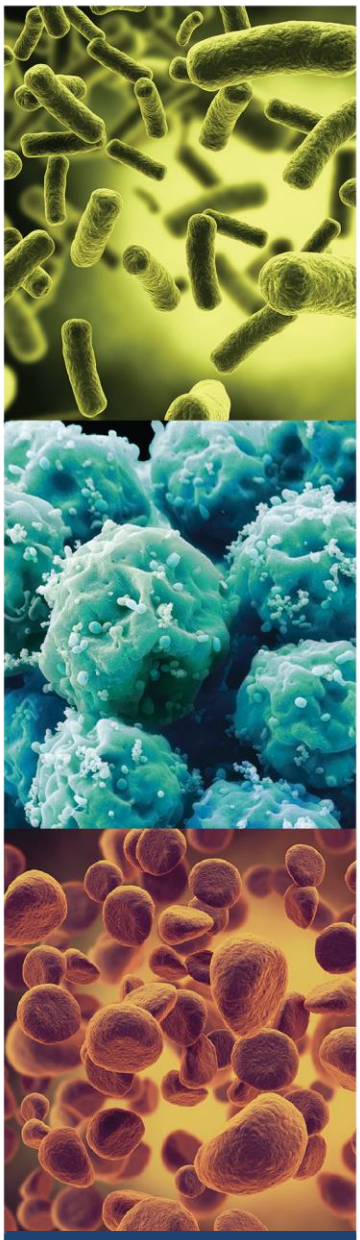
ImQuest BioSciences



ImQuest BioSciences is a preclinical contract research and development company that evaluates the potential of new and novel pharmaceutical products. We specialize in the development of drugs, vaccines and biologic products for the treatment and prevention of infectious disease, cancer and inflammatory disease.

Robert W. Buckheit, Jr., Ph.D.
Chief Scientific Officer
rbuckheit@imquestbio.com

HIV Antiviral Drug Development
Evaluation of HIV Priming Complex Inhibitors

Introduction

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.

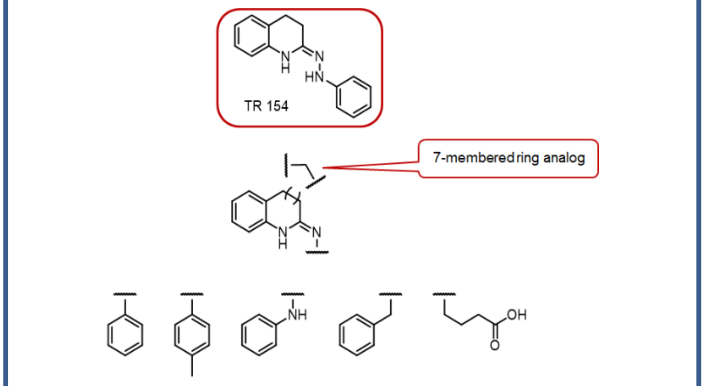
In addition to the 18 base pair duplex formed between the viral genome and the 5' end of human $tRNA^{Lys}_3$ at the viral primer binding site, a second region of interaction has been identified. This region has been shown to be essential for viral replication in both deletion and antisense characterization studies. These studies have validated this interaction as a target for therapeutic intervention.

To discover small molecule therapeutics, a screening assay design was created by Trana Discovery using synthetic oligonucleotides, as a mimic of the priming complex and integrating this into an AlphaScreen™ discovery detection platform.

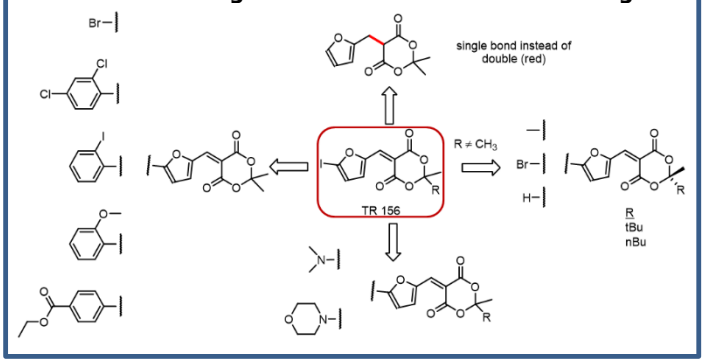
After screening a diverse set of 300,000 small molecules, two unique scaffolds were identified: TR 154 and TR 156 (see red-ringed structures in figure at right).

Biochemical and structural docking experiments by NMR 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.

TR 154 Analogs Selected for Additional Testing



TR 156 Analogs Selected for Additional Testing



Using the NMR information, Trana Discovery selected 18 analogs of these scaffolds for further testing. ImQuest BioSciences, in collaboration with Trana Discovery, evaluated the efficacy and toxicity of these analogs against the clinical clade B strain HT/92/599.

Methodology – PBMC Assays

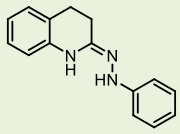
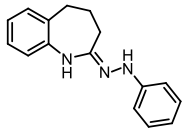
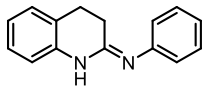
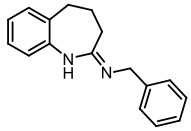
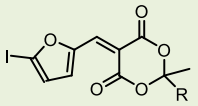
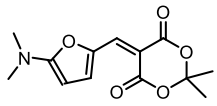
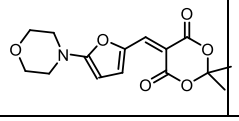
Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll hypaque gradient centrifugation from whole blood and activated with PHA. Following a seven day acute infection with the clinical clade B strain HT/92/599 in the presence of compound, supernatant reverse transcriptase (RT) activity was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

Results & Conclusion

Using molecular dynamic docking as a guide, eighteen analogs of the two unique scaffolds previously identified were selected. Seventeen were bioactive in the PBMC assay. Several of these showed good activity in the inhibition of viral replication in a PBMC assay without concomitant toxicity. See table at right.

Future work will entail synthesis of analogs to further refine the Structure Activity Relationship (SAR) and to enhance compound stability.

The PBMC assay used to produce the results reported herein was optimized by ImQuest BioSciences and is part of the ViroSens suite of tools for screening and evaluating antiviral drug candidates.

ID #	Structure	EC50 µM	TC50 µM	TI
TR 154		51.10	120.20	2.40
TR 452		3.30	114.00	34.24
TR 646		49.50	197.90	4.00
TR 789		89.50	175.00	1.96
TR 156		11.70	18.80	1.60
TR 662		302.70	>377	>1.25
TR 676		221.90	218.00	0.98

ImQuestSUCCESS

Select drug candidates with the highest probability of clinical success

The ImQuest*SUCCESS* preclinical services platform is used to critically evaluate the potential of a test compound and to assure that its efficacy, toxicity, and pharmaceutical properties are evaluated in a comprehensive and interactive way. Successful completion of platform objectives provides significant confidence in the potential of a test compound to transition to human clinical trials, enhances the robustness of drug development efforts and reduces the risk of expensive clinical development failures by the exclusion of candidates which are likely to fail during advanced preclinical and clinical development at early (and less expensive) time points.

7340 Executive Way, Suite R, Frederick, MD 21704
www.imquestbio.com | p: 301-696-0274 | f: 301-696-0381