Thesis Abstract

Title: Synthesis and Biological Evaluation of Carboxymethylsulfonyl Tethered Nucleosides: A Chemical Logic Directed Approach Towards Ribonuclease A Inhibition

The central thesis of DNA to RNA to protein is largely dependent on the action of RNA polymerases that synthesize RNA, and RNA depolymerases also known as ribonucleases (RNases), which degrade RNA. There exists a wealth of information on ribonucleases that have exhibited a multitude of activities including many medicinal applications. Ribonuclease A (RNase A) serves as a convenient model enzyme in the identification and development of inhibitors of proteins belonging to the ribonuclease superfamily. In RNase A, $P_0...P_n$, $R_0...R_n$ and and $B_0...B_n$ corresponds to phosphate, ribose and nucleobases of bound RNA, respectively (Figure 1). Most of the efficient inhibitors of RNase A are phosphate- and pyrophosphate- based nucleotides but have problems associated with transport through membranes. Therefore a different class of modified nucleosides was designed using chemical logic with respect to the active site of RNase A. In short it was expected that the acidic functional groups attached to this class of modified nucleosides would effectively bind to the positively charged P_1 site of RNase A. Using different synthetic strategies, a series of acidic nucleosides was synthesized with carboxymethylthio and carboxymethylsulfonyl groups attached at 2′, 3′ and 5′ sites of sugar ring (Scheme 1).

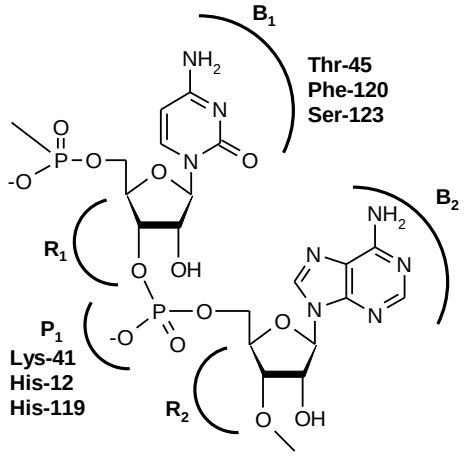
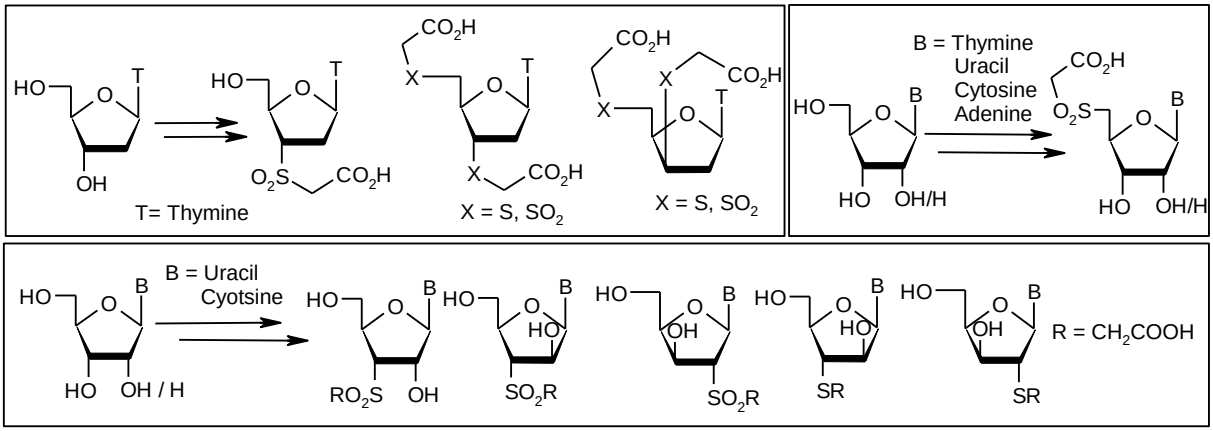


Figure 1: Active clefts of RNase A



Scheme 1

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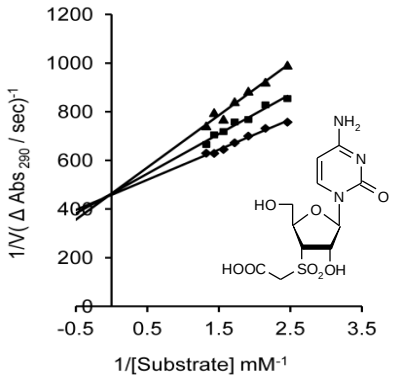

Figure 2: Representative Lineweaver-Burk plots for inhibition of RNase A by carboxymethylsulfonyl modified nucleoside

To determine the mode of inhibition and inhibition constants (K_i) of compounds these compounds steady state kinetic experiments were performed. The inhibition constants of potent inhibitors of RNase A, were determined from the Lineweaver-Burk plots (Figure 2). From the K_i values, it was concluded that carboxymethylthio and carboxymethylsulfonyl modified thymidine compounds were potent reversible competitive inhibitors of RNase A which was in agreement with the preliminary studies like agarose gel electrophoresis and precipitation assays.

Molecular docking studies were carried out to identify the probable binding sites of the inhibitors with RNase A. Inhibitor to protein docking has been performed considering various types of molecular interactions. In these studies, it was observed that nucleobases of the inhibitors bound

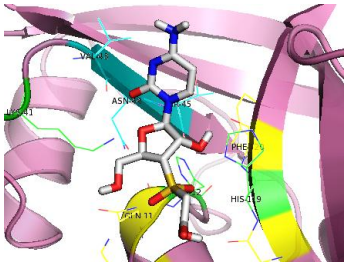

Figure 3: Representative docked pose of the carboxymethylsulfonyl modified inhibitor of figure 2 with RNase A.

to the B_1 site whereas, with an aid of extra H-bondings from the oxygen atoms of sulfone groups, -COOH groups reached to the active site (P_1) efficiently which resulted in effective inhibition of enzyme. Thus, extensive docking studies corroborated experimentally obtained data appreciably which in turn, strengthened our approach towards the design and synthesis of a new class of RNase A inhibitors.

In conclusion, carboxymethylsulfonyl and carboxymethylthio functionalized nucleosides have been synthesized on the basis of chemical logic which is exclusively dependent on pK_a and electrostatic interactions between enzyme and inhibitors. With a high acidity and anchoring ability of carboxymethylsulfonyl groups, nucleosides with -SO_2CH_2COOH moiety revealed better potencies with low inhibition constant (K_i) values. Effect of this group at 5′-site of nucleoside was also monitored which elicited good results. Finally, the role of 2′- and 3′-hydroxyl groups on inhibitory property was investigated. The ribo-analogs emerged as the most effective inhibitors (K_i = 1.75 µM) with preferentially far better interactions with enzyme than former inhibitors.

Keywords: Ribonuclease A, Inhibitors, Acidic nucleosides, Kinetics, Docking.