NS5A clinical candidates appear to have a low genetic barrier to resistance. The NS5A inhibitors evaluated to date were generally well tolerated and offer considerable promise for use in combination therapy that has the potential to cure chronic HCV infection in the absence of pegylated interferon-α and/or ribavirin.[83–85]

1.6 Mode of Action Studies with HCV NS5A Replication Complex Inhibitors

Detailed *in vitro* replicon studies have revealed that the mutations that confer resistance to HCV NS5A replication complex inhibitors, such as **1** and **3**, map to the N-terminal region of Domain I, involving amino acids 24–100.[1,65] Resistance analysis of clinical samples obtained from SAD and MAD studies of daclatasvir (**1**) have corroborated these *in vitro* findings.[86] Although there are differences in the exact mutation composition of different resistant genotypes, there is a broad overlap in the specific locale that are hot-spots for the emergence of resistance mutations, which is suggestive of a common mode and, possibly, region of interactions between NS5A and its putative inhibitors. Although residues 28–31, which is a key resistance mutation region for both G-1a and G-1b viruses, was not captured in the X-ray structural studies of Domain I, it is noteworthy that the mutation Y93H, which is a clinically relevant mutation common for G-1a and G-1b, lies in the dimer interface region of both X-ray structures.

To gain additional insight into the mode of interaction between **1** and the protein, two models for the G-1b NS5A Domain I were constructed using the Rice dimer (1ZH1.pdb, model 1) or the Love dimer (3FQM.pdb, model 2) and the NMR structure of the amphipathic N-terminal α-helix, amino acids 1–31.[9,10,87] The respective missing amino acids were modeled in so as to complete each monomer. Mapping of the primary resistance mutations observed in clinical studies for both G-1a and/or G-1b patients on to the model indicates a clustering of mutation sites that is presumed to indicate the putative inhibitor binding site. Daclatasvir (**1**) was hand-docked into the putative binding site symmetrically across the dimer interface of each model. Docking into the model constructed based on the Rice dimer produced the best fit based on the physical properties of both ligand and protein and also the location of resistance mutations. The hydrophobic regions of **1** – the biphenyl core, pyrrolidine and valine side chain moieties – are surrounded by hydrophobic amino acids (residues M28 and L31), while the polar imidazole and carbamate moieties deployed on each end of the inhibitor are in a position to form hydrogen bonds with the monomeric units (Figure 1.13). It is believed that these inhibitors may interact with the NS5A dimer and induce a conformational change that is not functionally viable. Most interestingly, a recent report suggests that disruption of dimerization may not be the mode of action for **1** and related analogs.[11,88]