to induce membrane permeability, although 2B is now thought of as the mature viroporin effector molecule.[12,13,264] Enterovirus 2B is a class 2 viroporin with two helical TMDs separated by a stretch of highly polar residues. Enterovirus 2B fused to maltose binding protein forms tetramers with a radius of $\sim$6 Å capable of permeabilising vesicles[264] and multimerisation has also been observed in mammalian cells.[269,270] In agreement, molecular modelling of channel complexes predicts tetrameric pores of 5–7 Å radius with a lumen lined by a stretch of three lysines followed by a serine.[271] Expression of enterovirus 2B within cells leads to elevated cytosolic Ca^{2+}, which alters vesicle trafficking, induces apoptosis and leads to eventual cell lysis, reminiscent of a membrane-active toxin.[13] Localisation to the Golgi is necessary for this to occur as 2B from distantly related picornaviruses, such as hepatitis A virus, are ER localised and do not alter Ca^{2+} levels. Recently, enterovirus 2B was shown to activate the NLRP3 inflammasome,[272] revealing an as-yet uncharacterised role for viroporins influencing immune activation. Interestingly, the 2B protein of EV71 was recently proposed to act as a chloride channel rather than a Ca^{2+} channel.[273] Accordingly, a chloride channel inhibitor, DIDS (4,4′-diisothiocyano-2,2′-stilbenedisulfonic acid), both inhibited chloride conductance of EV71 2B expressed in *Xenopus* oocyte membranes and retarded the spread of EV71 in cell culture. Direct channel conductance of other 2B proteins has not been characterised, making it possible that they all act to raise Ca^{2+} levels *via* an indirect effect on chloride ion homeostasis and providing an opportunity to apply chloride channel inhibitors as therapeutics. No structural information exists for 2B and no other examples of 2B-specific compounds have been described at the time of writing.

Another picornavirus protein capable of inducing membrane permeability is the VP4 capsid component.[15] This small protein is retained on the inside of the virion particle until uncoating during virus entry, where it becomes externalised and inserted into the endosomal membrane. Here, in cooperation with VP1, it is thought to enable the passage of viral RNA into the cytosol.[274] Such pore-like activity is at the very extreme of the pore–channel dualism spectrum for viroporin function; however, VP4 forms defined channels that do not disrupt membranes and induce discrete channel events in artificial bilayers.[14] Furthermore, VP4 channels can be reconstituted with recombinant protein *in vitro* and their activity is amenable to assessment *via* liposome dye release assays.[15] Hence expanded screens may yield small molecules capable of interfering in this process.

9.3.2 Coronavirus (CoV) E, 3a and ORF8a Proteins

Coronavirus (CoV) infection was brought to the fore in the early part of the century following the outbreak of severe acute respiratory syndrome (SARS), which was subsequently directly linked to the zoonotic transfer of the SARS CoV.[275,276] Although this infection has not re-emerged to date, much research has focused on its molecular biology and the coronavirus field has been reinvigorated as a result. However, CoV also represent significant agricultural