Compound **61**, with three carbons between the carbamate oxygen and the P2-quinoline, proved to have very modest activity of 2000 nM in a genotype 1b NS3/4A enzyme inhibition assay[90] (Table 7.9). Incremental lengthening of the linker, however, afforded dramatic improvements with optimized activity of 8.5 nM in the case of the C_5-linked **63**. The improved enzyme inhibition of **63** also translated to a corresponding improvement in genotype 1b cell-based replicon activity.[91] The point of attachment of the macrocyclic linker on the P2-quinoline was critical to activity, as demonstrated by the synthesis of the corresponding 5-substituted derivative **74** (Figure 7.12), which proved much less active (K_i 4400 nM). Synthesis of an acyclic analog, **75**, demonstrated that potency enhancement, particularly in the replicon assay, could be achieved through a P2–P4 macrocyclization strategy.

Replacement of the P1 carboxylic acid functionality in **63** with the cyclopropylacylsulfonamide[65] to afford **76** (Figure 7.12) led to a subnanomolar inhibitor of NS3/4A protease ($K_i < 0.016$ nM). Disappointingly, given the critical need for liver exposure, oral administration of **76** to rat at 5 mg kg^{-1} p.o. provided low (0.2 μM) compound levels in the liver at 4 h with barely detectable plasma exposure (Table 7.10). In a surprising result, when the P3 *n*-butyl residue was replaced with the isomeric *tert*-butyl group, the resultant inhibitor **77**, which had a very similar *in vitro* activity profile, was effectively partitioned into the liver with a tissue concentration at 4 h of 3.9 μM, although plasma levels were unimproved. The dramatic impact of this minor structural change on liver levels strongly suggested that uptake was *via* an active transporter-mediated process.

An additional attraction of this class of P2–P4 macrocycles relative to the P1–P3 structural class is the potential to use a range of macrocyclization

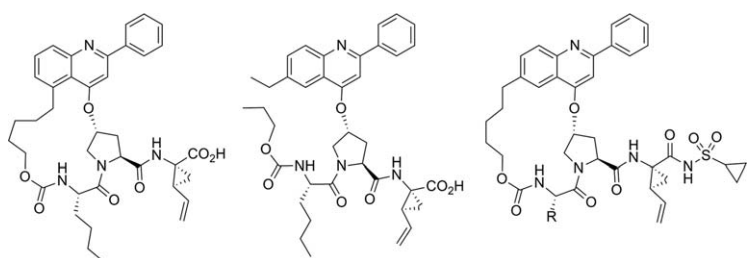

Figure 7.12 Phenylquinoline analogs.

Table 7.10 Liver exposure of compounds **76** and **77**.[a]

Compound	*C_{max} (nM)*	*Plasma AUC 0–4 h (μM h)*	*4 h liver concentration (μM)*
76	7	0.006	0.2
77	6	0.01	3.9

[a]Compounds dosed at 2 mg kg^{-1} p.o. in PEG400 ($n = 3$).