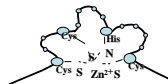


Mono- and Combination Therapy Efficacy of Unique Antiviral Agents that Target the HIV-1 Nucleocapsid Protein (NCp7)

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Abstract

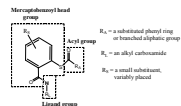
The highly conserved p7 nucleocapsid protein (NCp7) of HIV-1 is a target for the development of new antiviral agents based on its broad range of function in virus replication and the ability of inhibitors to selectively interact with the fingers and eject the coordinated zinc, inhibiting virus replication and rendering the virus noninfectious. The highly conserved NCp7 protein of HIV contains two copies of the zinc finger motif Cys(X)2Cys(X)4His(X)4Cys (CCHC). NCp7 plays pivotal roles during both early and late phases of HIV-1 replication, being required for the functioning of the reverse transcriptase, integrase and protease, as well as the selection and packaging of the RNA genome into maturing virions. Mutations in the Zn chelating and/or non-chelating residues have been shown to result in loss of NCp7-mediated functions, rendering the virus non-infectious. Thus, the central role of the NCp7 protein and the absolute requirement for intact NCp7 protein and Zn fingers during HIV replication makes this protein an attractive target for drug development. Diverse sets of electrophilic compounds that react with cysteine thiolates in the NCp7 protein or NCp7 protein precursors (p55agg and p160gag-pol) have been identified and evaluated. Although different in chemical composition all lead molecules lead to the ejection of Zn (II) ions bound within the structural Zn finger motifs of the NC protein and inhibit the replication of HIV. We have continued our efforts to increase the solubility, stability and potency of Ncp7 inhibitors and have recently identified a series of less hydrophilic, uncharged S-acyl 2-mercaptobenzamide thioester derivatives. In order to maximize the potential to identify the optimal compound configuration we developed a combinatorial chemistry approach to explore three spaces of the thioester chemotype. Using this model as a skeleton for synthetic variation allowed the use of substituents, which could potentially modify the reactivity of the thioester bond through electronic influences and steric hindrance. The results of these evaluations will be presented. In addition we have pursued studies evaluating the efficacy of Ncp7 inhibitors when used in combination with other anti-HIV agents. Significant antiviral synergy was observed when the compounds were used in combination with other anti-HIV agents, including attachment/fusion, reverse transcriptase and protease inhibitors and other Zn finger inhibitor chemotypes. The synergistic interaction of the test compounds appears to be mediated through antagonistic toxicity in which the combination extends the therapeutic index of the test molecules. The results of our *in vitro* combination assays with several potent Zn-finger inhibitors will be presented.



Possible Targets of Zinc Finger Inhibitors

- Reverse Transcriptase
- Integrase
- Tat-Independent Transcription
- Virus Assembly and Maturation

Combinatorial Template for Thioesters



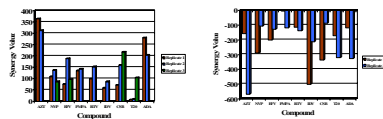
Methods

- **HIV/SIV Cytotoxicity Assay:** Following a six day acute infection of CEM-SS cells with the RF strain of HIV-1 in the presence of compound, cell viability was measured spectrophotometrically (450/650 nm absorbance) using XTT dye reduction. The same assay format was used to measure cytoprotection of 174xCEM cells infected with SIVmac251 in the presence of compound.
- **PBMC/Monocyte Assay:** Peripheral blood mononuclear cells were isolated by ficoll hypaque gradient centrifugation from whole blood and activated with PHA. Monocytes were further purified by adherence to plastic and washing to remove lymphocytes. Following a seven day incubation, supernatant reverse transcriptase (RT) activity or p24 antigen expression by ELISA was measured to quantify virus replication. Cell viability was determined by XTT dye reduction.
- **Latent Infection Assay:** The effect of compounds on virus expression from TNF- α induced, latently infected cells were performed using U1 cells. Cultures were incubated for 3 days and virus expression was measured by supernatant RT activity. Cell viability was determined by XTT dye reduction.
- **Combination Therapy Assay:** MacSynergy II was used to analyze a checkerboard concentration matrix in the HIV cytoprotection assay for efficacy and toxicity in CEM-SS cells. Combinatorial results are expressed as μM^2 or volume of the area under the curve at the 95% confidence level. Above 50 μM^2 range is defined as synergistic, < 50 to 50 is additive and below < 50 is antagonistic.

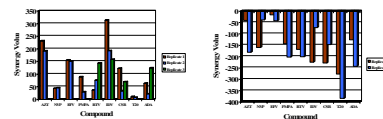
Efficacy and Toxicity of PATE Compounds

Compound	Structure	Cytotoxicity		PBMTC			
		IC ₅₀	IC ₉₀	IC ₅₀	IC ₉₀		
YS1332D		1.2	156	310	1.8	248	111
AG-991		0.82	93	113	1.2	>200	>167
AG-998		2.3	318	38	631	>100	>118
AG-996B		3	122	43	1.3	>200	>182
AG-996A		3.1	>200	65	6.8	169	225

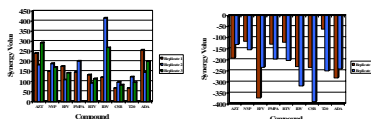
Combination Efficacy and Toxicity of AG9908



Combination Efficacy and Toxicity of AG9968



Combination Efficacy and Toxicity of AG9901



Summary of Combination Efficacy Results

Compound	AG991	AG998	AG996A	AG996B	YS1332D
AZT	HS	HS	HS	HS	HS
Nevirapine	HS	HS	HS	A	S
Efavirenz	HS	HS	HS	A	HS
PMPA	HS	HS	A	HS	A
Rilovavir	HS	HS	S	A	HS
Indinavir	HS	S	HS	HS	HS
CSB	S	HS	AS	HS	HS
T20	S	A	A	A	A
ADA	HS	HS	S	S	HS

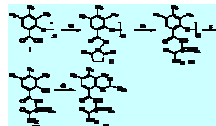
S: synergistic; HS: highly synergistic; A: additive; An: antagonistic; HAn, highly antagonistic

Summary of Combination Toxicity Results

Compound	AG991	AG998	AG996A	AG996B	YS1332D
AZT	HAn	HAn	An	A/An	HAn
Nevirapine	HAn	HAn	An	A	HAn
Efavirenz	HAn	HAn	A	HAn	HAn
PMPA	HAn	A	HAn	An	An
Rilovavir	HAn	HAn	HAn	HAn	HAn
Indinavir	HAn	HAn	HAn	HAn	HAn
CSB	HAn	HAn	HAn	HAn	HAn
T20	HAn	HAn	HAn	A	HAn
ADA	HAn	HAn	HAn	HAn	An

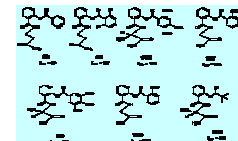
S: synergistic; HS: highly synergistic; A: additive; An: antagonistic; HAn, highly antagonistic

Synthesis of S-Acyl 2-Mercaptobenzamide Thioester Derivatives



Reagents/conditions: a) Hydrosulfonylation (DICTHF, 2-POM/25C; b) H₂N(CH₂)_nC(=O)NH₂ (DMF/25C; c) TCEP.HCl/Et₃N/DMF/H₂O(1:1)/25C; d) RACOCl/DMA/25C

Lead Thioester Compounds



Anti-HIV Activity of Lead Thioester Compounds

Compound	IC ₅₀ (nM)	IC ₉₀ (nM)	IC ₅₀ (nM)	IC ₉₀ (nM)	IC ₅₀ (nM)	IC ₉₀ (nM)	IC ₅₀ (nM)	IC ₉₀ (nM)
AG-991	0.82	93	113	1.2	>200	>167		
AG-998	2.3	318	38	631	>100	>118		
AG-996B	3	122	43	1.3	>200	>182		
AG-996A	3.1	>200	65	6.8	169	225		
YS1332D	1.2	156	310	1.8	248	111		

Note: Thioester compounds are in order of low to high serum t_{1/2}.

Summary

•Combinations of PATEs with either AZT, indinavir or ADA yielded the highest overall level of synergistic anti-HIV activity.

•Combinations of PATE or PATE-derivatives with Azodicarbonamide (ADA) resulted in decreased cytotoxicity and synergistic to highly synergistic antiviral activity, suggesting that formulations of multiple Zn finger inhibitors may be as advantageous as Zn finger inhibitors with other antiviral agents.

•Although synergistic reductions in cytotoxicity were observed, NVP and T-20 were the least compatible (lower overall synergism or additivity) when combined with the PATEs.

•Comparison of the results obtained from combination of the PATE and PATE-derived chemotypes with common antiviral compounds, results in the following rank ordering of the PATEs (most potent to least potent): AG-9901 > YS1332D > AG9908 > AG996A > AG996B.

•S-Acyl 2-mercaptobenzamide thioester derivatives demonstrate efficacy in a variety of anti-HIV assays (acute infection with laboratory and clinical strains, including multi-drug resistant virus, HIV-2 and SIV)

•Lead thioester compounds (19, 52, 89 122) were chosen based on antiviral potency (EC₅₀ < 5 μM), low cytotoxicity (TC₅₀ > 125 μM) and stability in serum (t_{1/2} > 100min)

Acknowledgements

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