initiation step of RNA synthesis. In the replicon cell-based assay, **61** inhibited gt1a/1b at single-digit nanomolar concentrations ($EC_{50} = 9/5$ nM). Using the replicon system, HCV variants that harbor mutations which confer resistance to HCV-796 were isolated at several amino acid residues, mapping a new binding pocket adjacent to the enzyme active site. Sensitivity to major C316Y mutant in close proximity to the catalytic triad of the polymerase (GDD motif) in particular was rationalized using X-ray structural data.[90] Binding of HCV-796 analogs to NS5B was found to induce a conformational change in the R200 hinge site, revealing a new pocket that only partially overlaps with that of benzothiadiazines (palm site 1 binders), as depicted in Figure 8.1.[9a] HCV-796 was evaluated in a chimeric mouse model of HCV infection and, unlike its predecessors, produced a $2\log_{10}$ reduction in HCV titers.[91]

In 2006, HCV-796 was advanced into a Phase 1 proof-of-concept clinical trial and a modest $1.4\log_{10}$ reduction in viral load was obtained for a 1000 mg bid dose in gt1 HCV patients. Furthermore, on-treatment rebound was observed in this monotherapy trial, with the C316Y mutant emerging in half of the patients. In combination with PegIFN/RBV, a more robust antiviral effect was obtained, with $2.6–3.2\log_{10}$ reductions in virus for gt1 patients. Unfortunately, soon after Phase 2 trials were initiated with HCV-796 in combination with SOC, clinically significant liver enzyme elevations were noted in 8% of patients following 8 weeks of therapy. Development of this compound was subsequently terminated owing to safety concerns.[90]

In the last few years, there seems to have been renewed interest in this class of palm site 2 NS5B inhibitors, as several patent applications have been filed by companies (*e.g.*, Abbott Laboratories, Biota, Bristol-Myers Squibb, GlaxoSmithKline, Presidio Pharmaceuticals) claiming analogs of the benzofurancarboxamide chemotype. One such compound, from Presidio Pharmaceuticals (PPI-383, structure undisclosed), was recently presented as a next-generation NS5B non-nucleoside inhibitor with potent replicon activity against major HCV genotypes 1a/b, 2a, 3 and 4a ($EC_{50} = 3.0–13$ nM).[92] Resistant variants with decreased susceptibility to PPI-383 were isolated and encoded S365T/A and C316Y mutants, consistent with inhibitors targeting the palm site 2 binding site. Unlike many NS5B allosteric inhibitors, PPI-383 is only moderately protein bound (66%) and exhibited good oral bioavailability in preclinical animal species and plasma half-lives consistent with once- or twice-daily dosing in humans. Time will tell if these new derivatives are able to provide good efficacy and, at the same time, address the safety issues encountered during the development of HCV-796.

8.3.5 Covalent NS5B Inhibitors

Imidazopyridines, shown several years ago to possess potent cell-culture activity against pestivirus replication (*e.g.*, BVDV, **62**, Figure 8.16), were optimized to a series of specific HCV replication inhibitors through a collaboration between Gilead Sciences and the Rega Institute in Leuven. Key structural features necessary for selectivity and high anti-HCV potency in the