Interdomain Communication in HCV Polymerase Abolished by Small-Molecule Inhibitors

Stefania Di Marco, Cinzia Volpari, Licia Tomei, Sergio Altamura, Steven Harper, Frank Narjes, Uwe Koch, Michael Rowley, Raffaele De Francesco, Giovanni Migliaccio, Andrea Carfí, *Istituto di Ricerche di Biologia Molecolare, "I.R.B.M. P. Angeletti", Via Pontina Km 30.600, 00040, Pomezia (Rome), Italy.* E-mail: stefania_dimarco@merck.com

The hepatitis C virus (HCV) polymerase is required for replication of the viral genome and is a key target for therapeutic intervention against HCV. We have determined the crystal structures of the HCV polymerase complexed with two indole-based allosteric inhibitors at 2.3 Å and 2.4 Å resolution. The structures show that these inhibitors bind to a site on the surface of the thumb domain. A cyclohexyl and phenyl ring substituents, bridged by an indole moiety, fill two closely spaced pockets whereas a carboxylate substituent forms a salt bridge with an exposed arginine side chain. In the apoenzyme, the inhibitor binding site is occupied by a small alpha-helix at the tip of the N-terminal loop that connects fingers and thumb domains. Thus, these molecules inhibit the enzyme by preventing formation of intramolecular contacts between these two domains and consequently precluding their coordinated movements during RNA synthesis. Our structures identify a novel mechanism by which a new class of allosteric inhibitors inhibit the HCV polymerase and open the way to the development of novel antiviral agents against this clinically relevant human pathogen. Furthermore, the structures reveal a mechanism of inhibition, with the inhibitor displacing part of the fingertip loop anchoring fingers to the thumb, which may be relevant also for the inhibition of other viral RNA dependent RNA-polymerases.

Keywords: HCV, NS5B, polymerase