mutants (referred to as palm site 1). The quinolone portion of the inhibitor fits in a tight pocket which is lined by residues from the thumb, palm and finger domains and restricts substitution at the C5 and C6 positions. While small- to medium-sized substitution was tolerated at C5, the effect on NS5B inhibition was negligible. On the other hand, C6 tolerated greater variety and allowed optimization of replicon potency.

Hydrogen-bonding interactions between the benzothiadiazine core and the protein were probed through modification of the core itself. Removal of acidic H-bond donors led in all cases to inactive compounds, suggesting that critical direct or water-mediated interactions with the protein were affected or a presumed bioactive planar conformation of the ring systems was no longer favorable. Indeed, analysis of structural data revealed the presence of several conserved water molecules, mediating important H-bonding interactions between the inhibitor and protein. In addition, an edge-to-face π-interaction between the benzothiadiazine ring system and a phenylalanine residue (F193) was apparent. Whereas the free state of benzothiadiazines locks the two ring systems into coplanarity through internal hydrogen bonds, this conformation appears to be somewhat distorted when bound to protein.

Overall, analog **46** provided the best potency profile, inhibiting gt1a, 1b and 2a NS5B with comparable efficiency ($IC_{50} = 10–49$ nM), while the compound was significantly less potent towards the 3a genotype (60% inhibition at 10 μM).[70] Analog **46** exhibited a favorable combination of high oral bioavailability and low intrinsic clearance in preclinical animal studies with favorable distribution to the liver in rats (liver/plasma ratio ≥2–5-fold). Benzothiadiazines had relatively low volumes of distribution and were extensively bound to human plasma proteins (>99%). In the presence of human serum albumin, the $EC_{50}$ of **46** increased ~350-fold. Analog **46** was evaluated in a 4 day toxicology study in rats at doses up to 300 mg $kg^{-1}$ $d^{-1}$ with no adverse findings and was advanced into preclinical development. The outcome has not been disclosed and there are no reports of this analog progressing into clinical trials.[70] Subsequent SAR studied on the benzothiadiazine portion of inhibitors was particularly productive at the C7 position. For example, addition of an acetamide chain through an ether linkage provided further potency enhancements that could in part compensate for some of the observed potency shifts in high-protein replicon assays and also improve overall developability of the compounds (*e.g.*, **47**). Unfortunately, no further information is currently available on the GSK benzothiadiazine program.[71]

### *8.3.3.2 Benzothiadiazines from Abbott*

Several companies have followed suit on benzothiadiazine NS5B leads; among them, Abbott Laboratories have published on the discovery, development and clinical evaluation of its own versions of this chemotype. Initial reports by Abbott described the effect of replacing the C8 carbon of the quinoline ring system by a nitrogen atom and linking the aliphatic chain at N1 through an oxygen or nitrogen atom. Quinolone and 1,8-naphthyridone cores were shown