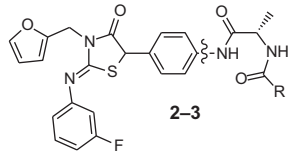

Compd.	R	G-1b EC_{50} (nM)
2	OCH_2Ph	580
3	CH_2Ph	6.0
4	OCH_2Ph	110
5	CH_2Ph	2.3

Figure 1.4 SAR highlights of thiazolidinone series.

inhibitor (Figure 1.4). It is noteworthy that over one million compounds were assayed in the HTS effort and only this single chemotype class met the stringent screening criteria to qualify as a suitable lead structure. Carbamate **2** exhibited moderate HCV potency (G-1b $EC_{50} = 0.58\,\mu M$), very good HCV specificity (BVDV EC_{50} $>50\,\mu M$) and a CC_{50} of $>100\,\mu M$.[19,20]

In order to identify the HCV protein that carbamate **2** might be targeting, passage of a G-1b replicon system through increasing concentrations of the compound resulted in a resistant phenotype that was >10-fold less sensitive to the inhibitor. After confirming that the mutation that caused the resistance phenotype was associated with viral RNA and not cellular RNA, sequence analysis of viral RNA from resistant cell lines was conducted and two dominant mutations were identified in the Domain I region of NS5A (Y93H and Y93C). Either mutation, when introduced individually into a G-1b replicon, was sufficient to confer the observed resistant phenotype and no cross-resistance was observed with inhibitors targeting alternative HCV mechanisms. This resistance analysis was the first indication that the thiazolidinone chemotype might be engaging the NS5A protein.

Preliminary structure–activity relationship (SAR) studies directed at establishing the fidelity of the lead revealed that there was a preference for the *S* stereochemistry at the amino acid moiety and that changing the benzyl carbamate to phenylacetamide, as in amide **3**, effected a $\sim$100-fold potency enhancement, an SAR observation that was recapitulated in the analogous proline series (see amides **4** and **5**). The SAR survey of the iminothiazolidinone region of the lead molecule revealed that variation of the substituent pattern also modulated potency; a >10-fold dynamic potency range that was dependent on structure was noted. However, the patterns of SAR associated with this region were less discrete than that of the amino acid moiety. Resistance selection with amide **3** yielded additional mutations in NS5A Domain I (L31V and Q54L) that resulted in a 9–60-fold potency loss and were cross-resistant to amide **2**, suggesting commonality of the inhibitory mechanism between these two molecules, despite the difference in their resistance mutations.

At this juncture, it became apparent that this thiazolidinone chemotype was exhibiting chemical instability in certain organic solvents and in the replicon medium.[21] Careful analysis of degradation products revealed that when **3** is stored in dimethyl sulfoxide (DMSO) under ambient conditions, it undergoes an oxidative rearrangement to afford the thiohydantoin **8**, which was inactive in the replicon assay, EC_{50} $>20\,\mu M$ (Scheme 1.1). Incubation of **3** in the replicon