screening against a binding site with a high proportion of negatively charged amino acids. Moreover, in a few cases, prescreen filtering used more sophisticated pharmacophores or SMARTS patterns to enrich the database in compounds with desired interacting features, for example, features that could provide hydrogen bonds to a kinase hinge region or electrostatic interactions with catalytic residues of an oxidoreductase.

- Before commencing the actual prospective screen, several of the virtual screeners of Table 7.3 carried out validation docking runs. For two of these, the validation involved confirming that the chosen docking protocol could successfully recapitulate the docking mode for a known protein/ligand crystal structure. A larger number of the virtual screeners salted a small number of known actives into the search database and carried out test docking runs; parameters of the docking protocols and score cutoffs for hit selections were fine-tuned to ensure that these known hits were recovered high in the docking hit list.
- Once the prospective screens were completed and potential hit lists compiled, all of the virtual screeners of Table 7.3 used alternative means for winnowing the lists to remove potential false positives. At the very least, in virtual screens across all of the activity bins in Table 7.1, the top docking hits were visually inspected in the protein environment, keeping only those compounds that were predicted to make interesting interactions with the protein. For those screens that produced submicromolar hits, most of the screeners used either alternative scoring strategies (e.g., consensus scoring, quantification of specific desired protein/ligand interactions, removal of compounds with high strain energy), subsequent more computationally intensive virtual screening protocols (e.g., fast rigid docking followed by slower flexible docking, docking followed by energy-based refinement), or some combination of both.
- Not all of the methodological details for these screens were conducive to true success; at least three compounds in Table 7.3 exhibit properties that would warrant orthogonal confirmatory assays to ensure that inhibition was due to the desired mechanism. For example, compound **1** and a close analog were reported as extremely potent inhibitors of the aspartyl proteases cathepsin D and plasmepsin; these acridine-containing compounds were identified by fluorescence resonance energy transfer (FRET) assay.[44] However, no data were provided that would allow assessment of any intrinsic fluorescence of the putative inhibitors nor of any possible interference with the FRET signal, even though acridine itself fluoresces[142] as do substituted versions of acridine such as quinacrine.[143] Compound **3**, ellagic acid, was reported to be a 40 nM, ATP-competitive inhibitor of protein kinase CK2 based on a phosphorylation inhibition assay using ^{33}P-labeled ATP[49]; virtual and experimental screening results were reported for only this single compound. This same compound has also been reported to be an inhibitor of AmpC β-lactamase under detergent-free assay conditions[94,144]; after further mechanistic characterization, these authors concluded that ellagic acid was a detergent-resistant promiscuous aggregator and that this aggregation behavior was responsible for inhibition in assays of β-lactamase, chymotrypsin, malate dehydrogenase, and cruzain. At a reported IC_{50} of 40 nM, ellagic acid may well be inhibiting through some mechanism other than aggregation, but the data reported[49] do not allow for assessment one way or the other. Finally, compound **7**, diiodosalicylic acid, was reported as a 99 nM inhibitor of 20α-HSD; activities for the related compounds aspirin and salicylic acid were reported to be 21 and 7.8 μM, respectively.[47] All three analogs are known metal chelators,[145,146] and the apparent SAR for inhibition of 20α-HSD is in line with pK_a trends for acetylsalicylic acid, salicylic acid, and diiodosalicylic acid. In the absence of additional data to the contrary, it is therefore equally plausible to hypothesize that either metal-chelate forms of each analog or the pure compounds themselves were responsible for inhibition.

Of course, one might follow absolute best practice for docking-based virtual screening – targeting a well-characterized protein system, searching a database of chemically reasonable molecules, carrying out detailed validation studies, postscoring docking hits at a higher level of theory – and still not be successful in experimentally identifying compounds with submicromolar activity. As a specific case in point, Barriero et al.[136] report virtual screening efforts directed at the identification of novel nonnucleoside inhibitors of HIV-1 reverse transcriptase, a target represented in Table 7.3. These researchers first carried out a similarity search to identify compounds similar to known NNRTIs, and then docked these similarity hits and the known actives into the NNRTI binding site. Docking hits were rescored using molecular mechanics and an implicit solvation model; six of the twenty top-scoring hits were purchased and assayed, but no active compounds resulted from that experimental assay. Visual inspection of the six docking hits gave these researchers some confidence that one of those six hits represented a promising scaffold and that the predicted interactions between protein and putative ligand were favorable for activity. Barriero et al. therefore committed synthetic resource to follow up on that scaffold through synthesis of a small number of analogs that differed in substitution pattern on two phenyl rings. That at-risk gamble based on a gut-instinct assessment of prediction reliability paid off in this particular instance; of the newly synthesized analogs, at least one was a submicromolar anti-HIV agent with an EC_{50} of 310 nM. Although in this particular case the original virtual screen produced no active inhibitors, in general following best practice and carrying out one's screens carefully is likely to at least