contributes to the stable conformation of the active site and the deprotonation of S70 (Pimenta et al. 2014). The mutation S130G is quite rare ($n = 2$), but it is characterized by the highest decrease in sensitivity to all three inhibitors, and the K_i values are enhanced by 2–3 orders of magnitude compared with K_i of TEM-1 (Thomas et al. 2005) (Figure 6.7).

The substitutions R275L/Q and N276D are rarely found in β-lactamases isolated from clinical samples as single mutations ($n = 3$ and 1, correspondingly) (Figure 6.4). These residues are located on H11 α-helix rather far from the active site. It explains non-marked effects on inhibition; at the same time substitutions R275Q and N276D were revealed to be involved in the stabilization of the enzyme (Osuna et al. 2002).

Increased interaction between D276 and R244 due to forming a salt bridge between the positive charge of arginine and the negatively charged carboxyl of aspartate (Brown et al. 2010) neutralizes positive electrostatic potential of R244 guanidinium group and results in the decreased affinity of the mutant for β-lactams and inhibitors (Swarén et al. 1999; Brown et al. 2010).

6.3.5 Combinations of Key Mutations in IRT TEM-Type β-Lactamases (2br)

Combinations of the key IRT mutations are less common than the key ESBL mutations. They are found only in 12 TEM-type β-lactamases and include mainly combinations of M69/R275 and M69/N276 substitutions (Figure 6.4). In general, an additive effect of combinations of mutations is observed: in double mutants, K_i is one order higher compared to the effect of single M69 substitutions (Figure 6.7). This is revealed mainly toward the resistance to clavulanic acid.

6.3.6 Combinations of Key ESBL and IRT Mutations in CMT TEM-Type β-Lactamases (2ber)

β-Lactamases with a confirmed CMT phenotype (2ber) involve 10 enzymes, which combine the key mutations of two types (ESBL and IRT) (Figure 6.4). The combination of M69L(I), R164S(H), and N276D is found in three enzymes, and the remaining combinations are found only in single β-lactamases (Figure 6.5). The analysis of changes in the catalytic properties of these mutants shows that ESBL and IRT mutations have a negative effect on each other: the efficiency of hydrolysis of various β-lactams and resistance to inhibitors decrease in all CMT enzymes in comparison with the corresponding ESBL and IRT enzymes, and the magnitude of this effect depends on combinations of mutations (Figures 6.6 and 6.7).