suggesting that motavizumab may continue development as a therapeutic product.

2.4.3 RSV Nanobody (F-VHHb)

Ablynx developed a functional antibody for RSV that is based on their 'nanobody' technology of heavy chain-only antibodies.[44] These nanobodies, derived from camelids and fish, show improved stability and high affinity and may bind to epitopes that are not accessible to regular monoclonal antibodies. Indeed, the Ablynx fusion inhibitor is the most potent antibody described so far, with an EC_{50} of 0.056 nM, and efficacy in mice ($-2\log_{10}$) was observed after intranasal delivery 4 and 24 h post-inoculation.[45] The viral load drop was even higher when dosed 48 h after inoculation; however, the authors cautioned that carry-over of the antibody into the assay may have affected the data, a common issue with plaque-based viral load assays (see Table 2.1). Ablynx projects their RSV nanobody (F-VHHb) to be intranasally qd or bid.[46]

2.5 Small Molecule Fusion Inhibitors

2.5.1 J&J 2408086 and TMC-353121

The discovery of TMC-353121 dates back to a screening campaign in 1990 by Johnson & Johnson (J&J). A cellular assay based on cytopathic effects (CPE) was used and a benzimidazole series moved into a hit-to-lead optimization. Starting with potency in the double-digit micromolar (EC_{50}) range (see **3**, Scheme 2.2), empirical medicinal chemistry optimization afforded significantly more potent compounds with EC_{50}s in the picomolar range that appeared in 2005 in the patent literature.[47–50]

A time-of-addition assay established the entry mechanism of action (MOA) of this class of compounds. Resistant mutations in the F protein further clarified the antiviral target as the fusion protein (S398L, K394R/ S398L, D486N).[12,51]

Early compounds in this series, such as JNJ-2408086 (**4**), had unexpected long tissue retention of 153 h in the lungs, which was considered a safety concern, especially since this was not accompanied by a long plasma half-life. The structural features responsible for the tissue retention were systematically explored and found to be based on the highly polar and cationic ethylenediamine side chain. This observation is in line with the observation that many cationic lipophilic compounds tend to have elevated lung exposure, a property that may seem beneficial for a respiratory disease such as RSV. It is, however, far from clear how well lung tissue exposure correlates with viral load drop for inhibitors that interfere with an extracellular target such as the fusion protein. Replacement of the highly basic ethylenediamine side chain with a morpholinopropylene side chain reduced the lung tissue half life to an acceptable 14 h; however, the potency decreased by over 1000-fold back to high nanomolar