culture, to more potent indole-6-carboxylic acid derivatives and, more recently, conformationally restricted polycyclic analogs. This class of allosteric inhibitors was shown through biochemical studies to inhibit viral replication at the initiation stage of RNA synthesis by preventing the formation of a productive polymerase:RNA primer–template complex. Structural studies suggest that this effect results from inhibitors binding to the thumb domain, competing with an interaction with the protein loop that extends from the finger domain, thus interfering with the formation of an enclosed active site, which is thought to be important for RNA synthesis. BILB 1941 is the first compound in this class to provide proof of concept for this mechanism of action in genotype 1 HCV-infected patients. Among NS5B allosteric inhibitors, those that bind to thumb pocket 1 (finger-loop inhibitors) display an attractive cross-genotype profile, with comparable potency observed against genotypes 1, 3, 4, 5 and 6 (within threefold) but somewhat larger shifts against genotype 2 (15–30-fold). Consistent with clinical observations, the *in vitro* resistance signature of this class of allosteric inhibitors is characterized by mutations at residues P495 and P496. Today, three thumb pocket 1 inhibitors (BI 207127, BMS-791325 and TMC647055) are progressing through clinical evaluation and may become potential combination partners with complementary DAAs in all-oral interferon-free regimens.

8.3.2 Thumb Pocket 2 Inhibitors

The existence of a second allosteric pocket in the thumb domain of NS5B (thumb pocket 2) was initially revealed in 2002 in a patent from Agouron Pharmaceuticals describing the X-ray structure of the dihydropyrone derivative **31** (Figure 8.8) in complex with the enzyme.[47a,b] In 2003, Shire Biochem described the crystal structure of a different chemotype (phenylalanine derivative **32**) bound to the same hydrophobic pocket, 30–35 Å away from the polymerase active site and, subsequently, a thiophenecarboxylic acid derivative, **33**, bound to a gt2a NS5B.[47c,d] These two findings served as starting points for two successful programs that each led to the discovery of development candidates (filibuvir and lomibuvir, respectively).

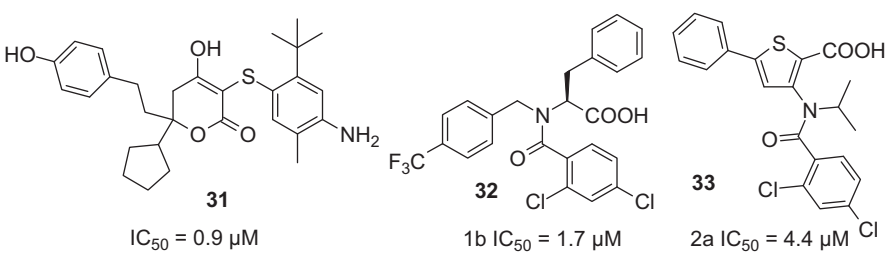

Figure 8.8 Thumb pocket 2 starting points from Agouron and Shire Biochem.