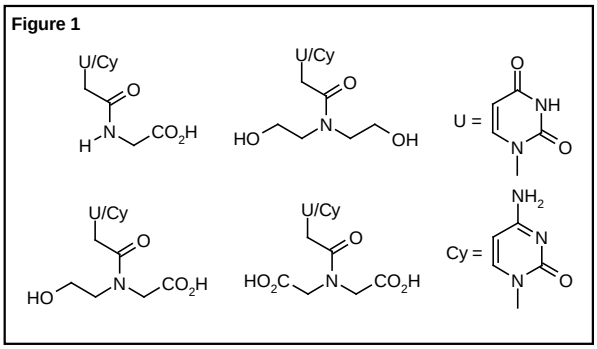
ABSTRACT

Target Ribonuclease A: Synthesis and Biological Evaluation of Nucleoside-derived Inhibitors

Ribonuclease family of enzymes catalyze the degradation of RNA into smaller components. Effective inhibition of their enzymatic activity has become a topic of growing interest with the realization that the biological activities manifested by several members of this family are critically dependent upon their ribonucleolytic activity. Ribonuclease A (RNase A) is a representative member of this family that works at the junction of the transcription and translation processes, thereby maintaining the cellular RNA levels. Structural homology among the various members of this communion permits the use of Ribonuclease A (RNase A) as a model system to explore the structure- activity relationships.

In our continuous hunt for suitable non-nucleotide inhibitors of RNase A, a gamut of different approaches has been adopted in the current study. A series of uracil and cytosine-derived carboxylated acyclonucleosides have been synthesized as potent RNase A inhibitors (Figure 1). Agarose gel based assay qualitatively indicated inhibition of the enzyme, which was further confirmed by inhibition kinetics. Binding of inhibitors to RNase A resulted in perturbation of the secondary structure of the enzyme. Modified acyclonucleosides were also used as ligands to synthesize a couple of copper complexes as probable inhibitors of RNase A. Agarose gel based assay revealed that copper complexation markedly improves the inhibitory potency of the parent acyclonucleosides. Steady state kinetics experiments confirmed that the copper complexes were mixed type reversible inhibitors of the enzyme.

Figure 1



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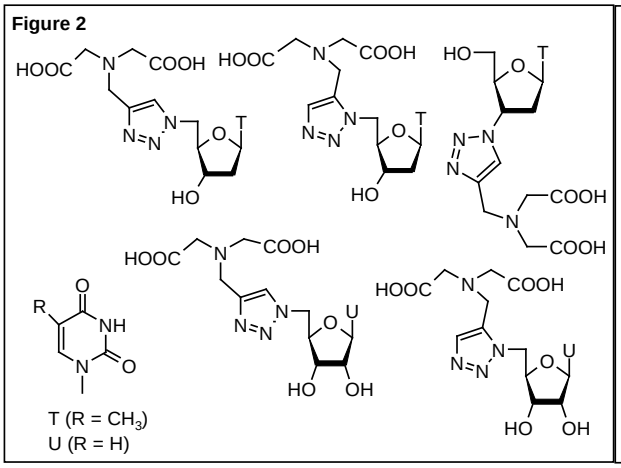
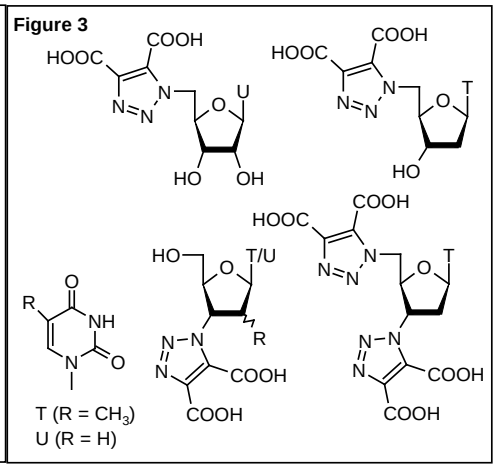
1, 4- and 1, 5-disubstituted 1, 2, 3-triazole-linked nucleoside carboxylic acids were synthesized and employed as potent RNase A inhibitors (Figure 2). The biophysical studies revealed the 1, 4-regioisomers to be more potent RNase A inhibitors in comparison to the corresponding 1, 5-derivatives. Docking studies showed that the inhibitors were in close proximity to the active site of RNase A, resulting in reversible competitive inhibition. Successful application of disubstituted triazole-linked nucleoside carboxylic acids as RNase A inhibitors prompted us to synthesize nucleoside carboxylic acids linked through 1, 4, 5-trisubstituted triazole ring (Figure 3). The inhibitory properties of the synthesized molecules were explored using several experimental as well as theoretical methods. Agarose gel based assay and kinetics studies revealed the relative inhibitory potencies of the different nucleoside derivatives. Docking studies showed that the molecules were binding at the active site of the enzyme, resulting in efficient inhibition of RNase A in a reversible competitive fashion. 3', 5'-bistriazole tetracarboxylic acid derivative of thymidine was found to be the most potent inhibitor of the series.





Keywords: Ribonuclease A, Inhibitors, Acyclonucleoside, Complex, Triazole, Kinetics, Docking.