Various models have been proposed to explain the role of CypA in the replication of HCV RNA.[66] The finding that *in vitro* resistance to Cyp inhibitors, although slow to emerge, is associated with mutations in both NS5A and NS5B supports the biochemical demonstration of an interaction between these proteins and CypA.[67] However, knockdown of CypA inhibited replication of a full length HCV genome significantly more effectively than the subgenomic replicon system, suggesting that CypA is involved in additional interactions with HCV proteins.[60] Gallay and co-workers provided evidence in support of a model whereby CypA, pre-residing in crude replication complex membrane fractions, serves to associate NS5A–NS5B into an assembling replication complex and that CsA depletes this compartment of CypA, thereby preventing replication.[68] Clues towards an understanding of the role that PPIase activity might play in HCV RNA replication were presented by Bartenschlager and co-workers in a study of the rate of HCV polyprotein processing.[60] The demonstration that a resistance mutation to the Cyp inhibitor DEBIO-025 (alisporivir), occurring near the cleavage site of the NS5A–NS5B polyprotein, results in delayed processing of the protein and impaired replication activity.[60] A role for CypA in the conformational reorganization of multiple regions of the HCV polyprotein can be envisaged that might account for the high barrier to resistance exhibited by Cyp inhibitors due to the need for multiple virus mutations to appear to escape the need for Cyp PPIase activity.

Interestingly, it has been described that treatment of replicon-bearing Huh cell lines with Cyp inhibitors (alisporivir and SCY-635) causes a secretion of CypA and CypB from the cell into the supernatant.[69] This release leads to a depletion of intracellular Cyp levels which may play a role in controlling replication of HCV RNA. Finally, a large-scale siRNA experiment involving Cyp binding compounds provided evidence that NIM-811 (**4**) reduces viral replication *via* inhibition of multiple Cyps and pathways.[64]

The discovery that Cyp inhibition was sufficient to prevent HCV RNA replication quickly led to renewed interest in NIM-811 owing to its prior demonstration of potent Cyp binding activity, oral bioavailability and potential for low nephrotoxicity.[70] While originally discovered as part of an anti-HIV program, NIM-811 was found to be a more potent inhibitor of HCV replication than CsA in a HCV replicon assay, and further, co-treatment with interferon-α led to clearance of the virus.[71] Initial structure–activity relationship (SAR) data presented by Novartis supported a correlation between binding affinity for CypA and activity in HCV replicon assays and highlighted the attractive profile of NIM-811.[72]

NIM-811, at various dose levels, in combination with Peg-IFN/ribavirin was investigated in clinical studies of HCV genotype 1 infected patients over the course of 4 weeks of therapy; however, no subsequent clinical studies have been described.[65]

The most clinically advanced Cyp inhibitor for HCV, alisporivir ([D-MeAla]^3[EtVal]^{4}CsA, **7**, Figure 11.5), was also originally identified as a potent inhibitor of CypA PPIase with good activity against HIV-1