this class of compounds further to identify interferon inducers for the treatment of HCV.

An early compound from this series that was investigated clinically for HCV is resiquimod, also known as R-848.[35] Resiquimod is more potent than imiquimod and is suited to oral administration based on pharmacokinetic parameters. Whereas imiquimod is selective for TLR-7, resiquimod is a dual agonist of TLR-7 and TLR-8.[36] Like TLR-7, TLR-8 also is known to sense ssRNA but the receptor has some differences in the nature of the response that it initiates. The distribution of TLR-8 is different: it is predominantly expressed in myeloid dendritic cells and monocytes, which do not produce high levels of interferon.[9] Additionally, TLR-8 signals primarily through NF-κB, resulting in the production of cytokines such as TNF-α that have a more pro-inflammatory signature than the antiviral interferon-type of signature of TLR-7. Hypotheses have been presented that suggest that TLR-8 agonist activity may result in more side effects than TLR-7 activity, although definitive data to support this notion are lacking.

Resiquimod was evaluated in a placebo-controlled Phase 2 clinical trial in HCV patients to establish proof of concept for antiviral activity.[35] It was administered orally twice per week for 4 weeks at 0.01 and 0.02 mg kg^{-1}. In this key clinical study, resiquimod was found to have antiviral efficacy, with mean viral RNA reductions of $\sim 0.4 \log_{10}$ and $1.3 \log_{10}$ units for the 0.01 and 0.02 mg kg^{-1} doses, respectively, with maximum reductions occurring at about 24 h. At the higher dose, 1/11 subjects achieved a viral load reduction of $>3 \log_{10}$ units. The antiviral effects were transient and the virus levels rebounded after 24 h. The 0.01 mg kg^{-1} dose was well tolerated, but some subjects discontinued treatment at the higher dose. Adverse events were dose related and correlated with serum resiquimod levels and serum levels of IFN-α. Based on this study, resiquimod appeared to have a steep dose response both for antiviral activity and for side effects.

In order to address some of the issues with resiquimod, a second-generation compound, R-852, which later became PF-4878691 (**12**, Figure 10.7), was advanced to clinical trials by Pfizer.[37] PF-4878691 is selective for TLR-7, which would address any detrimental issues that might be associated with TLR-8 activity. In order to model the therapeutic window of PF-4878691, *in vitro* experiments were conducted in which human PBMCs were stimulated with PF-4878691 and the supernatants were evaluated for cytokine levels and antiviral activity in the HCV replicon. These experiments suggested that there would be a separation between antiviral effects and the induction of proinflammatory cytokines and served to guide the dose levels in clinical trials.

In a Phase 1 clinical study, PF-4878691 was administered orally to healthy volunteers at doses of 3, 6 and 9 mg twice per week for 2 weeks. PF-4878691 induced biomarkers of immune and IFN responses, such as mRNA level for the interferon-stimulated gene OAS-1. A surrogate biomarker of antiviral activity was the *in vitro* inhibitory activity of serum from these subjects against the HCV replicon. Serum from subjects administered 3 mg did not have activity greater than the placebo controls. There was a dose-dependent increase in