Combination of key mutations in double mutants results generally in strong additive effects on catalysis, leading to higher hydrolysis efficiency (Figure 6.6). For example, the addition of E104K to R164S results in a 30-fold increase in catalytic efficiency for ceftazidime compared with the R164S enzyme. The addition of E104K to the G238S enzyme results in a 75-fold increase in the efficiency for the same antibiotic compared with the G238S enzyme. Apparently, mutations of residues 104 and 240 located in the loops near the catalytically important α-helix and β-sheet contribute to additional changes in the active site conformation and improve the orientation of cephalosporins with bulky substituents due to additional ionic interactions (Page 2008). The loop between the S3 and S4 β-strands in TEM-52 (G238S/E104K) was shown to move by 2.8 Å with respect to its position in TEM-1, and the side chain of E240 was shifted from the active site (Orencia et al. 2001). In this respect, it has been suggested that E240K and E104K might act as stabilizing mutations that can compensate for the destabilizing effect of R164 and G238 substitutions (Raquet et al. 1995).

Combinations of R164S/G238S and E104K/E240K not found together in double mutants may show negative effects on the hydrolysis in triple mutants. For example, catalytic efficiency toward cefotaxime drops markedly (by 30-fold) in triple mutant TEM-134 (E104K/R164S/G238) compared with double mutant TEM-3 (E104K/G238S) (Perilli et al. 2007). While the residues are not in direct contact (more than 10 Å apart), it was found that both G238S and R164S introduce conformational changes in the Ω-loop, resulting in nonoptimal conformations (Dellus-Gur et al. 2015).

6.3.3 Key Mutations in IRT TEM-Type β-Lactamases (2br)

The TEM-type β-lactamases with the IRT phenotype (2br), which are characterized by resistance to the inhibitors with the β-lactam ring (clavulanic acid, sulbactam, and tazobactam), involve 38 enzymes. These inhibitors compete with β-lactams for the binding in the active site of β-lactamase. Unlike antibiotics, the acyl-enzyme complex with inhibitors is stable and characterized by a very low deacylation rate defined by the value of k_3 (Figure 6.3). TEM-type β-lactamases that have substitutions of residues M69, S130, R244, R275, and N276 are resistant to these inhibitors. In general, the total number of β-lactamases with mutations of these residues is substantially less than enzymes with mutations related to the ESBL phenotype. Apparently, this is due to the significantly smaller amount of inhibitors used in clinical practice as compared with antibiotics.

6.3.4 Single Key Mutations in IRT TEM-Type β-Lactamases (2br)

Single key mutations in TEM-type β-lactamases isolated from clinical samples most often occur in residues R244 (substitutions C/G/H/L/S, $n = 12$) and M69 (substitutions I/L/V, $n = 8$) (Figures 6.4 and 6.5).