 compounds yielded 40 hits, active in biochemical and antiviral assays.
- The two most active hits were shown to interact with the target RNA sequence by NMR.
- Using molecular dynamic docking as a guide, eighteen analogs (that were not in the original library) of the two most active hits were selected.
- Seventeen of these were bioactive in the PBMC assay with a few having good therapeutic indices.
- Future work will entail synthesis of analogs to further refine the structure activity relationship (SAR) and to enhance compound stability.

Acknowledgements

The authors wish to thank Southern Research, ImQuest Biosciences, Technical University of Lodz Poland and North Carolina State University for their technical support. Funding for this research was performed under the DAIDS, NIAID contract N01-AI-70042; Roger Miller, Project Officer and North Carolina Biotechnology Center.