in various stages of clinical investigation. Two of these molecules, erlotinib (Tarceva®, Genetech, Inc. and OSI Pharmaceuticals, Inc. San Francisco, USA) and lapatinib (Tykerb®, GlaxoSmithKline plc. Brentford, U.K.), share a common 4-anilinoquinazoline core, yet their ErbB inhibition profiles and mechanisms of action are clearly differentiated on the basis of biochemical and crystallographic studies. For example, while erlotinib is a potent and selective inhibitor of EGFR only ($K_i^{app} = 0.4$ nM), lapatinib exhibits potent activity against both EGFR and ErbB2, with estimated K_i^{app} values of 3 and 13 nM, respectively. In addition to its dual kinase activity profile, lapatinib can be distinguished further from erlotinib in that it has a prolonged off-rate from its kinase targets compared to the very fast off-rate from EGFR of erlotinib. This translates to a half-life of dissociation of 300 minutes for the lapatinib–EGFR complex. Importantly, in cellular washout experiments this slow off-rate correlates with a prolonged inhibition of receptor tyrosine phosphorylation in tumor cells (see Section 11.6 for additional information).

An evaluation of the binding mode of lapatinib, based on the crystal structure of the compound in complex with EGFR, suggests a rationale for its long target residence time compared to other 4-anilinoquinazoline inhibitors. Not surprisingly, the quinazoline ring was observed to be hydrogen-bonded to the flexible hinge region between the *N*- and *C*-terminal lobes of the kinase, but there are variations in the key hydrogen-bonding interactions compared to those revealed in the erlotinib–EGFR structure. These differences indicate that lapatinib binds to a relatively closed form of this binding site, whereas erlotinib binds to a more open form. In addition, the ATP-binding pocket of the lapatinib–EGFR complex has a larger back pocket than the apo-EGFR or erlotinib-EGFR structures owing to a shift in one end of the C-helix. This enlarged back pocket accommodates the 3-fluorobenzyloxy group of lapatinib (Figure 11.8). The structural change is significant because it results in the loss of a highly conserved Glu738–Lys721 salt bridge which is an important regulatory mechanism of kinases, functioning to ligate the phosphate groups of ATP. The net result of these structural differences is that the activation loop in the lapatinib–EGFR structure adopts a conformation that is reminiscent of that found in inactive kinases. In contrast, the erlotinib–EGFR structure in Figure 11.8 displays the activation loop in an active conformation. These effects provide a potential molecular rationale for the prolonged residence time of lapatinib on its target which in turn may result in the observed duration of drug activity in cells. In total, these elegant structural and biochemical studies have important implications for the discovery of novel, targeted signal transduction inhibitors and suggest that subtle differences in kinase inhibitor structure can have a profound impact on the binding mode, kinetics, and cellular activity.

11.5.2 Structure-Based Design of HIV Protease Inhibitors

Perhaps the greatest impact of structure-based design on the identification of novel medicines has been in the treatment of AIDS, the etiologic agents of which are human immunodeficiency virus type 1 and type 2 (HIV-1 and HIV-2). These retroviruses encode relatively simple genomes consisting of three open reading frames (ORFs): *gag*, *pol*, and *env*. The *gag* gene encodes the structural capsid, nucleocapsid, and matrix proteins, while the *env* gene is processed by multiple alternative splicing events to yield regulatory proteins. The *pol* ORF encodes the essential viral enzymes necessary for viral replication: reverse transcriptase (RT), integrase, and protease (PR). HIV-1 PR is an aspartyl protease which is required for proteolytic processing of the Gag and Gag-Pol polyprotein precursors to yield the viral enzymes and structural proteins, and it is absolutely indispensable for proper virion assembly and maturation. For this reason it has been an important target for the discovery of anti-HIV therapeutics, and indeed there are at least eight drugs in current clinical use whose antiviral mode of action is by potent inhibition of the HIV protease (Figure 11.9).

One of the major driving forces behind the rapid progress in the identification of HIV protease inhibitors to combat AIDS has been the intense investigation of the structure of the enzyme, particularly in complex with a number of different inhibitors. HIV-1 PR is a homo-dimer comprising