This strategy of hierarchical filtering starting with a mapping of candidate molecules onto a binding site-derived pharmacophore, followed by a similarity analysis with known ligands using either FlexS (3301, SEAL (254–256), or FeatureTrees (357, 358); and concluded by flexible docking with FlexX, which meanwhile was applied to three other proteins in the same laboratory. For t-RNA guanine transglycosylase, thermolysin, and aldose reductase, novel micromolar to submicromolar lead structures could be discovered. Most challenging in this context is aldose reductase because it performs pronounced induced fit changes upon ligand binding. Crystal structure analysis of a micromolar hit retrieved by virtual screening clearly revealed known and new areas of induced fit adaptation. The crystal structure obtained with this hit provides a good starting point for further lead optimization.

The de *novo* design of inhibitors of the bacterial enzyme DNA gyrase, a well-established antibacterial target (381), is another example for successful structure-based virtual screening, reported by Roche (16). HTS performed on the proprietary compound library provided no suitable lead structures. Therefore, a new rational approach was developed to discover potential lead structures using structural information of the ATP binding site in subunit B of the enzyme. At the onset of the project, the crystal structures of DNA gyrase subunit B complexed with a substrate analog and two inhibitors were available. In the buried part of the pocket they all donate a hydrogen bond to an aspartic acid side-chain and accept one from a conserved water molecule. As a design concept, the formation of these two key hydrogen bonds has been defined as mandatory. As an additional requirement, a lipophilic portion forming hydrophobic interactions with the enzyme was demanded. A new assay was established to allow for the detection of weakly binding inhibitors. A computational search of the ACD (376) and the Roche Compound Inventory identified hits having low molecular weights and matching the above-mentioned criteria. Relying on the results of the in *silico* screening Based on docking with LUDI and a pharmacophore search with CATALYST (356)] 600 compounds were tested initially. Then, close analogs of the first series of hits were assayed, resulting in a total screen of 3000 compounds. This provided 150 hits, clustered into 14 chemical classes. Seven of these classes could be demonstrated as novel DNA gyrase inhibitors competing for the ATP binding site. Subsequent structure-based optimization resulted in inhibitors with potencies equal to or up to 10 times better than those of known antibiotics.

6 OUTLOOK

The first docking programs were introduced about 20 years ago, and the publication of the first generally applicable scoring functions dates back about 10 years. Since then, much experience has been gained in developing and applying docking algorithms, using scoring functions, and assessing their accuracy. Significant progress has been made over the last few years and it appears as if there are now docking tools available to address a variety of goals with considerable accuracy, from the precise and detailed analysis of binding interactions for a small set of ligands up to a fast screening of large compound collections. Similarly, scoring functions are currently available that can be applied to a wide range of different proteins and consistently yield a considerable retrieval of active compounds. As a consequence, the pharmaceutical industry increasingly uses virtual screening to identify possible leads.

In fact, structure-based design is now established as an important approach to drug discovery complementing HTS (3821, although HTS has a number of serious disadvantages. It is expensive (383) and it leads to many false positives and a disappointingly small number of real leads (384, 3851, particularly if screening is performed on a member of a new protein class. Also, not all assays are easily amenable to HTS requirements. Finally, despite the library sizes of several million entries available to the pharmaceutical industry, these compound collections do not approach the size and diversity needed to even approximately cover the chemical space of drug-like organic molecules. Accordingly, focused design of novel compounds and com-