induce an L-shaped bioactive conformation of ligands in the free state, which complemented the protein bioactive binding pocket as revealed in X-ray structures.[26] While diamide derivatives such as **10** were excellent inhibitors of the polymerase *in vitro*, their modest potency in the cell-based replicon compromised further progression of the benzimidazole class. Replacement of the benzimidazole left-hand side of compounds, such as **10,** by the isosteric *N*-methylindole **11** depicted in Figure 8.4, increased cell activity by 20–30-fold. A large array of indole diamides bearing variations at C2, amino acid linker and the right-hand side capping group were subsequently synthesized, eventually leading to the discovery of **15** (Figure 8.5).[27] Compound **15** displayed comparable enzymatic and cell-based potency (IC_{50} and $EC_{50} \approx 50$ nM), reflecting improved cellular permeability compared with benzimidazole analogs. The C2 furyl substituent was replaced with a more drug-like 2-pyridyl moiety, which also provided a significant decrease in lipophilicity and improved solubility.

Whereas Caco-2 permeability for this compound was excellent and predictive of good oral absorption (Caco-$2_{A \rightarrow B} = 39 \times 10^{-6}$ cm s^{-1}), metabolic stability upon incubation with human and rat liver microsomes was only modest (HLM/RLM $t_{\frac{1}{2}} = 50$ and 17 min, respectively) and compounds in this series showed disappointing oral pharmacokinetics in rats.[27] As poor plasma exposures following oral dosing were in part due to rapid clearance of compound, metabolite ID studies were performed and hydroxylation of the cyclohexyl ring was identified as a central metabolic pathway for 3-cyclohexylindole analogs.[28] Fortunately, replacement of the cyclohexyl moiety with the less lipophilic cyclopentyl group provided compounds with significantly improved *in vitro* metabolic stability and only modest decrease in potency.

High-throughput parallel synthesis was used once more to provide a sparse matrix of 3-cyclopentylindole inhibitors that harbored modifications at C2, the central α,α-disubstituted amino acid and the right-hand side acidic capping group. Out of 110 combinations, 84 compounds were identified with $EC_{50} < 150$ nM in the cell-based replicon. Following Caco-2 permeability profiling and metabolic stability assessment in human and rat liver microsomes, 71 compounds were selected for oral absorption studies in rats. This was accomplished through screening of compounds in cassette mode, where mixtures of 3–4 compounds were orally administered to animals and plasma exposure was measured at 1 and 2 h time points. This rapid screening provided a short list of compounds that were then evaluated as single compounds in rats, dogs and monkeys, culminating with the discovery of BILB 1941 (**16**, Figure 8.4) as a development candidate.[28] BILB 1941 is a specific and reversible inhibitor of HCV NS5B polymerase with good cell-based potency in gt1a/1b replicons ($EC_{50} = 153$ and 84 nM, respectively). BILB 1941 displays an attractive pan-genotype profile in chimeric replicon assays with moderate (up to threefold) shifts in genotypes 3a, 4a, 5a and 6a, while a more significant shift (14–32-fold) was observed for gt2a/2b.[29] BILB 1941 is highly protein bound (98–99%), but a modest threefold EC_{50} shift was measured in replicon assays performed in the presence of serum. The PK profile in rats, monkeys and dogs was favorable, with