Structure-based design of novel P2-P4 macrocyclic inhibitors of hepatitis C NS3/4A protease

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INTRODUCTION

Approximately 170 million people worldwide are chronically infected with hepatitis C virus (HCV),[1] a (+)-strand RNA virus of the Flaviviridae family, which is spread primarily by direct contact with human blood.[2] HCV causes chronic liver disease, including cirrhosis and hepatocellular carcinoma.[3] At present, HCV is a leading cause of death in HIV co-infected patients[4] and is the most common indication for liver transplantation.[5] In the United States alone, data from death certificates suggest that there are 10,000 to 12,000 deaths annually due to hepatitis C.[6]

Unlike HIV, HCV can be "cured"; that is, patients can achieve a sustained virologic response (SVR), in which the virus remains undetectable after termination of therapy. The current standard of care for the most prevalent genotype 1 infection is a regimen of pegylated interferon (IFN) plus ribavirin for 48 weeks.[7] Due to limited efficacy (only about half of genotype 1 patients are able to achieve SVR at twenty-four weeks post-therapy) and significant side effects (e.g., injection site inflammation, flu-like symptoms, depression, and anemia), many patients discontinue treatment. Thus, there is a significant need to improve efficacy, reduce the duration of treatment, and develop an IFN-free regimen with a more convenient route of administration.

DRUG DESIGN TARGET

Several promising antiviral targets for HCV have emerged in recent years.[8] As with HIV, most efforts have focused on inhibiting the key viral enzymes (see Figure 14.1). Inhibitors of one such target, HCV NS3/4A, have perhaps shown the most dramatic antiviral effects.[9,10] The full-length NS3/4A protein is comprised of an N-terminal trypsin-like serine protease (residues 1–180), a C-terminal NTPase/helicase (residues 189–626), and a fifty-four residue NS4A cofactor. The NS3/4A serine protease is responsible for *cis* cleavage at the NS3/4A junction, as well as *trans* cleavage at the NS4A/4B, NS4B/5A, and NS5A/5B junctions[11] and is essential for viral replication.[12] Clinical proof of concept for NS3/4A protease inhibitors has also been demonstrated, both for rapidly reversible noncovalent protease inhibitors such as BILN-2061 (**1**)[13] and for slowly reversible covalent serine-trap inhibitors such as VX-950 (telaprevir, **2**),[14] shown in Figure 14.2.

INITIAL MODELING

Examination of published views of a close analog of **1** bound to the 1–180 protease domain of NS3 protease[15] suggests that the P2 thiazolylquinoline portion of the inhibitor lies on a relatively featureless enzyme surface with binding interactions that provide little apparent basis for the dramatic potency derived from that moiety (>30,000-fold) in a related series of tripeptide inhibitors.[16] As the crystal structure of this complex was unavailable, we recapitulated the binding pose by creating a model of **1** in the NS3/4A protease domain active site, based on the published view and employing a previously deposited NS3/4A protease domain crystal structure (PDB identifier 1JXP).[17] Figure 14.3 illustrates the solvent-exposed positioning of the P2 thiazolylquinoline in the model of **1** bound to the NS3/4A protease domain. Based on this view of binding to the relatively shallow, solvent-exposed, protease domain pocket, developing a tight-binding, drug-like inhibitor of NS3/4A protease was once likened to the probability of success of a climber scaling a featureless dome-shaped rock with few, if any, hand- or toe-holds.

In an effort to reconcile the bound pose with the observed structure/activity profile, we chose to model **1** bound to the full-length NS3/4A protein, including the significantly larger helicase domain, to determine what role the helicase might play in inhibitor binding. No full-length NS3/4A structures with inhibitors bound are currently available. Consequently, a published *apo* enzyme structure[18] of a single-chain form of NS3/4A was used as the starting point (Figure 14.4). In this structure, the six C-terminal residues (DLEVVT) of the helicase domain occupy the NS3 protease active site, forming twelve hydrogen bonds and creating a contact surface of ~500Å^2. To mimic the conformational change required to permit inhibitor binding, these six residues were deleted. Additionally, to accommodate the model of **1**, the crystallographic conformation of some protein side chains (e.g., R155 and Q526)