13.4 Efflux Pump Inhibitors

Proteins present in membranes that remove antibiotics from bacteria, called efflux pumps, can be responsible for high levels of resistance especially if overexpressed. These efflux pumps reduce or restrict the intracellular concentrations of antibiotics and are often responsible for the failure of infection therapies. MDR efflux pumps transport a wide range of substrates that are structurally dissimilar, while other pumps can transport substrates with a degree of specificity toward a class of molecules or according to their physicochemical properties (Blair et al. 2015). The ability of pumps to extrude a wide range of drugs from its interior prevents the use of drug modification approaches to overcoming the resistance by efflux, and alternative approaches have to be employed. One approach would be to identify compounds that evade efflux pump or to develop inhibitors of efflux that would restore the efficacy of antibiotics (Figure 13.1a). Therefore, efflux pumps were considered as attractive targets where the inhibition by an efflux pump inhibitor (EPI), delivered together with an antibiotic drug, should result in the increased potency as well as the enhanced spectrum of activity of the antibiotic.

Early efforts in the discovery of such agents were focused on *in vitro* screening of antibiotics in combinations with natural products, other drugs, or synthetic analogues that resulted in some hit molecules that acted as broad-spectrum EPIs (Lomovskaya and Bostian 2006). EPIs such as MC-207,100 (Phe-Arg-ß-naphthylamide [PAßN]), reserpine, GG918, INF55, INF277, verapamil (Scheme 13.2), and their analogues never reached clinic due to their poor pharmacokinetic or toxicological profiles or unfavorable molecular properties.

Substrates that are more susceptible for efflux by the same efflux pump are assumed to have a common map of pharmacophore features despite their chemical structures dissimilarity, which may potentially be applied to EPI acting on the same pump. Initial analysis of the molecular similarity of structurally unrelated EPIs using MIPSIM software revealed that overall 3D distributions of their molecular interaction potentials overlap considerably with the similar spatial orientation of aromatic rings (Zloh and Gibbons 2004).

That indicates that the pharmacophore approach may be useful in EPI discovery. The *S. aureus* NorA efflux pump, responsible for resistance to the quinolone drugs, is considered as a good target for the development of EPIs, especially when the efflux is the only mechanism of resistance that affects the therapy. A set of compounds with the known inhibitory activities of this efflux pump served as a training set for developing a pharmacophore model (Figure 13.4). The training set included both active and inactive compounds while comprising nine different scaffolds to ensure that chemical diversity is considered during *in silico* screening for new EPI candidates.