THE IDENTIFICATION OF HCV POLYMERASE INHIBITORS: A SHOW CASE OF MODERN DRUG DISCOVERY

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An estimated 3% of the world population (~170 million) suffers from chronic HCV infection that causes a progressive liver disease which can lead to fatal conditions such as cirrhosis or hepatocellular carcinomas. The leading cause for liver transplants in the US results from HCV infection, and some 10,000 deaths occur annually in that country that can be linked directly to the disease. Current therapies for HCV are based on combinations of pegylated interferon (IFN-α) and ribavarin, a broad spectrum antiviral agent. They are sub-optimal for a significant portion of the patient population, who fail to achieve a sustained response (50% in the case of the predominant genotype 1) or can not tolerate the adverse side effects associated with this treatment. Therefore, the discovery of specific antiviral agents would be of great benefit to HCV patients in general. In an effort to discover novel anti-HCV chemotherapeutics, two essential virally-encoded enzymes, NS3 serine protease and NS5B polymerase, were targeted in our drug discovery effort.

We recently reported the discovery of BILN 2061, a macrocyclic tripeptide inhibitor of NS3 protease that effectively reduced HCV RNA plasma levels in a short term study with HCV genotype 1 infected patients. NS5B polymerase inhibitors provide a complementary approach that likewise, offers opportunities for the development of novel HCV therapies. Screening of our corporate collection using a modified NS5B construct allowed the identification of specific benzimidazole 5-carboxamide derivatives originating from a large combinatorial screening library. These compounds were shown to specifically inhibit productive RNA binding to the polymerase through a novel and specific mechanism. NMR techniques were used to confirm interaction of compounds with the polymerase in the absence of RNA, and to determine the solution conformation of a bound inhibitor. Photo-affinity labeling experiments identified a putative binding region for these inhibitors which is located in the upper-thumb domain of the enzyme. The evolution of this class of compounds through a synergistic interaction between medicinal and combinatorial chemists allowed the rapid identification of derivatives which showed activity in a cell-based assay of *subgenomic* HCV RNA replication (replicons). Selection of resistant replicons using these analogs supported the existence of an allosteric binding site for this class of inhibitors. Further developments resulted from successful soaking of compounds into protein crystals and 3D-structure determination of inhibitors bound to NS5B using X-ray crystallography. These experiments confirmed the location of an allosteric binding pocket and provided insight into the mechanism by which this class of compounds inhibits polymerase activity. Using the cell-based replicon system, we also demonstrated that a combination of HCV protease and polymerase inhibitors are complementary in cell culture models of HCV RNA replication in suppressing the emergence of resistant variants, and expand the repository of potential treatments for chronic HCV infection.