complex, as attempts to generate bespoke compounds targeting the peripheral site on *de novo* models, although successful, could not improve on the potency of rimantadine.[63]

Screening-based alternatives to rational development have been employed for p7 with some success. Genotype 1b channels were tested in a large-scale liposome dye release screen of 3520 compounds by investigators at Boehringer Ingelheim.[62] This identified multiple hits and also validated this assay system as a robust means of identifying novel inhibitors with low sensitivity to fluorescence artefacts. However, these compounds have not been pursued to date. In addition, BIT225 was selected from a bacterial toxicity screen in cells expressing genotype 1a p7.[87] BIT225 inhibits 1a p7 at 100 μM *in vitro* and was shown to display a cell culture IC_{50} of ~300 nM against the related pestivirus bovine viral diarrhoea virus (BVDV). Given the varied susceptibility of HCV isolates to p7 inhibitors, it is perhaps surprising that BIT225 displays efficacy against the highly sequence-divergent BVDV p7, although, like rimantadine, this may indicate that a binding site is present across multiple HCV genotypes to which the drug binds with reasonable efficacy. In addition, whether BIT225 adopts the same mode of action against p7 proteins as it does for Vpu is unknown. However, recent studies on p7 from the related pestivirus classical swine fever virus (CSFV) revealed marked differences between this and the HCV protein, making it all the more surprising that BIT225 displays efficacy in this system.[41] Data for BIT225 in HCV cell cultures are yet to be presented and no resistance information for this or other amilorides such as HMA, is available to assign a potential mode of action. Based on Vpu studies,[211,258] binding may occur at a lumenal site involving Ser21, yet this has not been analysed *in silico* or otherwise. However, results from early clinical trials appear encouraging (see below), making BIT225 a first step towards targeting p7 clinically for HCV treatment.

9.2.3.5 *Clinical Use of p7 Inhibitors: Past, Present and Future*

The majority of prototype p7 inhibitor trials have concerned amantadine alongside SOC and were conducted prior to the identification of p7 as a viroporin. Many patient studies of amantadine (usually 200 mg d^{-1}) for 12 weeks in combination with SOC have revealed little improvement compared with dual therapy, as assessed by sustained virological response (SVR) at week 60, 12 weeks after completing a 48 week course of interferon/ribavirin. However, meta-analyses stratifying results for patients who had previously failed treatment – usually genotype 1 HCV – did reveal a measurable benefit of the triple therapy regimen.[84,85] Amantadine monotherapy studies measuring the kinetics of patient responses revealed rapid viral rebound in a matter of days[259] and, when given with SOC, a loss of clinical benefit was observed upon withdrawal of amantadine at 12 weeks, where improved patient responses reverted to the SOC controls.[260] Similarly disappointing results were obtained using a nonylated imino sugar p7 inhibitor based on *N*N-DNJ, UT-231b, developed between United Therapeutics and researchers in Oxford