more common M423 variants (3–12-fold shifts). The safety, tolerability and antiviral activity of GS-9669 were recently investigated in gt1 HCV patients. A median viral load decrease of 3.25–4.09$\log_{10}$ was observed for all tested doses (50, 100 and 500 mg bid and 500 mg qd) following 3 days of treatment in both gt 1a and 1b subjects.[68] The potency, pharmacokinetic and safety profile of GS-9669 support once-per-day dosing and use in combination with other DAAs.

8.3.3 Palm Site 1 Inhibitors

8.3.3.1 Benzothiadiazines from GlaxoSmithKline

One of the first literature reports describing the discovery of non-nucleoside NS5B polymerase inhibitors was published in 2002 by the GlaxoSmithKline group. Benzothiadiazine derivative **45** (Figure 8.11), identified as a screening hit, was disclosed as a potent and specific inhibitor of HCV NS5B with submicromolar potency in cell-based replicon assays ($IC_{50} = 0.10\,\mu M$; $EC_{50} = 0.5\,\mu M$).[69]

Resistance selection experiments were performed in the replicon and mutations encoding amino acid changes at a palm site residue close to the interface with the thumb domain (M414) were found to be sufficient to confer resistance to the benzothiadiazine chemical class.[69b] As for other allosteric inhibitors, benzothiadiazines were found to interfere with initiation of RNA synthesis rather than elongation, regardless of whether replication was *de novo* or primer–template dependent. Compounds were non-competitive with respect to NTP substrates and could bind independently to *apo*-NS5B or RNA–NS5B complex and thus did not require disruption of NS5B–RNA interactions to mediate inhibition.[69c] SAR studies performed on the quinoline portion of **45** led to the discovery of analogs with significantly improved antiviral potency.[70] The nature of the N1 substituent (length, sterics and polarity) was found to be critical for NS5B inhibition, with a cyclopropylethyl chain providing optimum potency over polar, aromatic or heterocyclic substituents. X-ray crystal structures of several benzothiadiazine inhibitors in complex with NS5B were obtained and confirmed binding in proximity to the interface between palm and thumb domains, in agreement with the previously selected M414-resistant

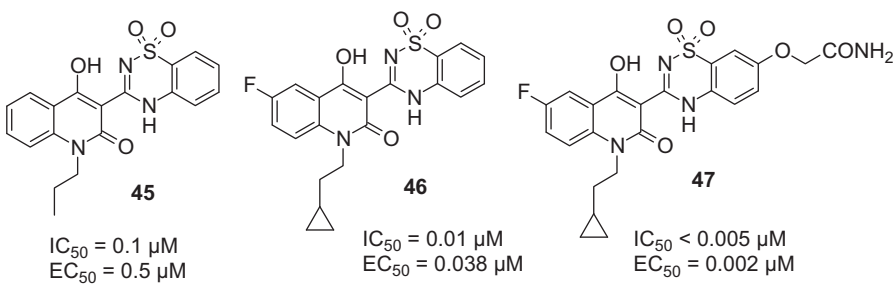

Figure 8.11 Benzothiadiazine palm site 1 NS5B inhibitors from GlaxoSmithKline.