replication.[73] Demonstration that the compound was ~10-fold more potent against HCV replication than CsA led to it being investigated further. Alisporivir binds to CypA in a similar manner as CsA and potently inhibits the PPIase activity of CypA with a K_i of 0.34 nM.[73] *In vitro* studies using an IL-2 reporter assay in Jurkat T-cells or a murine/human mixed lymphocyte proliferation assay demonstrated that alisporivir is up to 7000-fold less immunosuppressive than CsA, consistent with the alisporivir–CypA complex being a weak inhibitor of CaN.[74] Using an HCV genotype 1b subgenomic replicon system, alisporivir was shown to be significantly more effective than CsA at suppressing viral RNA replication (IC_{50} 0.04 μM *versus* 0.3 μM for alisoprivir and CsA, respectively) over the course of 72 h of treatment.[75] Importantly, alisporivir was shown to be equally effective against replicons derived from HCV genotypes 1–4 and to be active against the JFH-1 infectious system.[75] Combinations of alisporivir with interferon, ribavirin, the NS3 protease inhibitor telaprevir, 2′-*C*-methylcytosine (a nucleoside NS5B inhibitor) or JT-16 (a non-nucleoside NS5B inhibitor) showed additive to slightly synergistic activity against HCV RNA replication with no cytotoxicity toward the host cell.[75] Alisporivir retains wild-type levels of inhibitory activity against replicons resistant to NS5B polymerase (nucleoside and non-nucleoside) and NS3 protease inhibitors and, likewise, these inhibitors retained potency against alisporivir-resistant replicon cell lines, indicating no cross-resistance between Cyp inhibitors and the primary viral target inhibitors.[75] Generation of low-level (about fivefold) *in vitro* resistance to alisporivir was difficult to achieve, requiring a high number of passages, consistent with a very high viral barrier to resistance.[76] Alisporivir was progressed to clinical investigation with initial studies being performed in HIV/HCV co-infected individuals where the compound showed a marginal reduction in HIV viral load; however, a significantly greater reduction in HCV RNA ($3.63\log_{10}$ reduction from baseline) was observed when dosed at 1200 mg bid.[69] Phase 2 clinical studies investigating the effect of alisporivir, at various dose levels, as monotherapy or in combination with Peg-IFN/ribavirin in HCV genotypes 1–4 individuals have been performed.[77] While the details of these clinical studies are beyond the scope of this chapter, alisporivir significantly increased the antiviral effectiveness of Peg-IFN/ribavirin therapy and the compound is currently under evaluation in expanded studies of HCV genotype 1- and 2/3-infected patients.[78]

SCY-635 (**8,** Figure 11.5) is a third Cyp inhibitor to be evaluated in clinical studies of HCV infection and, like NIM-811 and alisporivir, was originally discovered as part of an anti-HIV program.[79] SCY-635 potently inhibits the enzyme activity of CypA ($K_i = 2$ nM), has low immunosuppressive potential (1500-fold lower inhibition of IL-2 production by stimulated Jurkat T cells than CsA) and inhibits replication of a HCV genotype 1b-derived replicon (IC_{50} 0.1 μM) with no evidence of toxicity.[80] When evaluated in a clinical study of activity in HCV genotype 1-infected patients, SCY-635 given as monotherapy resulted in a modest decline of serum HCV RNA, with no evidence of resistant virus being detected.[81] However, the finding that the clinical activity of SCY-635 in HCV-infected patients is closely correlated with an SNP