Optimization proceeded at Boehringer Ingelheim with analogs such as **6**, in which the minimum core **5** was coupled to various amino acids to improve potency and address solubility issues associated with neutral amide substituents (*e.g.*, **2**).[15] The potency of analogs such as **6** allowed the design of a photo-affinity probe, which was used in cross-linking experiments with NS5B to establish that the binding site of benzimidazole inhibitors was located in the thumb region of the polymerase in proximity to Λ1 finger loop residues and a postulated regulatory GTP-binding site.[11a,16] This was the first piece of evidence that suggested the presence of an allosteric site at the top of the thumb domain. Intriguingly, however, the presumed bioactive conformation of inhibitors such as **6** could not be docked in this region of the enzyme, as it lacked suitable binding pockets in the *apo*-enzyme structure.[17]

In early, 2000, Japan Tobacco selected a compound for clinical evaluation (JTK-003, structure undisclosed) that presumably belonged to the benzimidazole class. The outcome of Phase 2 trials with this drug were never published, but around the same time, a cell-based replicon system was developed that reconstituted replication of subgenomic HCV RNA in cell culture.[18] The best inhibitors reported at the time by Japan Tobacco exhibited only weak antiviral potency in the replicon ($\sim 1\,\mu M$), which could explain in part why the development of JTK-003 was discontinued. SAR studies were extended to analogs bearing large lipophilic biphenyl substituents on the left-hand side, one of which (JTK-109, **4**, Figure 8.2) inhibited the 1b replicon at submicromolar concentration ($EC_{50} = 0.32\ \mu M$) but with a high EC_{50}/IC_{50} ratio of 19. Nevertheless, the compound displayed favorable pharmacokinetic (PK) and safety profiles and also good plasma-to-liver distribution in rats.[19] In 2003, JTK-109 was reported to have initiated a Phase 1 clinical trial, but the outcome was never reported. Development was likely stopped due to still modest replicon potency and unsatisfactory antiviral clinical efficacy, safety and/or unsatisfactory pharmacokinetics.

In Boehringer Ingelheim's series, the presence of two ionizable carboxylic acid functions in compounds such as **6** prevented cell permeability and activity in the replicon system. Replacement of one of the carboxylic acid functions, which was shown to act as an orienting rather than binding group[17] by a small heterocycle, provided derivatives such as **7**, which were active in cell culture at micromolar concentrations and provided the possibility for further optimization.[20]

Inhibitors such as **7** exhibited low solubility and high lipophilicity, which compromised their ability to progress as drug candidates. As a result, an effort was initiated to identify alternative right-hand sides with potential to confer superior drug-like properties. Figure 8.3 summarizes the knowledge that had been acquired so far on benzimidazole inhibitors (structure **8**), pointing out the role of some of the structural features embedded in the molecules.

Retaining the cyclohexyl ring as a potency anchor and the right-hand side carboxylic acid as a solubilizing group, linkers between the two were explored using high-throughput synthesis techniques. Ultimately, an (*R*)-alanine–cinnamic acid conjugate (*e.g.*, **9**) provided inhibitors with promising