Further substitution of the bridge with solvent-exposed basic side chains produced zwitterionic derivatives with excellent potency in cell culture (*e.g.*, **19**, $EC_{50} = 35\,nM$). Introduction of this scaffold-rigidifying element into JTK-109 analogs provided compounds with impressive potencies: IC_{50} and $EC_{50} < 10\,nM$ (*e.g.*, **20**).[35b] No further reports from Japan Tobacco have appeared since these initial findings.

8.3.1.2 Discovery of MK-3281

Other companies also reported similarly rigidified analogs of thumb pocket 1 NS5B inhibitors. The IRBM-Merck group in Italy reported tetracyclic indole derivatives containing a four-atom bridge and basic side chains (*e.g.*, **21**) in which the anilinic function of analogs such as **19** (considered as a potential liability from a drug safety point of view) was eliminated.[36a] Such tetracyclic indole derivatives featuring an eight-membered ring displayed potency comparable to that of previously published indole-*N*-acetamides such as **14,** but with improved PK properties in preclinical species relative to open-chain analogs. Indeed, analog **21** had a cross-species PK profile consistent with low clearance in humans and had good distribution to the liver target organ in rats (liver/plasma ratio = 16). In human hepatocytes, elimination was mainly through CYP3A4-mediated phase 1 metabolism (hydroxylation of the cyclohexyl ring). Despite the identification of glucuronide conjugates, the compound had low potential for covalent binding to human microsomal protein. The overall profile of **21** justified advancement as a preclinical candidate, but no report is available on the outcome of such activities for this compound.[36a]

Subsequent reports from the IRBM-Merck group focused on identifying analogs with improved potency. These included pentacyclic indole derivatives in which the number of rotatable bonds of tetracyclic analogs such as **21** was reduced by inclusion of the solvent-exposed basic side chain into a five-membered ring that was fused on to a benzazepine rather than a benzodiazepine framework (*e.g.*, **22**, Figure 8.6).[36b] In general, *trans*-annellated systems retained comparable or improved potency relative to tetracyclic systems and did not exhibit notable differences between low- and high-serum conditions in the cell-based replicon system. Attempts were made to optimize the inhibitor core itself and thienopyrrole-based inhibitors (*e.g.*, **23**) were also described, but no improvement was achieved over previously described analogs.[37] Ultimately, a tetracyclic benzoxazocine derivative (**24**, MK-3281) was selected for clinical development as it combined improved potency with good oral bioavailability in preclinical animal models. Furthermore, MK-3281 showed efficacy in a chimeric mouse model of HCV infection where a bid dose of $50\,mg\,kg^{-1}$ produced a $3.1 log_{10}$ drop in viremia. On the basis of this promising profile, the compound was evaluated in the HCV-infected chimpanzee model and was progressed into Phase 1 clinical trials.[38] For undisclosed reasons, however, the development of MK-3281 has been discontinued.