Hepatitis C virus (HCV) infection is one of the leading causes of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma. It has affected more than 170 million individuals worldwide. The current treatment with ribavirin and pegylated α-interferon is successful only on 50% of patients and, both drugs are indirect antivirals because they do not target a specific HCV protein or RNA element. The NS3 protein is a potential target for drug development, since it is composed of two domains (a serine-protease and a RNAhelicase/ATPase), both essential for virus replication and proliferation. In this study, the domains of NS3 were expressed and purified, and analysis the conformational properties and inhibitors screens were performed with approximately 400 compounds against NS3 protease and ATPase activities. The ATPase activity was performed using colorimetric assay which amount of inorganic phosphate released by ATP hydrolysis was measured. For inhibition of proteolytic activity, we evaluated the cleavage of a peptide labeled with 5-FAM/QXL by FRET assay (fluorescence resonance energy transfer assay). In our studies showed that 11 compounds were able to inhibit the ATPase activity (including mimetic peptide, quinones and mainly triazole compounds), and one compound mimetic peptide was able to inhibit protease activity with IC50% of 76±14µM on pure enzyme assay. These compounds will be tested in Huh-7 cells infected with the HCV replicon to evaluate inhibitory potential. Moreover, thermodynamic assay are being carried out to understand the conformational changes of the protease NS3 protein in presence of urea, NaCl, and bis-ANS.

Word Keys: Hepatitis, NS3, inhibitors
Supported by: FAPERJ, CNPq, PRONEX-RIO, OMS/TDR.