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Inhibitors of the peptidoglycan biosynthesis enzymes MurA-F

Martina Hrast, Izidor Sosič, Roman Šink, Stanislav Gobec *

Faculty of Pharmacy, University of Ljubljana, Aškerčeva 7, 1000 Ljubljana, Slovenia

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ABSTRACT

The widespread emergence of resistant bacterial strains is becoming a serious threat to public health. This thus signifies the need for the development of new antibacterial agents with novel mechanisms of action. Continuous efforts in the design of novel antibacterials remain one of the biggest challenges in drug development. In this respect, the Mur enzymes, MurA-F, that are involved in the formation of UDP-*N*-acetylmuramyl-pentapeptide can be genuinely considered as promising antibacterial targets. This review provides an in-depth insight into the recent developments in the field of inhibitors of the MurA-F enzymes. Special attention is also given to compounds that act as multiple inhibitors of two, three or more of the Mur enzymes. Moreover, the reasons for the lack of preclinically successful inhibitors and the challenges to overcome these hurdles in the next years are also debated.

1. Introduction

Bacterial peptidoglycan is a major component of the bacterial cell wall, and it provides rigidity and enables bacteria to survive in hypotonic environments. The peptidoglycan biosynthesis pathway is one of the best known processes in bacteria. According to the location of its biochemical reactions, peptidoglycan biosynthesis can be divided into three major stages: intracellular, membrane, and extracellular. As intact peptidoglycan is essential for bacterial survival, all of the peptidoglycan biosynthesis steps are considered to be important targets for the discovery of new antibacterial agents [1–3].

In the history of antibacterial drug discovery, the attention has mostly been focused on the late stages of peptidoglycan biosynthesis that are catalyzed by transpeptidases and inhibited by important antibiotics, such as penicillins and cephalosporins [4–6]. However, attention has recently shifted also towards the cytoplasmatic steps of peptidoglycan biosynthesis, and especially towards the Mur enzymes [2,3,7,8].

The Mur enzymes, MurA-F, catalyze the last six steps in the formation of the final cytoplasmic peptidoglycan biosynthesis precursor uridine 5′-diphosphate (UDP)-*N*-acetylmuramyl-pentapeptide (Fig. 1). MurA and MurB catalyze the formation of UDP-*N*-acetyl muramic acid (UDP-Mur*N*Ac) from UDP-*N*-acetyl glucosamine (UDP-Glc*N*Ac). First, MurA catalyzes the transfer of enolpyruvate from phosphoenolpyruvate to UDP-Glc*N*Ac. The resulting product, UDP-Glc*N*Ac-enolpyruvate then undergoes a reduction that is catalyzed by MurB. In the next steps, the Mur ligases (MurC-F) catalyze the sequential addition of L-Ala, D-Glu, and *meso*-diaminopimelic acid (in Gram-negative bacteria) or L-Lys (in Gram-positive bacteria), and the dipeptide D-Ala-D-Ala to UDP-Mur*N*Ac, to form the target UDP-Mur*N*Ac-pentapeptide [9,10].

The enzyme specificities, kinetic and catalytic mechanisms, and three-dimensional structures of all of the Mur enzymes are well described and have been thoroughly reviewed [2,9–15]. In this paper, we will present a comprehensive overview of small-molecule inhibitors that have been discovered in the field of the Mur enzymes. All of the most important inhibitors are presented here, with the emphasis on inhibitors that have been developed in the last decade. Hopefully, this careful insight will help those who are active in the antibacterial field to channel their inhibitor-discovery strengths in the correct direction, and hence towards the discovery of novel lead inhibitors of these important enzymes.

2. Inhibitors of MurA

The naturally occurring broad-spectrum antibiotic fosfomycin (compound **1**, Fig. 2) is a well-known inhibitor of MurA. It can form a covalent adduct with the active site residue (Cys115) of MurA, and thereby provide irreversible inhibition of MurA [16]. This inhibition of MurA by fosfomycin is time-dependent and it

Abbreviations: Glc*N*Ac, *N*-acetyl glucosamine; HTS, high-throughput screening; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; Mur*N*Ac, *N*-acetyl muramic acid; PRSP, penicillin-resistant *Streptococcus pneumoniae*; (Q)SAR, (quantitative) structure–activity relationship; UDP, uridine 5′-diphosphate; VRE, vancomycin-resistant *Enterococcus*.

* Corresponding author. Fax: +386 1 4258031.
E-mail address: stanislav.gobec@ffa.uni-lj.si (S. Gobec).

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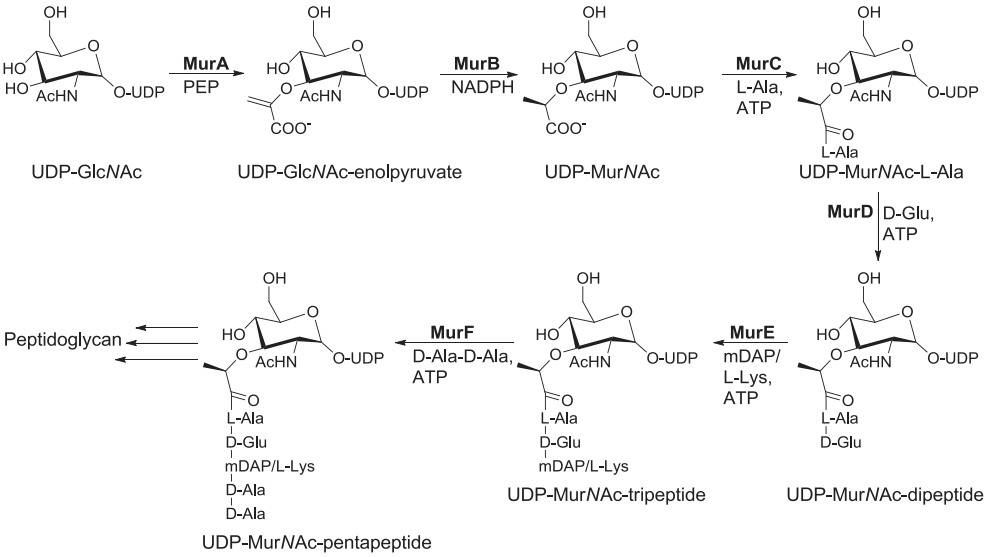

Fig. 1. Cytoplasmic steps of peptidoglycan biosynthesis catalyzed by the Mur enzymes.

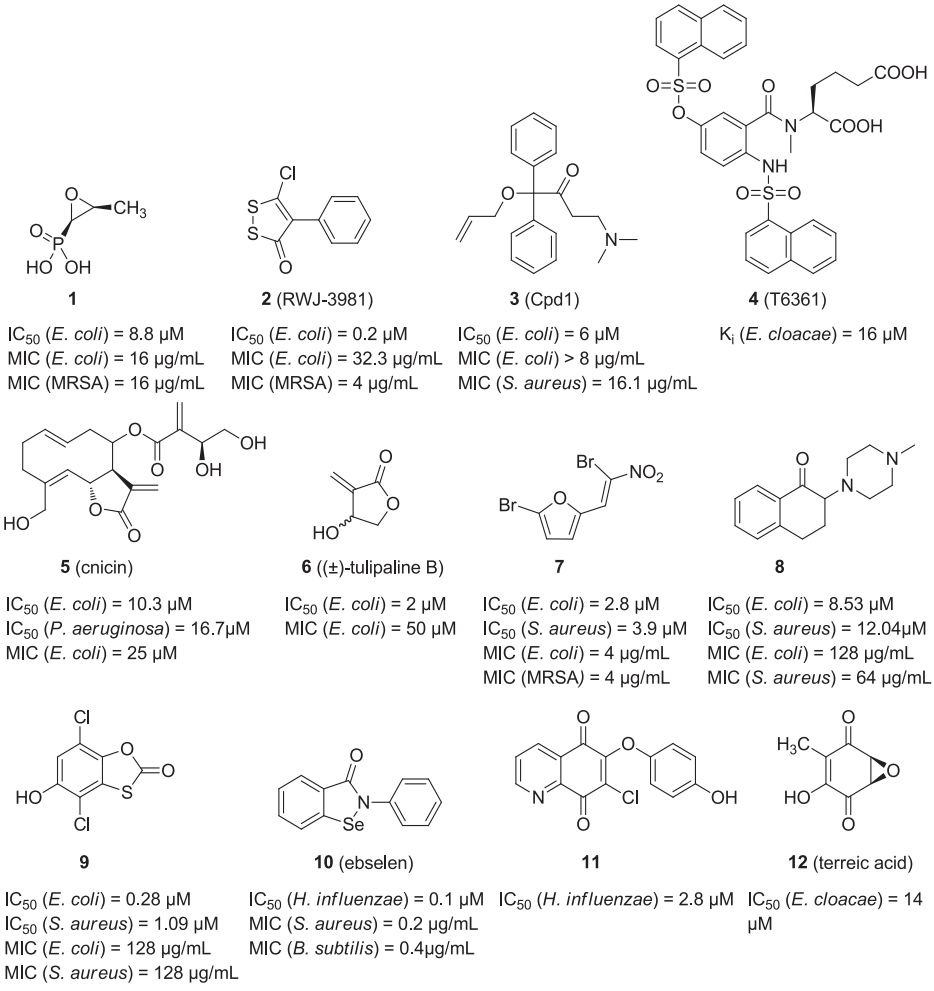

Fig. 2. Structures and biological activities of some structurally diverse inhibitors of MurA.

is enhanced in the presence of the nucleotide substrate UDP-GlcNAc [16]. Some pathogenic bacteria are intrinsically resistant to fosfomycin, such as *Mycobacterium tuberculosis*, *Chlamydia trachomatis* and *Borrelia burgdorferi*, as their corresponding Cys residue is changed into Asp [17]. In addition, fosfomycin resistance frequently occurs due to its reduced permeability, or to modification of the antibiotic

active site, or enzymatic modification of the fosfomycin itself [17]. Therefore, the development of structurally different inhibitors with new mechanism(s) of action is urgently needed.

First, by screening a chemical library of Johnson Pharmaceutical Research Institute, compound **2** (RWJ-3981, Fig. 2), pyrazolopyrimidine (RWJ-110192) and a purine analog (RWJ-140998) were identified as inhibitors of *Escherichia coli* MurA, with IC_{50} values from 0.2 μM to 0.9 μM. Ultrafiltration and mass spectrometry suggested that these compounds bind tightly to MurA at or near the active site, but not covalently. All three of these compounds showed minimum inhibitory concentrations (MICs) of 4 μg/mL to 32 μg/mL against *Staphylococcus aureus*; however, they also showed nonspecific inhibition of DNA, RNA and protein biosynthesis [18]. In a whole-cell peptidoglycan synthesis assay, compound **3** (Cpd1; Fig. 2) inhibited MurA in the low micromolar range. This inhibition of MurA activity by compound **3** required pre-incubation in the presence of UDP-Glc*N*Ac, similar to fosfomycin. Compound **3** shows modest antibacterial activity against several microorganisms (MIC, 8.1–32.3 μg/mL) [19].

Two derivatives of 5-sulfonoxy-anthranilic acid (compound **4**: T6361 (Fig. 2) and T6362) with IC_{50} values in the low micromolar range were discovered using high-throughput screening (HTS). The steady-state kinetics of MurA revealed that this inhibition by compound **4** is competitive with respect to UDP-Glc*N*Ac. The crystal structure of the MurA-T6361 complex, together with the fluorescence data, demonstrate that compound **4** blocks the transition from the open to the closed form of MurA, which is essential for its catalysis (Fig. 3A) [20]. By using phage display technology, a dodecapeptide inhibitor (HESFWYLPHHQSY) of MurA from *Pseudomonas aeruginosa* with an IC_{50} of 200 μM was identified. Kinetic analysis confirmed its competitive inhibition with respect to UDP-Glc*N*Ac [21].

Two sesquiterpene lactones, cnicin (compound **5**, Fig. 2) and cynaropicrin, have been shown to be potent, irreversible inhibitors of MurA from *E. coli* and *P. aeruginosa* [22]. The crystal structure of MurA with cnicin revealed that MurA catalyzes the formation of a covalent adduct between cnicin and UDP-Glc*N*Ac, thus forming a noncovalent 'suicide' inhibitor [23]. Although the antibacterial properties of sesquiterpene lactones are well known, it cannot be concluded that their antibacterial activities are exclusively due to inhibition of MurA, as there are numerous nucleophilic binding sites within the cell that can react with the electrophilic functional groups of these inhibitors. Furthermore, in the same study of Steinbach et al. [23], they identified 1-tuliposide B (compound **6**, Fig. 2) and lactonized aglycon (±)-tulipaline B as potent inhibitors of *E. coli* MurA. This inhibition is time-dependent, and the hydroxyl group of these compounds was found to be crucial for inhibition. Additionally, antibacterial activities of tulipaline B and its analogs have also been reported [24]. Recently, nitrovinylfuran derivatives and bromonitromethane (compound **7**, Fig. 2) were shown to have broad spectrum antibacterial activities, and were found to inhibit MurA from *E. coli*, *P. aeruginosa* and *S. aureus* in the low micromolar concentration range. However, enzymatic, antibacterial, cytotoxic and glutathione-reactivity data have indicated that these compounds interact with numerous proteins via their cysteine residues, thus making their further development unlikely [25].

Using HTS of a Novartis chemical library, two different classes of compounds were identified. First, 2-aminotetralone derivatives (e.g., compound **8**, Fig. 2) were found to be good inhibitors of *E. coli* and *S. aureus* MurA, with IC_{50} values in the low micromolar range, and to have antibacterial activity, with MICs between 8 μg/mL and 128 μg/mL. Based on structure–activity relationship (SAR) studies, it was proposed that its α-aminoketone moiety is responsible for either covalent binding or very tight noncovalent binding [26]. Moreover, a benzothioxalone series (e.g., compound **9**, Fig. 2) was found to have potent MurA inhibitory activity, with IC_{50} values between 0.25 μM and 18.54 μM. This inhibition of MurA was irreversible, and a radiolabeled inhibitor showed stoichiometric binding to MurA, which suggested covalent binding to Cys115. Some of these inhibitors show antibacterial activity against *S. aureus*, with MICs between 4 μg/mL and 128 μg/mL [27].

Novel inhibitors of *Haemophilus influenzae* MurA were identified using HTS of a chemical library from the Korea Chemical Bank. First, there were three inhibitors with IC_{50} values in the submicromolar range: ebselen (compound **10**, Fig. 2), thimerosal and thiram. These compounds covalently modify the active Cys177 in the open conformation of MurA, to induce a conformational change to a 'compact conformation' that might block the binding of UDP-Glc*N*Ac to the active site. These inhibitors can effectively inhibit the growth of several Gram-negative bacteria and of Gram-positive *S. aureus* [28]. Furthermore, compounds with quinoline (compound **11**, Fig. 2) or naphthoquinone scaffolds inhibited MurA in the low micromolar and submicromolar ranges. These inhibitors also formed covalent interactions with the cysteine residues in MurA; however, they do not show any antibacterial activity [29].

Terreic acid (compound **12**, Fig. 2) was shown to be a covalent inhibitor of *Enterobacter cloacae* and *E. coli* MurA. This inactivation of MurA is time-dependent and strongly depends on the presence of UDP-Glc*N*Ac. The crystal structure of the MurA dead-end complex with terreic acid revealed that the quinone ring is covalently

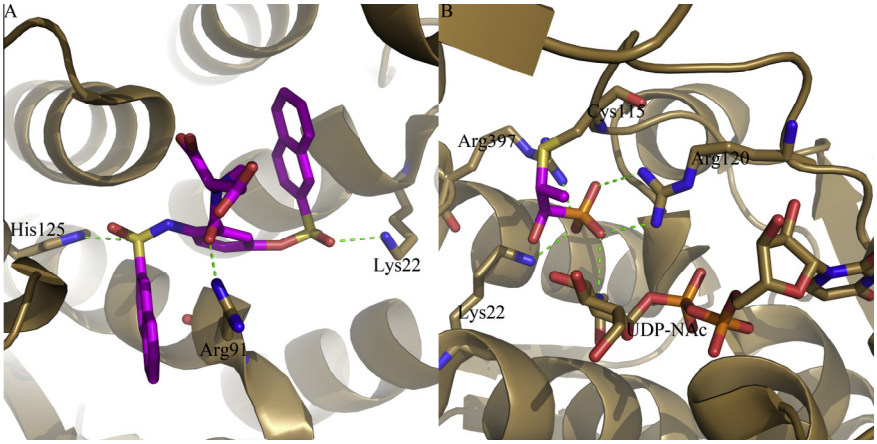

Fig. 3. (A) Binding mode of compound **4** (the MurA-T6361 complex, pdb code 1YBG) to MurA. (B) Binding mode of fosfomycin (compound **1**) in the active site of MurA (pdb code 3KR6).

attached to the thiol group of Cys115. Contrary to the MurA-fosfomycin complex, this MurA-terreic acid structure is in an open, UDP-Glc*N*Ac-free state, and has the Cys115-terreic acid adduct exposed to the solvent [30]. Compound **12** is known to have antibacterial activity; however, it was recently demonstrated that MurA is not the molecular target of terreic acid, and its antimicrobial effect acts through a different mechanism of action [31].

3. Inhibitors of MurB

Only a few inhibitors of MurB have been reported in literature to date. The 4-thiazolidinone derivatives (e.g., compound **13**, Fig. 4) were among the first reported MurB inhibitors, and these were designed to mimic the diphosphate moiety of enolpyruvate–UDP-Glc*N*Ac [32]. As their heterocyclic bioisosteric replacement, imidazolinone derivatives (e.g., compound **14**, Fig. 4) were synthesized and shown to have potent MurB inhibitory activity, and promising antibacterial activity against *S. aureus* [33]. Using HTS two structurally diverse inhibitors of *S. aureus* MurB were found (compounds **15** and **16**, Fig. 4), with K_d values in the submicromolar range [34].

Alkyl pyrazolidinedione analogs (e.g., compound **17**, Fig. 4) were identified as good inhibitors of *S. aureus* and *E. coli* MurB, and were shown to have modest antibacterial activity against Gram-positive bacteria, and particularly against penicillin-resistant *Streptococcus pneumoniae* (PRSP) [35]. In addition, a series of related pyrazolidinediones (e.g., compound **18**, Fig. 4) inhibit *S. aureus* and *E. coli* MurB in the low micromolar range, and also moderately inhibit MurA and MurC [36,37]. The crystal structure of one of these MurB-inhibitor complexes revealed the binding mode of these compounds in the MurB active site [37]. Moreover, these compounds inhibit peptidoglycan biosynthesis, as assessed by measuring the amount of soluble peptidoglycan accumulation after incubation of bacteria with these inhibitors [36]. All of these synthesized derivatives showed antibacterial activity against Gram-positive bacteria; however, when they were tested in the presence of 4% bovine serum albumin, their activity was lost, indicating their high protein binding properties [35–37].

A series of phenyl thiazolyl urea (e.g., compound **19**, Fig. 4) and carbamate derivatives were synthesized as inhibitors of MurA and MurB, with IC_{50} values in the low micromolar range. Many of these have demonstrated antibacterial activity against Gram-positive bacteria, including methicillin-resistant *Staphylococcus* aureus (MRSA), vancomycin-resistant Enterococcus (VRE) and PRSP. However, when tested in the presence of 4% bovine serum albumin, their MICs increased significantly [38].

4. Inhibitors of MurC

The first MurC inhibitors were phosphinate transition-state analogs, and the most potent (compound **20**, Fig. 5) inhibited MurC with an IC_{50} of 49 nM. It was established that the UDP moiety was crucial for this potent enzyme inhibition [39]. The precise biochemical characterization of this phosphinate inhibitor, compound **20**, showed that it acts as a mixed-type inhibitor with respect to all three of its enzyme substrates (i.e., ATP, UDP-Mur*N*Ac, L-Ala). In addition, it has been suggested that compound **20** forms dead-end complexes with multiple MurC enzyme states [40].

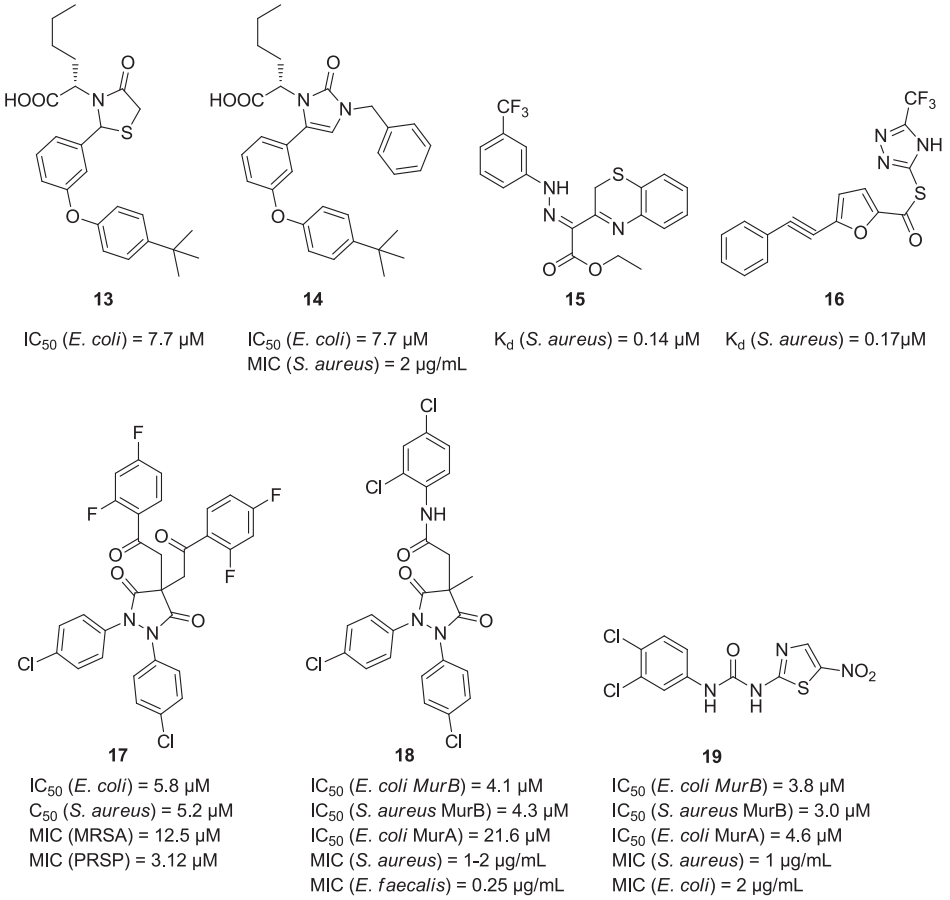

Fig. 4. Structures and biological activities of the MurB inhibitors, and the dual MurA/MurB inhibitors.

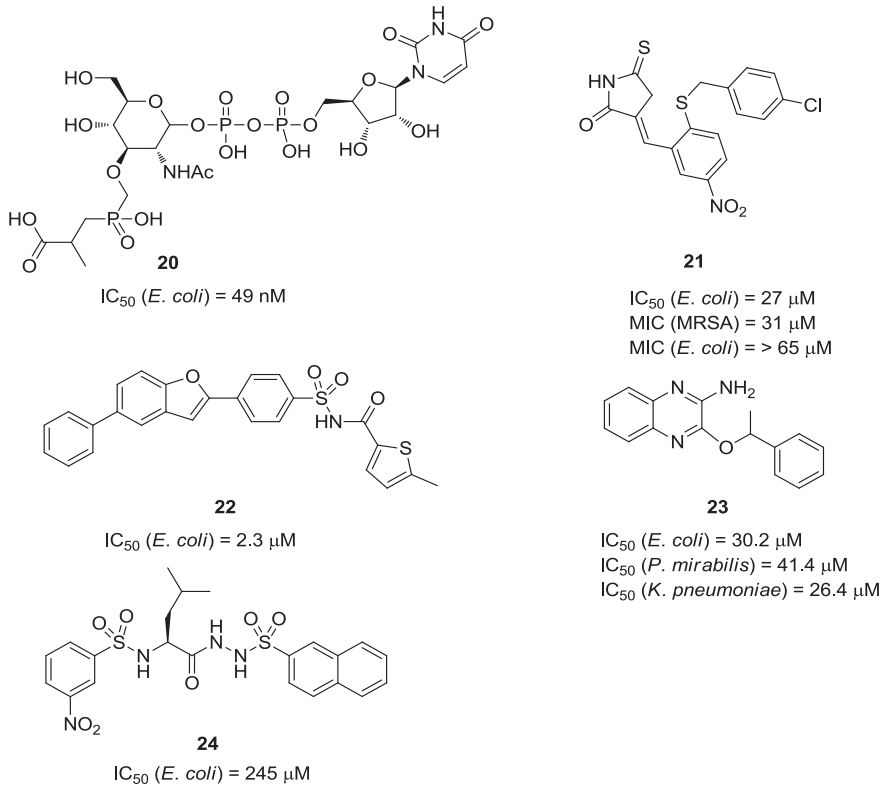

Fig. 5. Structures and biological activities of the MurC inhibitors.

Benzylidene rhodanines inhibit MurC with IC_{50} values ranging from 12 μM to 27 μM. These compounds have shown selective whole-cell activities against the Gram-positive MRSA, but not against the Gram-negative *E. coli*. Unfortunately, although compound **21** (Fig. 5) showed the best MIC against MRSA (31 μM), it was cytotoxic to mammalian CHO cells (IC_{50}, 29 μM), which indicates that it has a nonspecific mechanism of action [41]. Two peptide inhibitors of MurC from *P. aeruginosa* were discovered using a phage-display selection technique. However, these are weak inhibitors, with IC_{50} values of only 1.5 mM and 0.9 mM [10].

In 2004, AstraZeneca performed HTS of their chemical library and discovered a series of benzofuran acyl-sulfonamides as inhibitors of MurC. The most potent derivative (compound **22**, Fig. 5) showed time-dependent, partially reversible, low micromolar inhibition of MurC from *E. coli* (IC_{50}, 2.3 μM). However, it also showed high affinity binding to bovine serum albumin, which is most probably due to its highly lipophilic character [42]. As further studies on this type of compound have not been published since, it can be assumed that the problems of nonspecific off-target protein binding were never successfully resolved. A very promising effort in the development of potent MurC inhibitors was reported by Pfizer. They used HTS on MurC from *E. coli*, through which they identified a small-molecule reversible inhibitor (compound **23**, Fig. 5). Inhibition of MurC from *E. coli* by compound **23** (IC_{50}, 30.2 μM) was confirmed using nuclear magnetic resonance product detection and a kinetic-coupled enzyme assay. In competition assays, it was shown that compound **23** is a competitive inhibitor of ATP binding to MurC, with a K_i of 8.2 μM. Unfortunately, the activity of compound **23** was shown to be limited to MurC enzymes from enterobacterial species closely related to *E. coli* (e.g., *Proteus mirabilis*, IC_{50}, 41.4 μM; *Klebsiella pneumoniae*, IC_{50}, 26.4 μM), and not to be effective against MurC enzymes from more distant Gram-negative species (*H. influenza*, *Acinetobacter baylyi*, *P. aeruginosa*). Moreover, compound **23** had no effect on *E. coli* growth in a standard *in vitro* MIC assay [43].

Some other structural classes have also been explored as potential MurC inhibitors, such as acyl-sulfonohydrazide-based compounds. The design of these compounds was based on a hypothesis that an acyl-sulfonohydrazide moiety represents a potential diphosphate analog. In the assays, the majority of these compounds were poor MurC inhibitors. The most active from the series is shown in Fig. 5 (compound **24**; IC_{50} [*E. coli* MurC], 245 μM); however, this compound showed no antibacterial activity on two different *E. coli* strains [44].

There have been several examples in the literature where simultaneous inhibition of MurC and another Mur enzyme was observed. For instance, based on the work by Sim et al. [41], novel *N*-benzylidenesulfonohydrazide-based compounds were designed that showed concurrent MurC and MurD inhibitory activities. The best performing of these dual inhibitors was compound **25** (Fig. 6), with IC_{50} of 30 μM against both MurC and MurD. Unfortunately, the kinetic characterization and their high Hill coefficient indicated that the inhibitory activity of these *N*-benzylidenesulfonohydrazides most probably acts through nonspecific binding and/or aggregate formation [45].

On the basis of an in-house library screening, a series of compounds with an *N*-acylhydrazone scaffold were characterized as a new class of inhibitors of MurC and MurD from *E. coli*. A typical dual inhibitor is shown in Fig. 6 (compound **26**), which has IC_{50} values of 123 μM and 230 μM against MurC and MurD, respectively. On the other hand, compound **27** (Fig. 6) is a potent and selective inhibitor of MurC, with an IC_{50} of 32 μM. Although the antibacterial activities of these *N*-acylhydrazones were moderate (128 μg/mL), a correlation between their Mur ligase inhibition and *in vitro* antimicrobial activities was reported [46].

Recently, in a virtual screening study for potential ATP-competitive dual MurC/MurD inhibitors, a protocol of consecutive hierarchical filters yielded some weak dual inhibitors (e.g., compounds **28** and **29**, Fig. 6). Nevertheless, the new scaffolds that were discovered in this virtual screening provide solid starting points for

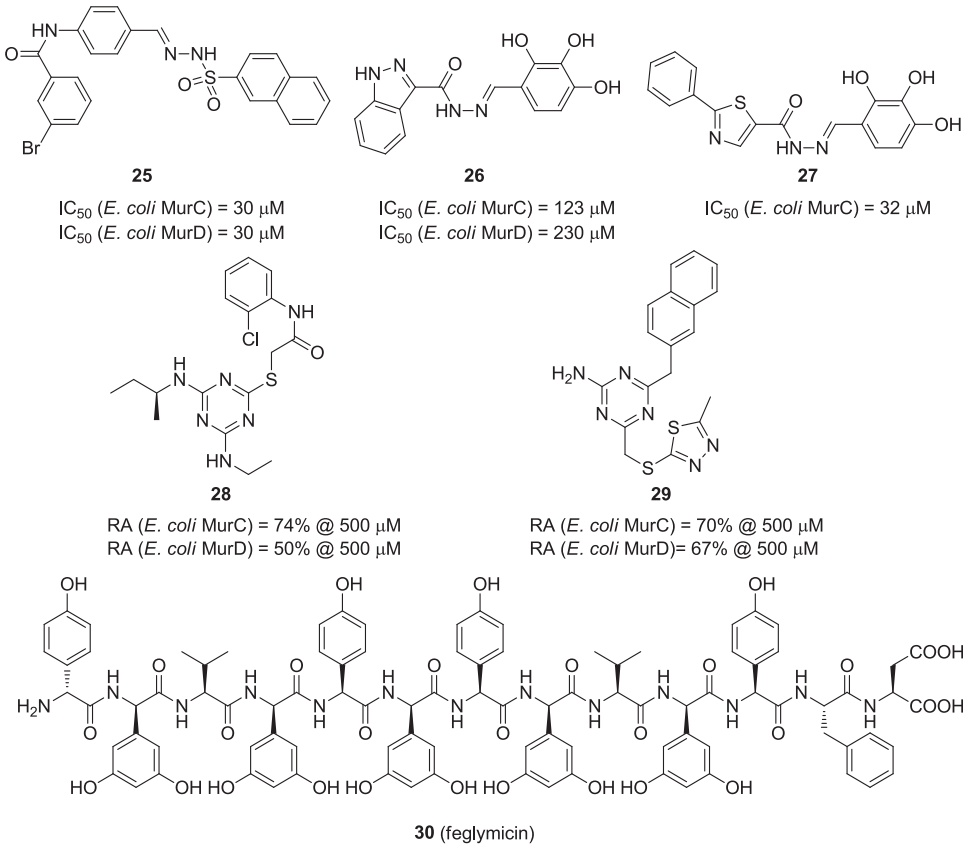

Fig. 6. Structural formulae of the dual MurC/MurD inhibitors, and feglymycin, a dual MurA/MurC inhibitor.

further development and optimization [47]. The group of Süssmuth identified feglymycin (compound **30**, Fig. 6), a 13-mer peptide, as a reversible inhibitor of MurA and MurC. The precise kinetic characterization of the feglymycin-based enzyme inhibition showed that it is a noncompetitive inhibitor of MurA from *E. coli* (K_i, 3.4 μM) and *S. aureus* (K_i, 3.5 μM). The antibacterial activity studies showed no activity against *E. coli*; most probably due to difficulties with the diffusion of feglymycin through the outer bacterial membrane [48].

5. Inhibitors of MurD

MurD has been targeted in several studies, and many structurally diverse inhibitors have been discovered and reviewed [9,49]. Peptides and several classes of nonpeptide inhibitors have been reported to date. Two cyclic nonapeptides were described with the amino-acid sequences CPAHWPHPC and CSAWSNKFC, which were shown to inhibit MurD from *E. coli* [50]. Small-molecule inhibitors can be divided into glutamic-acid-based inhibitors and inhibitors without a glutamic-acid motif [49]. These latter consist of several structural classes, as shown in Fig. 7.

The most potent macrocyclic inhibitor of MurD was discovered through computer-based molecular design: compound **31**, with an IC_{50} of 0.7 μM [51]. 9*H*-Xanthene derivatives and polycyclic inhibitors were discovered through structure-based virtual screening. Compound **32** was the most potent representative of these, as it inhibited *E. coli* MurD with an IC_{50} of 10 μM [52]. Benzene-1,3-dicarboxylic acid derivatives were also discovered through virtual screening, with compound **33** being the most potent (IC_{50}, 270 μM). In addition, compound **33** inhibited MurE from *E. coli* with an IC_{50} of 32 μM [53]. *N*′-Benzylidene-sulfono-hydrazides were designed and synthesized as diphosphate mimetics of the MurD nucleotide substrate UDP-Mur*N*Ac-L-Ala. When *E. coli* MurD was incubated with 500 μM of the best inhibitor from this series (compound **34**, Fig. 7), the MurD still retained 56% residual activity [44]. The sulfonohydrazone motif is the feature of another class of MurD inhibitors, where compound **35** is the most potent, with an IC_{50} of 30 μM [45].

The next class of MurD inhibitors are the glutamic-acid-based inhibitors. The early inhibitors that contained the glutamic acid motif were phosphinate transition-state analogs [54]. These still represent the series with the most potent inhibitors of MurD that have been published to date (e.g., compound **36**, Fig. 8). Further development of the phosphinate transition-state analogs resulted in simplified compounds with the phosphinodipeptide Ala-Ψ(PO_2-CH_2)-Glu structural motif [55]. The inhibitory activities of these compounds (e.g., compound **37**, Fig. 8) are in the micromolar range [55], and compound **37** also inhibited MurE [56].

As the glutamic acid moiety was determined to be the key structural motif for inhibition of MurD, variations to other parts of the transition-state have been thoroughly investigated, with sulfonamides being introduced here as another important class of MurD inhibitors. As with the phosphinate moiety, the sulfonamide moiety was integrated into the target compounds in order to mimic the MurD tetrahedral transition-state [57–59]. The early naphthalene sulfonamides were *N*-substituted glutamic acid derivatives [58,59], and compounds **38** (containing D-Glu) and **39** (containing L-Glu) both inhibited MurD from *E. coli.* However, the potency of the inhibitor containing the L-Glu enantiomer (compound **39**) was significantly lower than that of the D-Glu derivative (compound **38**) [58]. An important achievement of these thorough investigations into the inhibitory mechanisms here was the solution of the X-ray co-crystal structures of each of the L-Glu

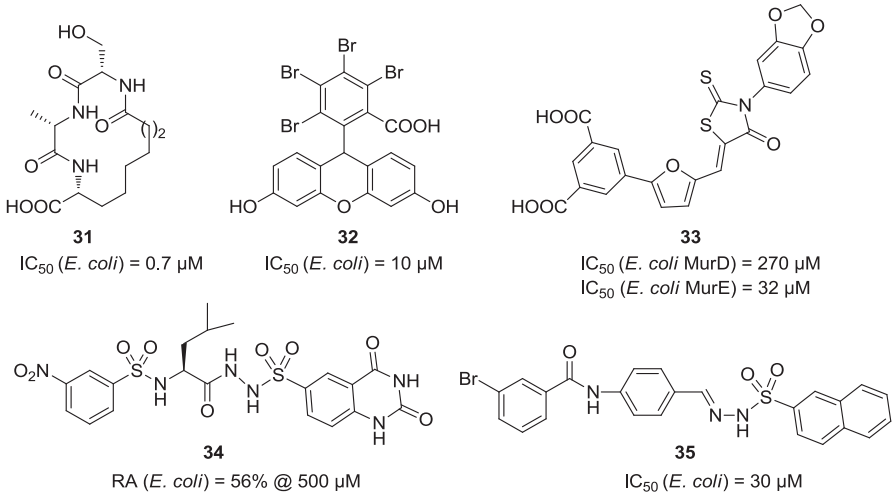

Fig. 7. Structures and biological activities of some diverse inhibitors of MurD and dual MurD/MurE inhibitors.

and D-Glu derivatives in the active site of MurD [58]. Optimization of these early naphthalene sulfonamides resulted in new inhibitors, among which the most potent (compound **40**, Fig. 8) for inhibition of MurD had an IC_{50} of 85 μM [59]. Binding energies of the naphthalene sulfonamides were calculated using the linear interaction-energy method and the calculated energies correlated very well with the experimentally obtained free energies [60]. Further analysis of the interactions between naphthalene sulfonamides and MurD were performed using NMR followed by molecular dynamics, which takes into account the ligand flexibility and its effects on particular ligand-enzyme contacts, thus explaining potential arguments for moderate inhibitory activities [61].

Further improvements to the naphthalene sulfonamides resulted in replacing the D-Glu moiety with more rigid cyclic counterparts, as shown for compounds **41** and **42** (Fig. 8, Fig. 9B) [62]. Incorporation of these more rigid mimetics in the position of D-Glu improved the inhibitory activities against MurD when compared to the parent compounds, thereby confirming the advantage of this conformational restriction [62]. The co-crystal structure of MurD with the more rigid inhibitor compound **41** revealed that both of the carboxylic acid groups occupy exactly the same binding sites as the D-Glu of the parent compound **40** (Fig. 8) [62]. These carboxylic groups of the more rigid glutamic acid mimetic form the typical interactions with Ser415, Leu416 and Phe422, and Lys348 and His183. The 2-cyano-4-fluoro phenyl ring occupies the uracil binding pocket of the nucleotide substrate, with a hydrogen bond between the cyano group and Thr36. Naphthalene sulfonamide inhibitors in a complex with MurD were also investigated by NMR, using $^{1}H/^{13}C$ hetero-nuclear single quantum correlation. The conformational and dynamic properties of these bound ligands and their binding interactions were examined using the transferred nuclear Overhauser effect, saturation transfer differences, and molecular dynamics. The discoveries here provided insights into the dynamic behavior of these ligand–MurD complexes and its influence on the ligand–enzyme contacts [61].

The benzylidene-2,4-thiazolidin-dione and 2-thioxothiazolidin-4-one substituted glutamic acids represent another large family of MurD inhibitors. Representative compounds are shown in Fig. 8. It was discovered that the most potent MurD inhibitor of the first of these series did not lose significant activity when D-Glu (compound **43**, Fig. 8) was replaced with L-Glu (compound **44**, Fig. 8) [63]. These benzylidene-2,4-thiazolidin-dione and 2-thioxothiazolidin-4-one substituted glutamic acids were further developed, with the substitution of the aromatic rings altered; this resulted in an *E. coli* MurD inhibitor (compound **45**, Fig. 8) with an IC_{50} of 45 μM [64]. Fine-tuning of the structures of these benzylidene-2,4-thiazolidin-dione and 2-thioxothiazolidin-4-one substituted glutamic acids also produced compound **46** (IC_{50}, 3 μM) [65,66]. Further optimization of this class of compounds then resulted in the introduction of a shorter linker between the glutamic acid part and the 2,4-thiazolidin-dione or 2-thioxothiazolidin-4-one parts of the molecule, although the inhibitory activity against MurD was not improved, as the most potent of these (compound **47**, Fig. 8) inhibited MurD from *E. coli* with an IC_{50} of 10 μM. An interesting concept around this series is that the most potent compound (**42**) contained L-Glu, while the D-Glu-containing derivative, compound **48**, was less active (IC_{50}, 45 μM) [67].

An important milestone in the development of these benzylidene-2,4-thiazolidin-dione and 2-thioxothiazolidin-4-one inhibitors was the solution of several X-ray crystal structures of MurD with some of these inhibitors in the active site, which thus revealed their binding modes [64–66,68]. The binding modes of all of the benzylidene-2,4-thiazolidin-dione and 2-thioxothiazolidin-4-one inhibitors are very similar. The representative binding mode of compound **49** is shown in Fig. 9B [69]. This binding mode revealed that the 2,4-thiazolidin-dione and 2-thioxothiazolidin-4-one rings are bound into the uracil binding site, for interactions with Arg35, Thr36 and Asp37. The carboxylic groups of the D-Glu part of the inhibitor occupy exactly the same positions as the carboxylic groups of UDP-Mur*N*Ac-L-Ala-D-Glu. The terminal carboxylic group of the D-Glu moiety interacts with Ser415, Leu416 and Phe422, while the α-carboxylic group is held in position through interactions with Thr321 and Lys348 directly, or with Lys115 and Lys348 via water molecules. No polar contacts between MurD and the central linker part of the inhibitors are seen. Only the π-π stacking of the aminophenyl ring with Phe161, and the hydrophobic interactions of the benzylidene ring with Gly73, are worth mentioning. A comparison of the crystal structures of a thiazolidin-dione inhibitor (PDB entry: 2X5O) [64] and UDP-Mur*N*Ac-L-Ala-D-Glu (PDB entry: 4UAG) [70] with *E. coli* MurD reveals that this thiazolidin-dione inhibitor is bound to the binding site of the product. In addition to the crystal structure, the main feature of compound **49** is its dual inhibitory activity against MurD and MurE from both *E. coli* and *S. aureus*, as well as its antibacterial activity against MRSA [69].

The MurD enzymes from different bacterial species share conserved residues that are essential for their catalytic activity; however, the overall similarities of their amino-acid sequences are very small. Therefore, it is not surprising that only a few compounds can inhibit MurDs across different species. For example, compounds **42**

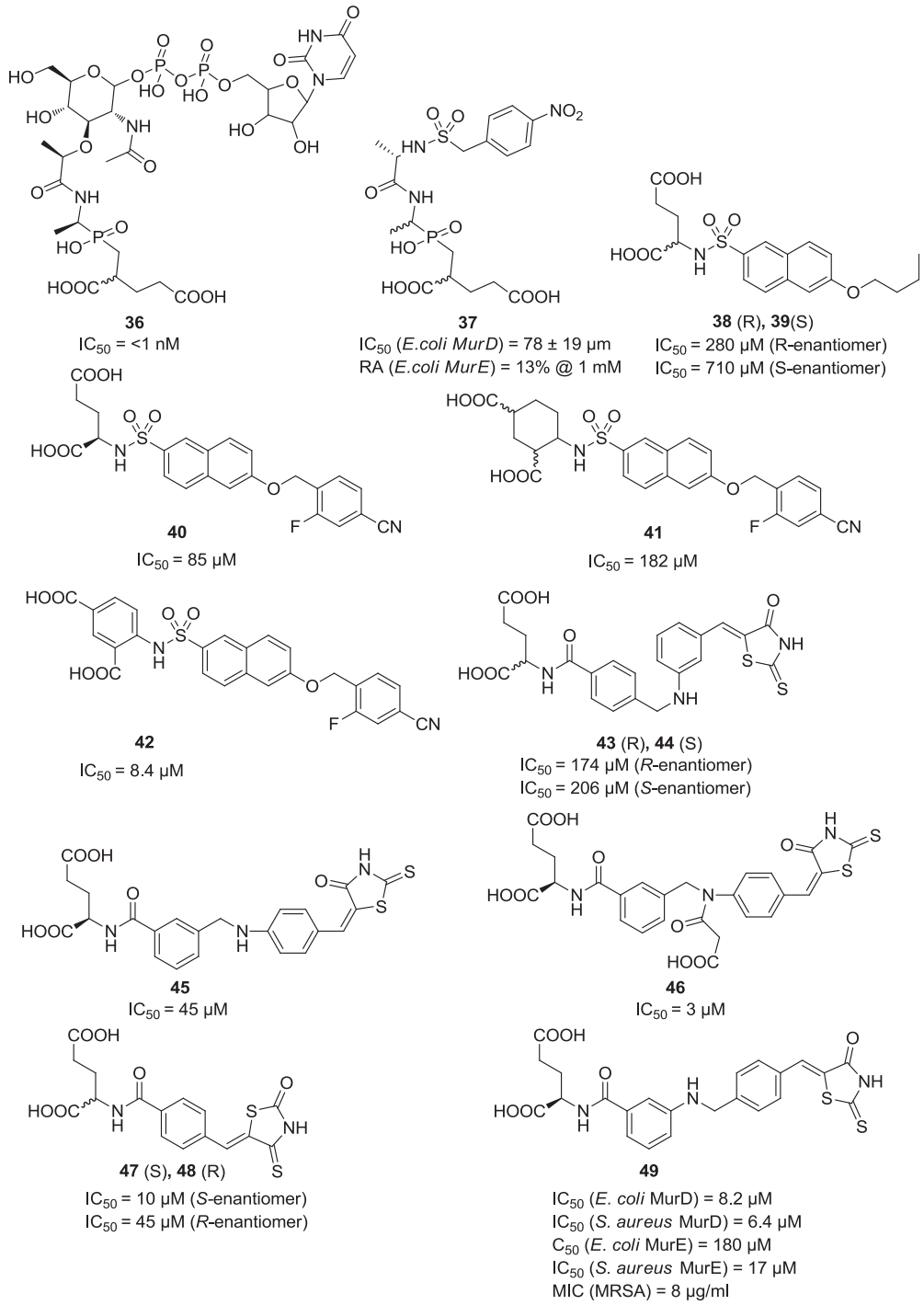

Fig. 8. Structures and biological activities of glutamic-acid-based inhibitors of MurD and dual MurD/MurE inhibitors.

and **45** (Fig. 8) can inhibit MurD from *S. aureus*, *S. pneumoniae*, *B. burgdorferi* and *M. tuberculosis* in the micromolar concentration range [71].

6. Inhibitors of MurE

A phosphinate inhibitor was the first inhibitor of MurE (compound **50**, Fig. 10), with an IC_{50} of 1.1 μM [72]. Some sulfonamides that are similar to the inhibitors of MurD described above can inhibit MurE (e.g., compound **51**, Fig. 10; IC_{50}, 181 μM) [57]. A cyclic peptide with the amino-acid sequence CQANLRSQC inhibited MurE from *S. aureus* [49]. Peptide inhibitors of MurE were also discovered, as the protein MurEp1 inhibited MurE with an IC_{50} of 500 μM [73]. 3-Methoxynordomesticine was discovered as an inhibitor of MurE from *M. tuberculosis* (compound **52**, Fig. 10) [74], and another inhibitor of MurE from *M. tuberculosis* (compound **53**, Fig. 10) was recently discovered, with an IC_{50} of 75 μM [75].

7. Inhibitors of MurF

The first MurF inhibitors were reported in 1998, by Miller et al. These were pseudo-tri-peptide and pseudo-tetra-peptide aminoalkylphosphinic acids, with the general structure of X-Lys-PO_2H-Gly-Ala.

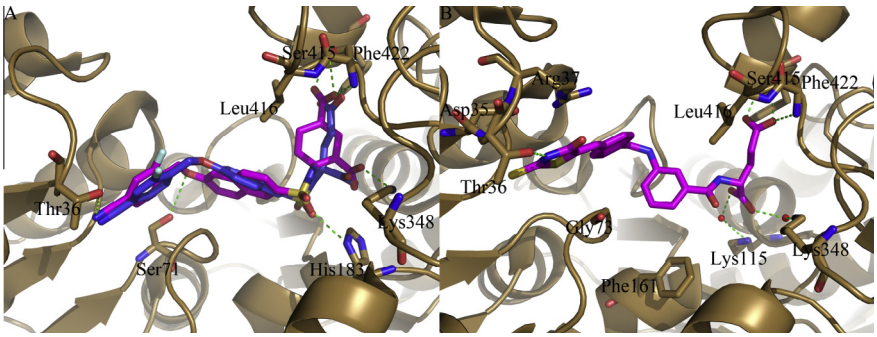

Fig. 9. (A) Binding modes of compounds **40** (blue) and **41** (magenta) in the active site of MurD. (B) Binding mode of compound **49** in the active site of MurD.

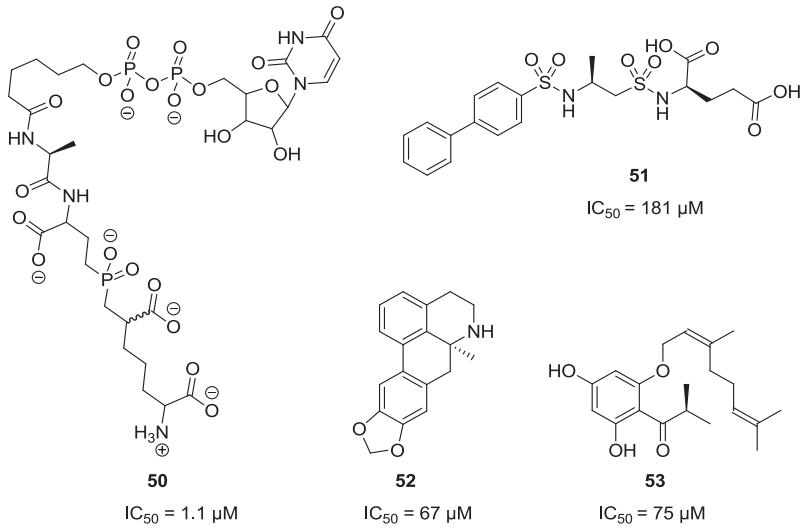

Fig. 10. Structures and biological activities of glutamic acid based inhibitors of MurE.

Kinetic assays with MurF from *E. coli* revealed that these transition-state analogs act as reversible competitive inhibitors, with K_i values from 200 μM to 700 μM [76].

One of the most encouraging sets of MurF inhibitors was published by Abbott Laboratories. Initially, using affinity selection screening technology, two very promising MurF hits (compounds **54** and **55**, Fig. 11) were identified, with IC_{50} values of 8 μM and 1 μM [77]. The binding modes of compounds **54** and **55** were confirmed with the determination of their co-crystal structures [78], and these data represented the basis for subsequent structure-based optimization that yielded compound **56** (IC_{50}, 22 nM) as the most potent MurF inhibitor [79,80]. Unfortunately, even the most potent compounds from this series did not show significant antibacterial activity, even in the presence of cell permeabilizers. Moreover, no activity was observed in *E. coli* AcrAB efflux-pump mutants. This all pointed to the possibility that MurF does not catalyze a rate-limiting step in this biosynthetic pathway [79]. It is also possible that compounds of this structural type bind nonselectively to other proteins inside the bacterial cell. Despite the lack of antibacterial activity of these compounds, several very recent attempts to obtain novel and more potent cyanothiophene-based leads have been described. Systematic structural modifications of the parent compounds **55** and **56** resulted in a comprehensive series of nanomolar inhibitors of MurF from *S. pneumoniae*, and micromolar inhibitors of MurF from *E. coli* and *S. aureus* (e.g., compound **57**, Fig. 11). The antibacterial activities of these compounds were also determined, and some of them showed antibacterial activities against *S. pneumoniae* at concentrations from 16 μg/mL to 64 μg/mL. Unfortunately, none of these were active against *E. coli* and *S. aureus* [81]. Very recently, a second generation of cyanothiophene-based derivatives with increased polarity was designed. The most potent inhibitors from this series have well-balanced inhibitory properties against both MurF from *S. pneumoniae* and MurF from *E. coli*. (e.g., compound **58**, Fig. 11). Compound **58** also has promising antibacterial activities against selected Gram-positive and Gram-negative strains (Fig. 11); however, at least a part of its antibacterial action is a consequence of membrane-damaging effects, as shown in an assay used to assess membrane integrity [82].

Several computational approaches were also initiated to discover new MurF ligase inhibitors. For example, extensive quantitative (Q)SAR modeling was used in the search for new inhibitors with modified physico-chemical properties [83]. In a similar attempt, new inhibitors were designed using a *de novo* approach. The activities of these promising hits were subsequently predicted using the established QSAR models; the predicted results could not, however, be confirmed experimentally [84]. In 2008, a pharmacophore modeling approach using a set of 39 known inhibitors led to the identification of three structurally novel micromolar inhibitors of MurF from *P. aeruginosa* that were retrieved from the NCI database. However, due to their nondrug-like structural properties, these do not represent promising starting points [85].

Structure-based virtual screening with MurF [52], and subsequent hit optimization, led to the discovery of a moderately potent 1,3,5-triazine (compound **59**, Fig. 11) [86]. Recently, a ligand-based approach led to the discovery of a MurF inhibitor with micromolar

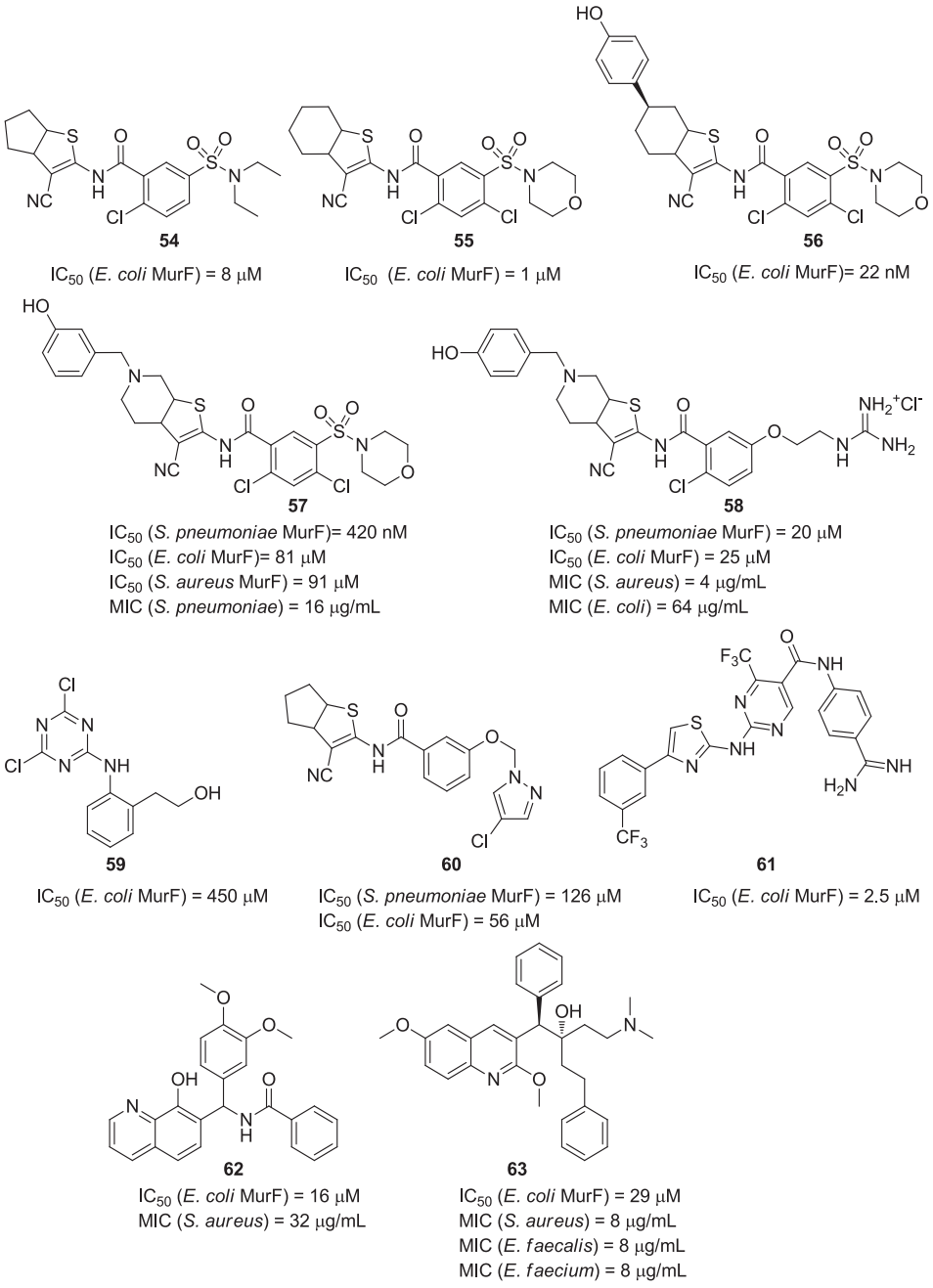

Fig. 11. Structures and activities of the MurF inhibitors.

activities against MurF from *S. pneumoniae* and *E. coli* (compound **60**, Fig. 11) [87]. A thiazolylaminopyrimidine series of MurF inhibitors from *E. coli* was identified in 2006 by Johnson & Johnson, with the use of an Mpl-based *in vitro* assay. The most potent from this series was compound **61**, with an IC_{50} of 2.5 μM (Fig. 11). Unfortunately, none of these synthesized compounds showed antibacterial activity [88]. Through the use of a MurF binding assay, a series of 8-hydroxyquinolines was identified that bound to the *E. coli* enzyme and inhibited its activity. One of these inhibitors (compound **62**, Fig. 11) also showed notable antibacterial activity [89]. Additionally, a pharmacophore model was constructed on the basis of these compounds, and this generated two diarylquinolines (e.g., compound **63**, Fig. 11) that can disrupt cell-wall biosynthesis via MurF inhibition. Compound **63** also has a MIC of 8 μg/mL against tested strains of *Enterococcus faecalis*, *Enterococcus faecium*, and *S. aureus* (both methicillin susceptible and resistant) [90].

8. Multiple inhibitors of the Mur enzymes

It is widely recognized that multiple ligands can be more beneficial therapeutically than single-target-specific ligands. The design of compounds that bind to more than one target is a demanding challenge, where the appropriately balanced affinities for the different targets and the preservation of the drug-like properties are necessary [91,92]. The efforts of several drug discovery programs to design multiple Mur inhibitors has yielded some compounds that show balanced inhibitory activities against the Mur enzymes. The Wyeth bacterial cell-wall program yielded more than 20 derivatives of 2-phenyl-5,6-dihydro-2*H*-thieno[3,2-*c*]pyrazol-3-ol (e.g., compound **64**, Fig. 12). These were prepared and evaluated as multiple inhibitors of *S. aureus* MurB, MurC and MurD. The compounds of this structural type also demonstrated promising antibacterial activities against Gram-positive bacteria, including

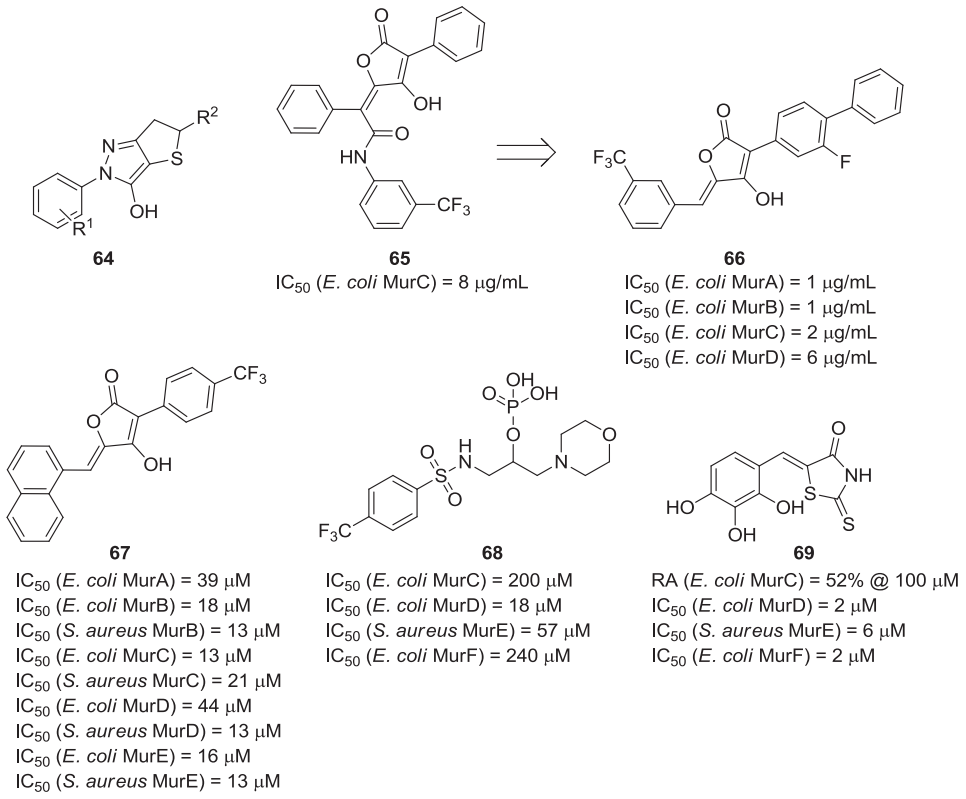

Fig. 12. Multiple inhibitors of the Mur enzymes.

MRSA, VRE and PRSP. However, their MICs increased to above 128 μg/mL in the presence of 4% bovine serum albumin [93].

Another effort to obtain a potent cell-wall biosynthesis inhibitor was based on a Wyeth in-house screening campaign of the Mur enzymes. In this way, a pulvinamide was discovered (compound **65**, Fig. 12; IC_{50} [MurC], 8 μg/mL) and this compound represented a basis for the preparation of more than 180 pulvinones for evaluation as inhibitors of MurA-D. Several derivatives showed Mur enzyme inhibition with IC_{50} values in the 1 μg/mL to 10 μg/mL range; in particular, the inhibition of MurC was in general more pronounced, whereas MurA and MurB were inhibited to lesser extents. Compound **66** (Fig. 12) showed inhibition that was well balanced for all of the Mur enzymes (IC_{50} values from 1 to 6 μg/mL). The majority of these derivatives also have antibacterial activities against Gram-positive organisms, including MRSA, VRE and PRSP strains [94].

In addition, Wyeth studies also identified the naphthyl tetronic acids as multiple inhibitors of Mur enzymes. These were demonstrated to be MurA-E inhibitors, with IC_{50} values in the micromolar range [95]. Moreover, the crystal structure of one of these compounds in the MurB active site was resolved, and thus their binding pattern has been carefully studied. Compound **67** (Fig. 12) showed the most balanced inhibitory activities against a panel of nine Mur enzymes. This compound also showed notable antimicrobial activities against *E. coli* and *S. aureus* species, with MICs of 2 μg/mL and 1–2 μg/mL, respectively.

In 2009, phosphorylated hydroxyethylamines were developed as inhibitors of MurC-F, and these represented promising hit compounds for further structural optimization of multiple Mur ligase inhibitors. These compounds inhibited the activities of the Mur ligases MurC-F at micromolar concentrations, and among these, compound **68** showed the most balanced inhibition of all four of these Mur enzymes (Fig. 12) [96].

Recently, several hydroxy-substituted 5-benzylidenethiazolidin-4-ones were synthesized and characterized as multiple inhibitors of the Mur ligases. The most potent of these (compound **69**, Fig. 12) was active against the MurD-F ligases, with well-balanced IC_{50} values, between 2 μM and 6 μM. Unfortunately, compound **69** and the other similar derivatives were found to be weak (>128 μg/mL) inhibitors of bacterial growth *in vitro* [68].

9. Discussion and concluding remarks

Due to the widespread emergence of bacterial resistance to antibiotics, it has become clear that new drugs with new mechanisms of action have to be developed rapidly [97–99]. In this context, attention has recently focused on some so-far underexploited antibacterial drug discovery targets: the Mur enzymes. We have presented here in this review many of the inhibitors that have been developed. Significant scientific data gathered during the past two decades clearly suggest good reasons why the Mur enzymes should be used in antibacterial inhibitor discovery: (i) they are genetically essential for bacterial survival; (ii) their X-ray crystal structures are available, they show similarities across bacterial species, and they are amenable for structure-based inhibitor design; (iii) they have no structural homology with the mammalian enzymes; (iv) their complex substrates and biochemical assays have become easily available also for academic groups; and (v) many inhibitors that have been developed to date demonstrate that the Mur enzymes are druggable [2,9,14].

The validity of MurA for antibacterial drug discovery has been clearly demonstrated by a small-molecule antibiotic in clinical use: fosfomycin. However, there are some important issues related to the other Mur enzymes that need to be kept in mind during the development of inhibitors, and that need be overcome in the future [100]. To date, to the best of our knowledge, there have been no reports of MurB-F inhibitors with antibacterial activity that have a clearly demonstrated mode of action (i.e., in terms of intracellular Mur inhibition). The reason for this is most probably related to the

poor bacterial cell-wall penetration properties of most inhibitors and/or their rapid extrusion from bacteria by the efflux pumps. Recently, possible solutions to enhance drug uptake across the bacterial cytoplasmic membrane have been suggested; e.g., by creating inhibitors that are in the form of zwitterions, or by using siderophores attached to compounds, to enable their delivery into bacteria (the 'Trojan horse' strategy) [101]. However, other possibilities to enhance bacterial penetration should also be investigated thoroughly. For example, it is still not known what physico-chemical properties are required for compounds to be able to penetrate into bacteria passively, which makes the design of intracellular inhibitors more difficult.

It has been speculated that the Mur enzymes are organized in a multi-enzyme complex *in vivo*, which allows for the channeling of intermediates and restricts the accessibility of inhibitors to the active sites of these enzymes. Also, it is known that the Mur enzymes are prone to feedback inhibition by the downstream products of their pathway. This all suggest that the goal of obtaining Mur inhibitors with good antibacterial activity has not been reached yet because more potent inhibitors of the Mur ligases (MurC-F) are needed to achieve this goal [100]. Indeed, drug-like (i.e., corresponding to the Lipinski Rule of 5) and potent nanomolar inhibitors have so far only been found for MurF. In addition, irreversible inhibitors are known only for MurA, and so the design of such compounds should be considered for the other Mur enzymes.

It is known that single-enzyme-targeting by inhibitors can be prone to development of resistance by bacterial mutations that alter the inhibitor binding site. To avoid this problem, inhibitors that simultaneously target two, three or even more of the Mur enzymes have been developed. At present these compounds are very early proof-of-concept molecular tools, but we believe that their further optimization and development will yield multiple Mur inhibitors with good antibacterial properties [91,92].

Acknowledgments

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References

[1] D.W. Green, The bacterial cell wall as a source of antibacterial targets, Exp. Opin. Therap. Targ. 6 (2002) 1–20.
[2] T.D.H. Bugg, D. Braddick, C.G. Dowson, D.I. Roper, Bacterial cell wall assembly: still an attractive antibacterial target, Trends Biotechnol. 29 (2011) 167–173.
[3] A. Gautam, R. Vyas, R. Tewari, Peptidoglycan biosynthesis machinery: A rich source of drug targets, Crit. Rev. Biotechnol. 31 (2011) 295–336.
[4] E. Sauvage, F. Kerff, M. Terrak, J.A. Ayala, P. Charlier, The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis, FEMS Microbiol. Rev. 32 (2008) 234–258.
[5] K.F. Kong, L. Schneper, K. Mathee, Beta-lactam antibiotics: from antibiosis to resistance and bacteriology, APMIS 118 (2010) 1–36.
[6] T. Schneider, H.G. Sahl, An oldie but a goodie – cell wall biosynthesis as antibiotic target pathway, Int. J. Med. Microbiol. 300 (2010) 161–169.
[7] A.R.M. Coates, G. Halls, Y. Hu, Novel classes of antibiotics or more of the same?, Brit J. Pharmacol. 163 (2011) 184–194.
[8] R. Wise, The urgent need for new antibacterial agents, J. Antimicrob. Chemother. 66 (2011) 1939–1940.
[9] H. Barreteau, A. Kovač, A. Boniface, M. Sova, S. Gobec, D. Blanot, Cytoplasmic steps of peptidoglycan biosynthesis, FEMS Microbiol. Rev. 32 (2008) 168–207.
[10] A. El Zoeiby, F. Sanschagrin, R.C. Levesque, Structure and function of the Mur enzymes: development of novel inhibitors, Mol. Microbiol. 47 (2003) 1–12.
[11] W. Vollmer, D. Blanot, M.A. De Pedro, Peptidoglycan structure and architecture, FEMS Microbiol. Rev. 32 (2008) 149–167.
[12] A.H. Katz, C.E. Caufield, Structure-based design approaches to cell wall biosynthesis inhibitors, Curr. Pharm. Des. 9 (2003) 857–866.
[13] J.v. Heijenoort, Recent advances in the formation of the bacterial peptidoglycan monomer unit, Nat. Prod. Rep. 18 (2001) 503–519.
[14] C.A. Smith, Structure, Function and dynamics in the Mur family of bacterial cell wall ligases, J. Mol. Biol. 362 (2006) 640–655.
[15] A.L. Lovering, S.S. Safadi, N.C.J. Strynadka, Structural perspective of peptidoglycan biosynthesis and assembly, Annu. Rev. Biochem. 81 (2012) 451–478.
[16] J.L. Marquardt, E.D. Brown, W.S. Lane, T.M. Haley, Y. Ichikawa, C.-H. Wong, C.T. Walsh, Kinetics, stoichiometry, and identification of the reactive thiolate in the inactivation of UDP-GlcNAc enolpyruvoyl transferase by the antibiotic fosfomycin, Biochemistry 33 (1994) 10646–10651.
[17] A. Castañeda-García, J. Blázquez, A. Rodríguez-Rojas, Molecular mechanisms and clinical impact of acquired and intrinsic fosfomycin resistance, Antibiotics 2 (2013) 217–236.
[18] E.Z. Baum, D.A. Montenegro, L. Licata, I. Turchi, G.C. Webb, B.D. Foleno, K. Bush, Identification and characterization of new inhibitors of the *Escherichia coli* MurA enzyme, Antimicrob. Agents Chemother. 45 (2001) 3182–3188.
[19] M.D.F.S. Barbosa, G. Yang, J. Fang, M.G. Kurilla, D.L. Pompliano, Development of a whole-cell assay for peptidoglycan biosynthesis inhibitors, Antimicrob. Agents Chemother. 46 (2002) 943–946.
[20] S. Eschenburg, M.A. Priestman, F.A. Abdul-Latif, C. Delachaume, F. Fassy, E. Schönbrunn, A novel inhibitor that suspends the induced fit mechanism of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA), J. Biol. Chem. 280 (2005) 14070–14075.
[21] J. Molina-López, F. Sanschagrin, R.C. Levesque, A peptide inhibitor of MurA UDP-N-acetylglucosamine enolpyruvyl transferase: The first committed step in peptidoglycan biosynthesis, Peptides 27 (2006) 3115–3121.
[22] A. Bachelier, R. Mayer, C.D. Klein, Sesquiterpene lactones are potent and irreversible inhibitors of the antibacterial target enzyme MurA, Bioorg. Med. Chem. Lett. 16 (2006) 5605–5609.
[23] A. Steinbach, A.J. Scheidig, C.D. Klein, The unusual binding mode of cnicin to the antibacterial target enzyme MurA revealed by X-ray crystallography, J. Med. Chem. 51 (2008) 5143–5147.
[24] K. Shigetomi, K. Shoji, S. Mitsuhashi, M. Ubukata, The antibacterial properties of 6-tuliposide B. Synthesis of 6-tuliposide B analogues and structure–activity relationship, Phytochemistry 71 (2010) 312–324.
[25] T. Scholz, C.L. Heyl, D. Bernardi, S. Zimmermann, L. Kattner, C.D. Klein, Chemical, biochemical and microbiological properties of a brominated nitrovinylfuran with broad-spectrum antibacterial activity, Bioorg. Med. Chem. 21 (2013) 795–804.
[26] C.J. Dunsmore, K. Miller, K.L. Blake, S.G. Patching, P.J.F. Henderson, J.A. Garnett, W.J. Stubbings, S.E.V. Phillips, D.J. Palestrant, J.D.L. Angeles, J.A. Leeds, I. Chopra, C.W.G. Fishwick, 2-Aminotetralones: Novel inhibitors of MurA and MurZ, Bioorg. Med. Chem. Lett. 18 (2008) 1730–1734.
[27] K. Miller, C.J. Dunsmore, J.A. Leeds, S.G. Patching, M. Sachdeva, K.L. Blake, W.J. Stubbings, K.J. Simmons, P.J.F. Henderson, J. De Los Angeles, C.W.G. Fishwick, I. Chopra, Benzothioxalone derivatives as novel inhibitors of UDP-N-acetylglucosamine enolpyruvyl transferases (MurA and MurZ), J. Antimicrob. Chemother. 65 (2010) 2566–2573.
[28] B.S. Jin, S.G. Han, W.K. Lee, S.W. Ryoo, S.J. Lee, S.W. Suh, Y.G. Yu, Inhibitory mechanism of novel inhibitors of UDP-N-acetylglucosamine enolpyruvyl transferase from *Haemophilus influenzae*, J. Microbiol. Biotechnol. 19 (2009) 1582–1589.
[29] S.G. Han, W.K. Lee, B.S. Jin, K.I. Lee, H.H. Lee, Y.G. Yu, Identification of novel irreversible inhibitors of UDP-N-acetylglucosamine enolpyruvyl transferase (MurA) from *Haemophilus influenzae*, J. Microbiol. Biotechnol. 23 (2013) 329–334.
[30] H. Han, Y. Yang, S.H. Olesen, A. Becker, S. Betzi, E. Schönbrunn, The fungal product terreic acid is a covalent inhibitor of the bacterial cell wall biosynthetic enzyme UDP-N-acetylglucosamine 1-carboxyvinyltransferase (MurA), Biochemistry 49 (2010) 4276–4282.
[31] S.H. Olesen, D.J. Ingles, Y. Yang, E. Schönbrunn, Differential antibacterial properties of the MurA inhibitors terreic acid and fosfomycin, J. Basic Microb. 54 (2014) 322–326.
[32] C.J. Andres, J.J. Bronson, S.V. D'Andrea, M.S. Deshpande, P.J. Falk, K.A. Grant-Young, W.E. Harte, H.-T. Ho, P.F. Misco, J.G. Robertson, D. Stock, Y. Sun, A.W. Walsh, 4-Thiazolidinones: novel inhibitors of the bacterial enzyme MurB, Bioorg. Med. Chem. Lett. 10 (2000) 715–717.
[33] J.J. Bronson, K.L. DenBleyker, P.J. Falk, R.A. Mate, H.-T. Ho, M.J. Pucci, L.B. Snyder, Discovery of the first antibacterial small molecule inhibitors of MurB, Bioorg. Med. Chem. Lett. 13 (2003) 873–875.
[34] R.W. Sarver, J.M. Rogers, D.E. Epps, Determination of ligand-MurB interactions by isothermal denaturation: application as a secondary assay to complement high-throughput screening, J. Biomol. Screen. 7 (2002) 21–28.
[35] K.M.K. Kutterer, J.M. Davis, G. Singh, Y. Yang, W. Hu, A. Severin, B.A. Rasmussen, G. Krishnamurthy, A. Failli, A.H. Katz, 4-Alkyl and 4,4′-dialkyl 1,2-bis(4-chlorophenyl)pyrazolidine-3,5-dione derivatives as new inhibitors of bacterial cell wall biosynthesis, Bioorg. Med. Chem. Lett. 15 (2005) 2527–2531.
[36] A.M. Gilbert, A. Failli, J. Shumsky, Y. Yang, A. Severin, G. Singh, W. Hu, D. Keeney, P.J. Petersen, A.H. Katz, Pyrazolidine-3,5-diones and 5-hydroxy-1H-pyrazol-3(2H)-ones, inhibitors of UDP-N-acetylenolpyruvyl glucosamine reductase, J. Med. Chem. 49 (2006) 6027–6036.
[37] Y. Yang, A. Severin, R. Chopra, G. Krishnamurthy, G. Singh, W. Hu, D. Keeney, K. Svenson, P.J. Petersen, P. Labthavikul, D.M. Shlaes, B.A. Rasmussen, A.A. Failli, J.S. Shumsky, K.M.K. Kutterer, A. Gilbert, T.S. Mansour, 3,5-Dioxopyrazolidines, novel inhibitors of UDP-N-acetylenolpyruvyl glucosamine reductase (MurB) with activity against Gram-positive bacteria, Antimicrob. Agents Chemother. 50 (2006) 556–564.
[38] G.D. Francisco, Z. Li, J.D. Albright, N.H. Eudy, A.H. Katz, P.J. Petersen, P. Labthavikul, G. Singh, Y. Yang, B.A. Rasmussen, Y.-I. Lin, T.S. Mansour, Phenyl

thiazolyl urea and carbamate derivatives as new inhibitors of bacterial cell-wall biosynthesis, Bioorg. Med. Chem. Lett. 14 (2004) 235–238.
[39] F. Reck, S. Marmor, S. Fisher, M.A. Wuonola, Inhibitors of the bacterial cell wall biosynthesis enzyme MurC, Bioorg. Med. Chem. Lett. 11 (2001) 1451–1454.
[40] S. Marmor, C.P. Petersen, F. Reck, W. Yang, N. Gao, S.L. Fisher, Biochemical characterization of a phosphinate inhibitor of *Escherichia coli* MurC, Biochemistry 40 (2001) 12207–12214.
[41] M.M. Sim, S.B. Ng, A.D. Buss, S.C. Crasta, K.L. Goh, S.K. Lee, Benzylidene rhodanines as novel inhibitors of UDP-N-acetylmuramate/l-alanine ligase, Bioorg. Med. Chem. Lett. 12 (2002) 697–699.
[42] D.E. Ehmann, J.E. Demeritt, K.G. Hull, S.L. Fisher, Biochemical characterization of an inhibitor of *Escherichia coli* UDP-N-acetylmuramyl-l-alanine ligase, Biochim. Biophys. Acta, Proteins Proteomics 1698 (2004) 167–174.
[43] L.E. Zawadzke, M. Norcia, C.R. Desbonnet, H. Wang, K. Freeman-Cook, T.J. Dougherty, Identification of an inhibitor of the MurC enzyme, which catalyzes an essential step in the peptidoglycan precursor synthesis pathway, Assay Drug Dev. Technol. 6 (2008) 95–103.
[44] R. Frlan, A. Kovač, D. Blanot, S. Gobec, S. Pečar, A. Obreza, Design, synthesis and *in-vitro* biochemical activity of novel amino acid sulfonohydrazide inhibitors of MurC, Acta Chim. Slov. 58 (2011) 295–310.
[45] R. Frlan, A. Kovač, D. Blanot, S. Gobec, S. Pečar, A. Obreza, Design and synthesis of novel N-benzylidenesulfonohydrazide inhibitors of MurC and MurD as potential antibacterial agents, Molecules 13 (2008) 11–30.
[46] R. Šink, A. Kovač, T. Tomašić, V. Rupnik, A. Boniface, J. Bostock, I. Chopra, D. Blanot, L.P. Mašič, S. Gobec, A. Zega, Synthesis and biological evaluation of N-acylhydrazones as inhibitors of MurC and MurD ligases, ChemMedChem 3 (2008) 1362–1370.
[47] T. Tomašić, A. Kovač, G. Klebe, D. Blanot, S. Gobec, D. Kikelj, L. Mašič, Virtual screening for potential inhibitors of bacterial MurC and MurD ligases, J. Mol. Model. 18 (2012) 1063–1072.
[48] S. Rausch, A. Hänchen, A. Denisiuk, M. Löhken, T. Schneider, R.D. Süssmuth, Feglymycin is an inhibitor of the enzymes MurA and MurC of the peptidoglycan biosynthesis pathway, ChemBioChem 12 (2011) 1171–1173.
[49] R. Šink, H. Barreteau, D. Patin, D. Mengin-Lecreulx, S. Gobec, D. Blanot, MurD enzymes: some recent developments, BioMol. Concepts 4 (2013) 539–556.
[50] T. Bratkovič, M. Lunder, U. Urleb, B. Štrukelj, Peptide inhibiotors of MurD and MurE, essential enzymes of bacterial cell wall biosynthesis, J. Basic Microbiol. 48 (2008) 202–206.
[51] J.R. Horton, J.M. Bostock, I. Chopra, L. Hesse, S.E.V. Phillips, D.J. Adams, A.P. Johnson, C.W.G. Fishwick, Macrocyclic inhibitors of the bacterial cell wall biosynthesis enzyme MurD, Bioorg. Med. Chem. Lett. 13 (2003) 1557–1560.
[52] S. Turk, A. Kovač, A. Boniface, J.M. Bostock, I. Chopra, D. Blanot, S. Gobec, Discovery of new inhibitors of the bacterial peptidoglycan biosynthesis enzymes MurD and MurF by structure-based virtual screening, Bioorg. Med. Chem. 17 (2009) 1884–1889.
[53] A. Perdih, A. Kovač, G. Wolber, D. Blanot, S. Gobec, T. Šolmajer, Discovery of novel benzene 1,3-dicarboxylic acid inhibitors of bacterial MurD and MurE ligases by structure-based virtual screening approach, Bioorg. Med. Chem. Lett. 19 (2009) 2668–2673.
[54] M.E. Tanner, S. Vaganay, J. van Heijenoort, D. Blanot, Phosphinate inhibitors of the D-glutamic acid-adding enzyme of peptidoglycan biosynthesis, J. Org. Chem. 61 (1996) 1756–1760.
[55] K. Štrancar, D. Blanot, S. Gobec, Design, synthesis and structure-activity relationships of new phosphinate inhibitors of MurD, Bioorg. Med. Chem. Lett. 16 (2006) 343–348.
[56] K. Štrancar, A. Boniface, D. Blanot, S. Gobec, Phosphinate inhibitors of UDP-*N*-acetylmuramoyl-L-alanyl-D-glutamate: L-lysine ligase (MurE), Arch. Pharm. 340 (2007) 127–134.
[57] J. Humljan, M. Kotnik, A. Boniface, T. Šolmajer, U. Urleb, D. Blanot, S. Gobec, A new approach towards peptidosulfonamides: synthesis of potential inhibitors of bacterial peptidoglycan biosynthesis enzymes MurD and MurE, Tetrahedron 62 (2006) 10980–10988.
[58] M. Kotnik, J. Humljan, C. Contreras-Martel, M. Oblak, K. Kristan, M. Hervé, D. Blanot, U. Urleb, S. Gobec, A. Dessen, T. Šolmajer, Structural and functional characterization of enantiomeric glutamic acid derivatives as potential transition state analogue inhibitors of MurD ligase, J. Mol. Biol. 370 (2007) 107–115.
[59] J. Humljan, M. Kotnik, C. Contreras-Martel, D. Blanot, U. Urleb, A. Dessen, T. Šolmajer, S. Gobec, Novel naphthalene-*N*-sulfonyl-D-glutamic acid derivatives as inhibitors of MurD, a key peptidoglycan biosynthesis enzyme, J. Med. Chem. 51 (2008) 7486–7494.
[60] A. Perdih, U. Bren, T. Šolmajer, Binding free energy calculations of N-sulphonyl-glutamic acid inhibitors of MurD ligase, J. Mol. Model. 15 (2009) 983–996.
[61] M. Simčič, M. Hodošček, J. Humljan, K. Kristan, U. Urleb, D. Kocjan, S.G. Grdadolnik, NMR and molecular dynamics study of the binding mode of naphthalene-*N*-sulfonyl-D-glutamic acid derivatives: novel MurD ligase inhibitors, J. Med. Chem. 52 (2009) 2899–2908.
[62] I. Sosič, H. Barreteau, M. Simčič, R. Šink, J. Cesar, A. Zega, S.G. Grdadolnik, C. Contreras-Martel, A. Dessen, A. Amoroso, B. Joris, D. Blanot, S. Gobec, Second-generation sulfonamide inhibitors of D-glutamic acid-adding enzyme: activity optimisation with conformationally rigid analogues of D-glutamic acid, Eur. J. Med. Chem. 46 (2011) 2880–2894.
[63] T. Tomašić, N. Zidar, V. Rupnik, A. Kovač, D. Blanot, S. Gobec, D. Kikelj, L.P. Mašič, Synthesis and biological evaluation of new glutamic acid-based inhibitors of MurD ligase, Bioorg. Med. Chem. Lett. 19 (2009) 153–157.
[64] N. Zidar, T. Tomašić, R. Šink, V. Rupnik, A. Kovač, S. Turk, D. Patin, D. Blanot, C. Contreras Martel, A. Dessen, M. Müller Premru, A. Zega, S. Gobec, L. Peterlin Mašič, D. Kikelj, Discovery of novel 5-benzylidenerhodanine and 5-benzylidenethiazolidine-2,4-dione inhibitors of MurD ligase, J. Med. Chem. 53 (2010) 6584–6594.
[65] T. Tomašić, N. Zidar, R. Šink, A. Kovač, D. Blanot, C. Contreras-Martel, A. Dessen, M. Müller-Premru, A. Zega, S. Gobec, D. Kikelj, L. Peterlin Mašič, Structure-based design of a new series of D-glutamic-acid-based inhibitors of bacterial UDP-*N*-acetylmuramoyl-L-alanine:D-glutamate ligase (MurD), J. Med. Chem. 54 (2011) 4600–4610.
[66] N. Zidar, T. Tomašić, R. Šink, A. Kovač, D. Patin, D. Blanot, C. Contreras-Martel, A. Dessen, M. Müller-Premru, A. Zega, S. Gobec, L.P. Mašič, D. Kikelj, New 5-benzylidenethiazolidin-4-one inhibitors of bacterial MurD ligase: design, synthesis, crystal structures, and biological evaluation, Eur. J. Med. Chem. 46 (2011) 5512–5523.
[67] T. Tomašić, A. Kovač, M. Simčič, D. Blanot, S.G. Grdadolnik, S. Gobec, D. Kikelj, L.P. Mašič, Novel 2-thioxothiazolidin-4-one inhibitors of bacterial MurD ligase targeting D-Glu- and diphosphate-binding sites, Eur. J. Med. Chem. 46 (2011) 3964–3975.
[68] T. Tomašić, N. Zidar, A. Kovač, S. Turk, M. Simčič, D. Blanot, M. Müller-Premru, M. Filipič, S.G. Grdadolnik, A. Zega, M. Anderluh, S. Gobec, D. Kikelj, L.P. Mašič, 5-Benzylidenethiazolidin-4-ones as multitarget Inhibitors of bacterial Mur ligases, ChemMedChem 5 (2010) 286–295.
[69] T. Tomašić, R. Šink, N. Zidar, A. Fic, C. Contreras-Martel, A. Dessen, D. Patin, D. Blanot, M. Müller-Premru, S. Gobec, A. Zega, D. Kikelj, L.P. Mašič, Dual inhibitor of MurD and MurE ligases from *Escherichia coli* and *Staphylococcus aureus*, ACS Med. Chem. Lett. 3 (2012) 626–630.
[70] J.A. Bertrand, G. Auger, L. Martin, E. Fanchon, D. Blanot, D. La Beller, J. van Heijenoort, O. Dideberg, Determination of the MurD mechanism through crystallographic analysis of enzyme complexes, J. Mol. Biol. 289 (1999) 579–590.
[71] H. Barreteau, I. Sosič, S. Turk, J. Humljan, T. Tomašić, N. Zidar, M. Herve, A. Boniface, L. Peterlin-Mašič, D. Kikelj, D. Mengin-Lecreulx, S. Gobec, D. Blanot, MurD enzymes from different bacteria: evaluation of inhibitors, Biochem. Pharmacol. 84 (2012) 625–632.
[72] B.Q. Zeng, K.K. Wong, D.L. Pompliano, S. Reddy, M.E. Tanner, A phosphinate inhibitor of the meso-diaminopimelic acid-adding enzyme (MurE) of peptidoglycan biosynthesis, J. Org. Chem. 63 (1998) 10081–10085.
[73] C. Paradis-Bleau, A. Lloyd, F. Sanschagrin, H. Maaroufi, T. Clarke, A. Blewett, C. Dowson, D.I. Roper, T.D.H. Bugg, R.C. Levesque, *Pseudomonas aeruginosa* MurE amide ligase: enzyme kinetics and peptide inhibitor, Biochem. J. 421 (2009) 263–272.
[74] J.D. Guzman, A. Gupta, D. Evangelopoulos, C. Basavannacharya, L.C. Pabon, E.A. Plazas, D.R. Munoz, W.A. Delgado, L.E. Cuca, W. Ribon, S. Gibbons, S. Bhakta, Anti-tubercular screening of natural products from Colombian plants: 3-methoxynordomesticine, an inhibitor of MurE ligase of *Mycobacterium tuberculosis*, J. Antimicrob. Chemother. 65 (2010) 2101–2107.
[75] W.K.P. Shiu, J.P. Malkinson, M.M. Rahman, J. Curry, P. Stapleton, M. Gunaratnam, S. Neidle, S. Mushtaq, M. Warner, D.M. Livermore, D. Evangelopoulos, C. Basavannacharya, S. Bhakta, B.D. Schindler, S.M. Seo, D. Coleman, G.W. Kaatz, S. Gibbons, A new plant-derived antibacterial is an inhibitor of efflux pumps in *Staphylococcus aureus*, Int. J. Antimicrob. Agent 42 (2013) 513–518.
[76] D.J. Miller, S.M. Hammond, D. Anderluzzi, T.D.H. Bugg, Aminoalkyl phosphinate inhibitors of D-Ala-D-Ala adding enzyme, J. Chem. Soc. Perkin Trans. 1 (1998) 131–142.
[77] K.M. Comess, M.E. Schurdak, M.J. Voorbach, M. Coen, J.D. Trumbull, H. Yang, L. Gao, H. Tang, X. Cheng, C.G. Lerner, O. McCall, D.J. Burns, B.A. Beutel, An ultraefficient affinity-based high-throughout screening process: application to bacterial cell wall biosynthesis enzyme MurF, J. Biomol. Screen. 11 (2006) 743–754.
[78] K.L. Longenecker, G.F. Stamper, P.J. Hajduk, E.H. Fry, C.G. Jakob, J.E. Harlan, R. Edalji, D.M. Bartley, K.A. Walter, L.R. Solomon, T.F. Holzman, Y.G. Gu, C.G. Lerner, B.A. Beutel, V.S. Stoll, Structure of MurF from *Streptococcus pneumoniae* co-crystallized with a small molecule inhibitor exhibits interdomain closure, Protein Sci. 14 (2005) 3039–3047.
[79] Y.G. Gu, A.S. Florjancic, R.F. Clark, T. Zhang, C.S. Cooper, D.D. Anderson, C.G. Lerner, J.O. McCall, Y. Cai, C.L. Black-Schaefer, G.F. Stamper, P.J. Hajduk, B.A. Beutel, Structure-activity relationships of novel potent MurF inhibitors, Bioorg. Med. Chem. Lett. 14 (2004) 267–270.
[80] G.F. Stamper, K.L. Longenecker, E.H. Fry, C.G. Jakob, A.S. Florjancic, Y.-G. Gu, D.D. Anderson, C.S. Cooper, T. Zhang, R.F. Clark, Y. Cia, C.L. Black-Schaefer, J. Owen McCall, C.G. Lerner, P.J. Hajduk, B.A. Beutel, V.S. Stoll, Structure-based optimization of MurF inhibitors, Chem. Biol. Drug Des. 67 (2006) 58–65.
[81] M. Hrast, S. Turk, I. Sosič, D. Knez, C.P. Randall, H. Barreteau, C. Contreras-Martel, A. Dessen, A.J. O'Neill, D. Mengin-Lecreulx, D. Blanot, S. Gobec, Structure-activity relationships of new cyanothiophene inhibitors of the essential peptidoglycan biosynthesis enzyme MurF, Eur. J. Med. Chem. 66 (2013) 32–45.
[82] M. Hrast, M. Anderluh, D. Knez, C.P. Randall, H. Barreteau, A.J. O'Neill, D. Blanot, S. Gobec, Design, synthesis and evaluation of second generation MurF inhibitors based on a cyanothiophene scaffold, Eur. J. Med. Chem. 73 (2014) 83–96.
[83] H.-L. Jiang, D.-L. Wu, X. Shen, W.-L. Zhu, D.-X. Kong, Comparison of three 3D-QSAR methods using a novel class of MurF inhibitors, J. Theor. Comput. Chem. 6 (2007) 63–80.

[84] S.A. Khedkar, A.K. Malde, E.C. Coutinho, Design of inhibitors of the MurF enzyme of *Streptococcus pneumoniae* using docking, 3D-QSAR, and *de-novo* design, J. Chem. Inf. Model. 47 (2007) 1839–1846.
[85] M.O. Taha, N. Atallah, A.G. Al-Bakri, C. Paradis-Bleau, H. Zalloum, K.S. Younis, R.C. Levesque, Discovery of new MurF inhibitors via pharmacophore modeling and QSAR analysis followed by in-silico screening, Bioorg. Med. Chem. 16 (2008) 1218–1235.
[86] I. Sosič, B. Štefane, A. Kovač, S. Turk, D. Blanot, S. Gobec, The synthesis of novel 2,4,6-trisubstituted 1,3,5-triazines: a search for potential MurF enzyme inhibitors, Heterocycles 81 (2010) 91–115.
[87] S. Turk, M. Hrast, I. Sosič, H. Barreteau, D. Mengin-Lecreulx, D. Blanot, S. Gobec, Biochemical characterization of MurF from *Streptococcus pneumoniae* and the identification of a new MurF inhibitor through ligand-based virtual screening, Acta Chim. Slov. 60 (2013) 294–299.
[88] E.Z. Baum, S.M. Crespo-Carbone, D. Abbanat, B. Foleno, A. Maden, R. Goldschmidt, K. Bush, Utility of muropeptide ligase for identification of inhibitors of the cell wall biosynthesis enzyme MurF, Antimicrob. Agents Chemother. 50 (2006) 230–236.
[89] E.Z. Baum, S.M. Crespo-Carbone, A. Klinger, B.D. Foleno, I. Turchi, M. Macielag, K. Bush, A MurF inhibitor that disrupts cell-wall biosynthesis in *Escherichia coli*, Antimicrob. Agents Chemother. 51 (2007) 4420–4426.
[90] E.Z. Baum, S.M. Crespo-Carbone, B.D. Foleno, L.D. Simon, J. Guillemont, M. Macielag, K. Bush, MurF Inhibitors with antibacterial activity: effect on muropeptide levels, Antimicrob. Agents Chemother. 53 (2009) 3240–3247.
[91] L.L. Silver, Multi-targeting by monotherapeutic antibacterials, Nat. Rev. Drug Discov. 6 (2007) 41–55.
[92] L.L. Silver, Polypharmacology as an emerging trend in antibacterial discovery, in: Polypharmacology in Drug Discovery, John Wiley & Sons Inc., 2012, pp. 167–202.
[93] Z. Li, G.D. Francisco, W. Hu, P. Labthavikul, P.J. Petersen, A. Severin, G. Singh, Y. Yang, B.A. Rasmussen, Y.-I. Lin, J.S. Skotnicki, T.S. Mansour, 2-Phenyl-5,6-dihydro-2H-thieno[3,2-c]pyrazol-3-ol derivatives as new inhibitors of bacterial cell wall biosynthesis, Bioorg. Med. Chem. Lett. 13 (2003) 2591–2594.
[94] S. Antane, C.E. Caufield, W. Hu, D. Keeney, P. Labthavikul, K. Morris, S.M. Naughton, P.J. Petersen, B.A. Rasmussen, G. Singh, Y. Yang, Pulvinones as bacterial cell wall biosynthesis inhibitors, Bioorg. Med. Chem. Lett. 16 (2006) 176–180.
[95] T.S. Mansour, C.E. Caufield, B. Rasmussen, R. Chopra, G. Krishnamurthy, K.M. Morris, K. Svenson, J. Bard, C. Smeltzer, S. Naughton, S. Antane, Y. Yang, A. Severin, D. Quagliato, P.J. Petersen, G. Singh, Naphthyl tetronic acids as multi-target inhibitors of bacterial peptidoglycan biosynthesis, ChemMedChem 2 (2007) 1414–1417.
[96] M. Sova, A. Kovač, S. Turk, M. Hrast, D. Blanot, S. Gobec, Phosphorylated hydroxyethylamines as novel inhibitors of the bacterial cell wall biosynthesis enzymes MurC to MurF, Bioorg. Chem. 37 (2009) 217–222.
[97] R.P. Bax, R. Anderson, J. Crew, P. Fletcher, T. Johnson, E. Kaplan, B. Knaus, K. Kristinsson, M. Malek, L. Strandberg, Antibiotic resistance - what can we do?, Nat Med. 4 (1998) 545–546.
[98] A.J. Alanis, Resistance to antibiotics: are we in the post-antibiotic era?, Arch Med. Res. 36 (2005) 697–705.
[99] S.J. Projan, P.J. Youngman, Antimicrobials: new solutions badly needed, Curr. Opin. Microbiol. 5 (2002) 463–465.
[100] L.L. Silver, Challenges of antibacterial discovery, Clin. Microbiol. Rev. 24 (2011) 71–109.
[101] I. Chopra, The 2012 Garrod Lecture: Discovery of antibacterial drugs in the 21st century, J. Antimicrob. Chemother. 68 (2013) 496–505.