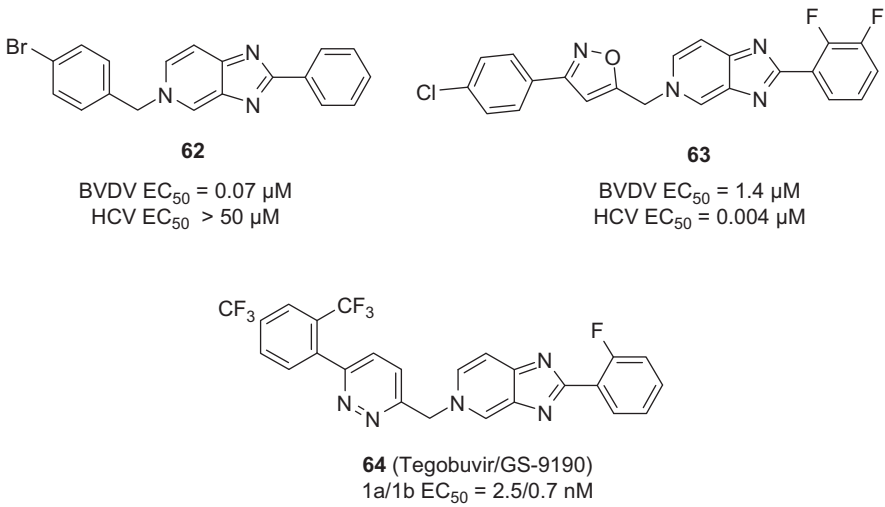

Figure 8.16 Imidazopyridine HCV replication inhibitors that interact with NS5B – discovery of tegobuvir (GS-9180).

low nanomolar range included the presence of a halogen atom at the *ortho*-position of the 2-phenyl ring and extension from the N5 position with aryl-substituted heterocycles (*e.g.*, **63**). Tegobuvir (**64**, formerly GS-9190) is a member of this class that was selected for clinical evaluation.[93]

Interestingly, although this class of highly potent HCV replication inhibitors did not inhibit any of the usual viral targets in biochemical assays, virus specificity and significantly reduced sensitivity to non-1 HCV genotypes (*e.g.*, gt2–6) strongly suggested that a host target was unlikely to be involved in its mechanism of action. Furthermore, selection experiments performed with the replicon led to the identification of NS5B mutants (C316Y, C445F, Y448H and Y452H) which conferred resistance to imidazopyridines and were not cross-resistant to other classes of HCV DAAs.[94] Although biophysical methods such as NMR, X-ray crystallography and photoaffinity labeling did not provide definitive evidence that inhibitors could bind to NS5B, several clues about the mechanism of action were gathered from mutational studies which suggested that the β-hairpin region of the thumb domain played an important role in the mechanism of inhibition. Based on computer docking experiments, SAR studies and the mutation signature of imidazopyridines, a putative binding site was proposed for the class that encompasses both palm sites 1 and 2.[94a] Interestingly, further understanding of the mechanism of action of tegobuvir was derived from metabolic studies, which suggested that the antiviral effect is mediated through a unique oxidative chemical activation pathway and subsequent interaction with NS5B to form a covalent complex. The formation of NS5B–tegobuvir covalent adducts was dependent on cellular glutathione levels and CYP1A activity. A mechanistic pathway was proposed that begins with CYP-mediated oxidation of the 2-fluorophenyl ring to produce a reactive