Scientific paper

Biochemical Characterization of MurF from *Streptococcus pneumoniae* and the Identification of a New MurF Inhibitor Through Ligand-based Virtual Screening

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Abstract

MurF is an essential bacterial enzyme that is involved in the last intracellular stage of peptidoglycan biosynthesis, and therefore it has the potential to be exploited as a target for the development of new antibacterials. Here, we report on the expression, purification and biochemical characterization of MurF from an important pathogen, *Streptococcus pneumoniae*. Additionally, ligand-based virtual screening was successfully used and a new hit compound with micromolar inhibitory activities against MurF enzymes from *S. pneumoniae* and *Escherichia coli* was identified.

Keywords: MurF, peptidoglycan, *Streptococcus pneumoniae*, ligand-based virtual screening

1. Introduction

The bacterial cell wall is a vital and essential structure as it provides structural support and protection from the outside environment, and it functions as a semi-permeable filter. Enzymes involved in cell-wall biosynthesis have long been successfully exploited as drug targets, and there are several classes of antibacterials that target these enzymes in clinical use. Unfortunately, resistance to those agents has become an ever-increasing problem.[1,2] Broad awareness of the growing resistance has spurred several different strategies to fight the arms race against bacteria. One of those strategies is to exploit new targets, and this is where MurF comes in.[3] MurF is an essential, widely conserved enzyme that is involved in the last intracellular stage of biosynthesis of bacterial peptidoglycan.[4,5] MurF catalyzes the addition of D-Ala-D-Ala to a UDP-Mur*N*Ac-tripeptide (UMtri), which is UDP-Mur*N*Ac-L-Ala-γ-D-Glu-L-Lys (UMtri-L-Lys) or UDP-Mur*N*Ac-L-Ala-γ-D-Glu-*meso*-DAP (UMtri-mDAP) in Gram-positive bacteria and Gram-negative bacteria, respectively.[4,5] The MurF enzymes from several bacterial species have been isolated and characterized;[6–8] moreover, several attempts to design inhibitors of these enzymes have been made.[9–14] The best inhibitors of MurF were reported by Abbott (Compound **1**, Figure 1) and were designed for the inhibition of MurF from *Streptococcus pneumoniae* ($MurF_{Sp}$). The published IC_{50} for compound **1** is 1 μM.[10,11] While the authors reported the expression and purification of $MurF_{Sp}$, they neglected to report if any attempt of characterization of MurF from *S. pneumoniae* was carried out.[10]

Virtual screening has become a useful method for rapid identification of hit compounds,[15–18] and it has had many successful applications in the discovery and design of antibacterials.[19] Three-dimensional (3D) ligand-based virtual screening methods are one of the fastest methodologies here, and if the starting compound (the 'query') is carefully selected, this can provide surprisingly good results.[17] Combining fast ligand-based methods with a vast resource of commercially available compounds in the

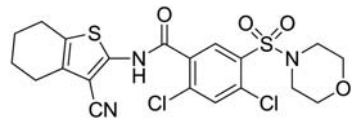

Figure 1. Compound **1**, a representative $MurF_{Sp}$ inhibitor identified by Abbott.

form of the constantly updated ZINC database[20] thus enables rapid exploration of the continuously expanding chemical space.

Herein, we report in detail the isolation and characterization of MurF from *S. pneumoniae*. Furthermore, we also report on a successfully used 3D ligand-based virtual screening campaign, which yielded a new micromolar inhibitor of two enzymes, MurF from *S. pneumoniae* and MurF from *Escherichia coli* ($MurF_{Ec}$).

2. Experimental

2. 1. Expression, Purification and Characterization of *S. pneumoniae* MurF

Materials. DNA restriction enzymes and synthetic oligonucleotides were purchased from New England Biolabs and Eurofins-MWG, respectively.

Bacterial strains and growth conditions. E. coli DH5α was used as the host for plasmids, and *E. coli* BL21(DE3)(pLysS) was used for the overproduction of the $MurF_{Sp}$ enzyme. The construction of the pET2160 vector from pET21d (Novagen) was as described previously.[21] The 2YT medium[22] was used for growing the cells, and their growth was followed by monitoring the optical density of the cultures at 600 nm (OD_{600}) using a Shimadzu UV-1601 spectrophotometer. Ampicillin and chloramphenicol were used at 100 μg · mL^{-1} and 25 μg · mL^{-1}, respectively.

DNA manipulation. PCR amplification of the *mur*F_{Sp} gene was performed in a Thermocycler 60 PCR machine (Bio-med) using Expand high-fidelity DNA polymerase. DNA fragments were purified using Wizard PCR preps DNA purification kits (Promega), and standard procedures were used for DNA digestion, ligation and agarose gel electrophoresis.[23] Plasmid isolation was carried out using kits purchased from Macherey-Nagel. *E. coli* cells were transformed with plasmid DNA by electroporation.

The MurF expression plasmid was constructed as follows. The *murF*$_{Sp}$ gene was amplified from the chromosome of the *S. pneumoniae* R6 strain by PCR using the primers SpnO3 (5'-CGCG**TCATGA**AATTAACAATC-CATGAAATTGCC-3') and SpnO4 (5'-CGCG**AGATCT-**CTTGTCTTCATTTTCTAAACTTTCTACCAACTTG-GC-3'), which were designed to incorporate BspH1 and BglII sites (in bold) at the 5' and 3' extremities of the gene (initiation codon underlined), respectively. The resulting fragment was digested with BspH1 and BglII and ligated between the compatible NcoI and BglII sites of the vector pET2160. The resulting plasmid, pET2160::*murF*$_{Sp}$, allowed expression of the corresponding protein with an Arg-Ser-His_6 C-terminal extension. DNA sequencing was performed to confirm the sequence of the cloned fragment.

Overproduction and purification of MurF$_{Sp}$. *E. coli* BL21(DE3)(pLysS) cells carrying the pET2160::*murF*$_{Sp}$ plasmid were grown at 37 °C in 2YT medium (1.0 L cultures) containing ampicillin and chloramphenicol. When the OD_{600} of the culture reached 0.8, isopropyl-β-D-thiogalactopyranoside was added at a final concentration of 1 mM and the incubation was continued overnight at 37 °C, with shaking. The cells were harvested at 4 °C and washed with cold 20 mM phosphate buffer, pH 7.2, containing 1 mM dithiothreitol (DTT) (buffer A). The cell pellet was resuspended in buffer A (12 mL), and the cells were disrupted by sonication in the cold (Bioblock Vibracell sonicator; model 72412). The resulting suspension was centrifuged at 4 °C for 30 min at 200,000 × *g* in a Beckman TL100 centrifuge, and the pellet was discarded. The supernatant was stored at –20 °C.

$MurF_{Sp}$ was purified on Ni^{2+}-nitrilotriacetate (Ni-NTA)-agarose following the manufacturer recommendations (Qiagen). Crude protein extracts were mixed for 1 h at 4 °C with the polymer pre-equilibrated in buffer A containing 300 mM KCl and 1 mM DTT. The washing and elution steps were performed with a discontinuous gradient of imidazole (20 mM to 200 mM) in buffer A containing 300 mM KCl and 1 mM DTT. $MurF_{Sp}$ was eluted with 200 mM imidazole, as judged by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The relevant fractions were pooled, concentrated by ultrafiltration (30,000 Amicon Ultra-15 centrifugal filter; Millipore) and dialyzed overnight against 100 volumes of buffer consisting of 20 mM Hepes, pH 7.4, 200 mM NaCl, 5 mM DTT and 0.05% NaN_3. The final preparations were stored at –20 °C after the addition of glycerol (10% final concentration).

SDS-PAGE analysis of the proteins was performed by the method of Laemmli and Favre.[24] Protein concentrations were determined by the method of Bradford,[25] with bovine serum albumin as the standard, or by amino-acid analysis with a Hitachi L8800 analyzer (ScienceTec), after hydrolysis of the samples in 6 M HCl at 105 °C for 24 h.

MurF$_{Sp}$ *characterization.* For the determination of the kinetic constants of $MurF_{Sp}$, the Malachite green method was used,[26] which detects the orthophosphate generated during the reaction. The mixture, with a final volume of 50 μL, contained 50 mM Hepes, pH 8.0, 50 mM $MgCl_2$, 0.005% Triton X-114, 600 μM D-Ala-D-Ala, 200 μM UMtri-L-Lys, 250 μM ATP and purified MurF from *S. pneumoniae* diluted in 50 mM Hepes and 5 mM DTT. The reaction mixture was incubated at 37 °C for 10 min and then quenched with 100 μL Biomol® reagent. The absorbance was read at 650 nm after 5 min. All of the experiments were run in duplicate. Identical assay conditions were used when the nucleotide precursor was UMtri-m-DAP. All of the initial velocity experiments were performed under these assay conditions using different concentrations of one substrate and fixed concentrations of the others. The data were fitted to the Michaelis equation, $v = V_{max}S/(K_m + S)$.

Inhibition assay. The compounds were tested for inhibition of the addition of D-Ala-D-Ala to either UMtri-L-Lys or UMtri-mDAP catalyzed by $MurF_{Sp}$ or $MurF_{Ec}$, respectively. The final volume of the mixture was 50 μL and it contained:

***S. pneumoniae* MurF:** 50 mM Hepes, pH 8.0, 50 mM $MgCl_2$, 0.005% Triton X-114, 100 μM D-Ala-D-Ala, 50 μM UMtri-L-Lys, 250 μM ATP, purified $MurF_{Sp}$ and 5% of either DMSO (assay control) or compound dissolved in DMSO.

***E. coli* MurF:** 50 mM Hepes, pH 8.0, 50 mM $MgCl_2$, 0.005% Triton X-114, 600 μM D-Ala-D-Ala, 100 μM UMtri-mDAP, 500 μM ATP, purified $MurF_{Ec}$[27] and 5% of either DMSO (assay control) or compound dissolved in DMSO.

The mixtures were incubated at 37 °C for 15 min and then quenched with 100 μL Biomol®reagent. After 5 min, the absorbance at 650 nm was measured. All of the experiments were run in duplicate. Residual activities (RAs) were calculated with respect to the assay control. The IC_{50} values were determined by measuring the residual activities at seven different concentrations.

2. 2. Computational

Virtual screening. Virtual screening was performed on a HP workstation with two quad core Intel Xeon 2.2 GHz processors, 8 GB of RAM, 320 GB and 1 TB hard drives, and a Nvidia Quadro FX 4800 graphic card, and it was running the current version of 64-bit Arch Linux.

The compound library for virtual screening was downloaded from the ZINC database.[20] The ZINC drug-like subset, which at the time contained 11 million compounds, was selected and downloaded in sdf form. The compound library was prepared with the Omega program (OpenEye Scientific Software Inc.)[28] to yield on average 152 different conformations per compound.

Ligand-based virtual screening was performed with ROCS (OpenEye Scientific Software Inc.)[29] using compound **1**, identified by Abbott, as a query. The conformation of compound **1** was extracted from the co-crystal structure of $MurF_{Sp}$ with compound **1** (PDB code 2AM1).[10] The previously prepared compound library of 11 million compounds with 152 conformations on average per compound was screened and the compounds were ranked according to the TanimotoCombo score. Forty of the highest ranked and available compounds were obtained and evaluated *in vitro*.

Molecular docking. The three-dimensional structures of compound **2** used for the docking experiments was prepared with OMEGA (OpenEye Scientific Software, Inc.).[28] Two hundred conformations of compound **2** were generated. FRED_receptor was used for the preparation of the MurF active site from the *S. pneumoniae* (PDB code 2AM1)[10] active site. The active site was defined as a box around the co-crystallized ligand with a volume of 5652 Å^3. Molecular probe was used for site detection. The volumes for the inner and outer contours were 78 Å^3 and 1578 Å^3, respectively. Chemgauss3 scoring function was used for the exhaustive search and optimization. Validation was done by re-docking of the co-crystallized ligand.

3. Results and Discussion

3. 1. Expression, Purification and Characterization of *S. pneumoniae* MurF

The $murF_{Sp}$ gene was inserted into the vector pET2160. The resulting plasmid pET2160::$murF_{Sp}$ enabled the expression of the corresponding protein with an Arg-Ser-His_6 C-terminal extension. The *E. coli* BL21(DE3) (pLysS) strain carrying the pET2160::$murF_{Sp}$ gene was used for the overproduction of the $MurF_{Sp}$ protein. The protein was purified on Ni-NTA-agarose and the relevant fractions were pooled, as judged by SDS-PAGE. This resulted in 11.5 mg enzyme per litre of culture. MALDI-TOF mass spectrometry analysis revealed peaks at *m/z* 25,805 and 51,617 for the $[M+2H]^{2+}$ and $[M+H]^+$ ions, respectively, which is in agreement with the calculated value (M_r 51,569).

A detailed investigation of the $MurF_{Sp}$ kinetic parameters was performed. The pH in all of the assays was set at 8, given the optimal pH range for the Mur ligases has been reported to be from 8 to 9.2.[30] The enzyme activity was measured indirectly by detecting orthophosphate generated during the reaction with Malachite green.[26] Initial velocity measurements were performed while keeping two of the substrates at constant saturating concentrations and varying the concentration of the third. Based on these experiments, the K_m values for UMtri-L-Lys, D-Ala-D-Ala and ATP were determined as 40 μM, 86 μM and 69 μM, respectively. The V_{max} of purified $MurF_{Sp}$ was 38 μmol min^{-1} mg^{-1}.

The maximum velocity for $MurF_{Sp}$ is between that of MurF from *Staphylococcus aureus* ($MurF_{Sa}$) (71 μmol min^{-1} mg^{-1})[8] and that of $MurF_{Ec}$ (16 μmol min^{-1} mg^{-1}).[6] The K_m values for ATP and their respective UDP-Mur*N*Ac-tripeptide were similar among all of these MurF orthologs, although the K_m for D-Ala-D-Ala was almost three-fold lower for $MurF_{Sp}$. As in the case of $MurF_{Sa}$,[8] but contrary to $MurF_{Ec}$,[31] no substrate inhibition by UDP-Mur*N*Ac-tripeptide was observed. Interestingly, there was a perceivable difference in the K_m values for both forms of the nucleotide precursor (L-Lys and *meso*-DAP); the K_m for UMtri-mDAP was estimated to be 240 μM, thereby showing that $MurF_{Sp}$ discriminates between these substrates, which was not observed for $MurF_{Ec}$.[31] Discrimination between these two forms of the UDP-Mur*N*Ac-tripeptide has previously been reported for MurF from *Chlamydia trachomatis*, with the preference being for UMtri-mDAP over UMtri-L-Lys.[7]

3. 2. Virtual Screening and Biochemical Evaluation of Hits

Three-dimensional, ligand-based, virtual screening was used to screen commercially available compounds for hit compounds with similar shapes, volumes and distributions of atom types (dubbed color in the OpenEye programs) to compound **1**. A drug-like subset that contained 11 million compounds at the time, was downloaded from the ZINC library.[20] To cover the conformational space of the compounds, the virtual library was pre-processed using the Omega program (OpenEye Scientific Software Inc.),[28] which yielded an average of 152 conformations per compound. Compound **1** was used as a query. The selection of the conformation of query compound is of great importance in 3D ligand-based virtual screening, and ideally this should be an active conformation.[17] Fortunately, compound **1** was co-crystallized with $MurF_{Sp}$ and so we were able to extract the bioactive conformation from the co-crystal structure (PDB code 2AM1)[10] and use it as a query. The ROCS program (OpenEye Scientific Software Inc.)[29] was used for the ligand-based virtual screening. Briefly, ROCS overlays screened compounds to the query structure and calculates shape similarities and the similarity of distribution of the atom types (color). Both similarities are calculated as Tanimoto indices. TanimotoCombo, as used here, is simply a sum of both the shape and color Tanimoto indices. Compounds from the virtual library were ranked according to the TanimotoCombo index, and subsequently 40 of highest ranked and available compounds were purchased and biochemically evaluated (Supplementary Information, Table 1) for inhibition of $MurF_{Sp}$ and $MurF_{Ec}$.

The results of the $MurF_{Sp}$ characterization were considered when setting up the assay, and the following concentrations of substrates were chosen: 100 μM D-Ala-D-Ala, 50 μM UMtri-L-Lys, and 250 μM ATP. The $MurF_{Ec}$ purification as well as assay were described previously.[12,27] To reduce the chance of false positive results due to compound aggregation, a surfactant was used in all of the assays (0.005% Triton X-114).[32] All 40 compounds were assayed for enzyme inhibition of both $MurF_{Sp}$ and $MurF_{Ec}$, although only compound **2** (3-((4-chloro-1*H*-pyrazol-1-yl)methoxy)-*N*-(3-cyano-5,6-dihydro-4*H*-cyclopenta[b]thiophen-2-yl)benzamide) showed activity on both. The IC_{50} values were 126 μM and 56 μM for $MurF_{Sp}$ and $MurF_{Ec}$, respectively.

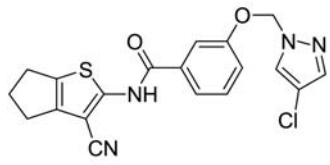

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Figure 2. Structure of compound **2**

Not surprisingly, compound **2** shares certain structural elements with query compound **1**. Both compounds have a three-ring system that is connected with short linkers. The 3-cyano-4,5,6,7-tetrahydrobenzo[b]thiophene from compound **1** is replaced with 3-cyano-5,6-dihydro-4H-cyclopenta[b]thiophene in compound **2**. In both compounds, the amide bond connects the thiophene with the phenyl moiety, which is dichloro-substituted in the case of compound **1**. The phenyl moiety is further connected via sulfonamide to morpholine in compound **1**, or via ether to chloropyrazole in compound **2**. Nonetheless, in spite of some structural similarity between these compounds, compound **2** expands the potentially useful chemical space of the cyanothiophene-type of MurF inhibitors.

To improve our understanding of the binding mode of compound **2**, it was docked into the $MurF_{Sp}$ active site (PDB code 2AM1; Figure 3), using the FRED program (OpenEye Scientific Software, Inc).[33] The active site was defined as a box around the co-crystallized compound **1**, with a volume of 5652 $Å^3$. The docking protocol was validated with successful re-docking of the co-crystallized compound **1**. The predicted binding pose was similar to that of the co-crystallized inhibitor **1**, as expected. For compound **2**, it was predicted to occupy the same part of the active site as compound **1**, with the three-ring systems of both compounds overlapping. In both cases, the nitrile moiety formed two H-bonds with the backbone nitrogens of Ala48 and Arg49. The amide nitrogen, which is also common to both structures, formed an H-bond with Thr330. The last shared structural element, the phenyl moiety, formed π-stacking interactions with Phe31. For the rest of compound **2**, no other interactions were predicted, except weak Van der Waals bonds. In contrast, one of the sulfonamide oxygens of compound **1** forms two H-bonds, one with Asn326 and the other with Asn328. Moreover, the morpholine oxygen of compound **1** forms a weak H-bond with the backbone

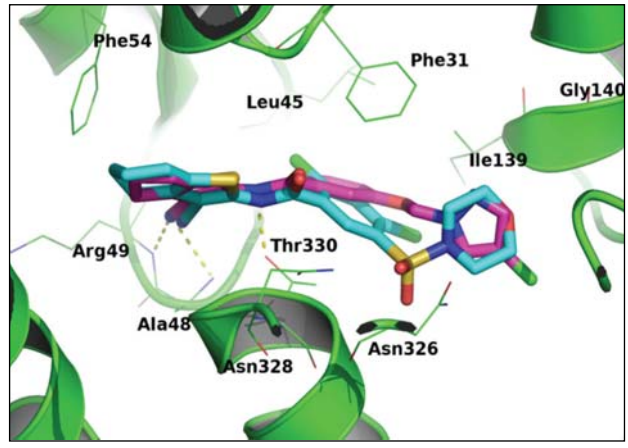

Figure 3. The predicted binding pose of compound **2** (dark grey). The co-crystallized compound **1** is shown as grey sticks, the relevant residues of $MurF_{Sp}$ active site are shown as lines.

nitrogen of Gly140.[10] The loss of three H-bonds might explain the somewhat higher IC_{50} of compound **2** compared to compound **1**.

4. Conclusions

MurF from *S. pneumoniae* was successfully expressed, purified and subsequently characterized. The kinetic parameters were determined: the K_m values for UMtri-L-Lys, D-Ala-D-Ala and ATP were 40 μM, 86 μM and 69 μM, respectively. These data extend our understanding of the MurF enzymes, and together with previous knowledge, this should facilitate the design of MurF inhibitors with potential broad-spectrum antibacterial activity. Additionally, it enabled us to set up an assay for inhibitor screening, with 40 compounds resulting from the ligand-based virtual screening campaign. These were assayed *in vitro*, and out of the 40 assayed compounds, compound **2** was identified as a hit compound. It showed micromolar inhibitory activities against $MurF_{Sp}$ and $MurF_{Ec}$, with IC_{50} values of 126 μM and 56 μM, respectively. Similar to the query compound **1**, compound **2** is a cyanothiophene-type MurF inhibitor, although it has different substituents, and so it further extends the potentially useful chemical space of this type of MurF inhibitors. Compound **2** thus represents a promising starting point for further development of new broad-spectrum antibacterials.

5. Acknowledgements

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6. Supporting Information

Complete results of biochemical evaluation of virtual hits and ^{1}H NMR spectrum of active compound.

7. References

1. I. Chopra, C. Schofield, M. Everett, A. O'Neill, K. Miller, M. Wilcox, J.-M. Frère, M. Dawson, L. Czaplewski, U. Urleb, and P. Courvalin, *Lancet Infect. Dis.*, **2008**, *8*, 133–139.
2. R. P. Wenzel and M. B. Edmond, *N. Engl. J. Med.*, **2000**, *343*, 1961–1963.
3. R. C. Moellering, *Int. J. Antimicrob. Agents*, **2011**, *37*, 2–9.
4. J. van Heijenoort, *Nat. Prod. Rep.*, **2001**, *18*, 503–519.
5. H. Barreteau, A. Kovač, A. Boniface, M. Sova, S. Gobec, and D. Blanot, *FEMS Microbiol. Rev.*, **2008**, *32*, 168–207.
6. K. Duncan, J. van Heijenoort, and C. T. Walsh, *Biochemistry*, **1990**, *29*, 2379–2386.
7. D. Patin, J. Bostock, I. Chopra, D. Mengin-Lecreulx, and D. Blanot, *Arch. Microbiol.*, **2012**, *194*, 505–512.
8. D. Patin, A. Boniface, A. Kovač, M. Hervé, S. Dementin, H. Barreteau, D. Mengin-Lecreulx, and D. Blanot, *Biochimie*, **2010**, *92*, 1793–1800.
9. 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, and B. A. Beutel, *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 267–270.
10. 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, and V. S. Stoll, *Protein Sci.*, **2005**, *14*, 3039–3047.
11. 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, and V. S. Stoll, *Chem. Biol. Drug. Des.*, **2006**, *67*, 58–65.
12. S. Turk, A. Kovač, A. Boniface, J. M. Bostock, I. Chopra, D. Blanot, and S. Gobec, *Bioorg. Med. Chem.*, **2009**, *17*, 1884–1889.
13. E. Z. Baum, S. M. Crespo-Carbone, B. D. Foleno, L. D. Simon, J. Guillemont, M. Macielag, and K. Bush, *Antimicrob. Agents Chemother.*, **2009**, *53*, 3240–3247.
14. M. Sova, A. Kovač, S. Turk, M. Hrast, D. Blanot, and S. Gobec, *Bioorg. Chem.*, **2009**, *37*, 217–222.
15. D. Krüger and A. Evers, *ChemMedChem*, **2010**, *5*, 148–158.
16. H. Geppert, M. Vogt, and J. Bajorath, *J. Chem. Inf. Model.*, **2010**, *50*, 205–216.
17. A. Nicholls, G. B. McGaughey, R. P. Sheridan, A. C. Good, G. Warren, M. Mathieu, S. W. Muchmore, S. P. Brown, J. A. Grant, J. A. Haigh, N. Nevins, A. N. Jain, and B. Kelley, *J. Med. Chem.*, **2010**, *53*, 3862–3886.
18. P. Ripphausen, B. Nisius, L. Peltason, and J. Bajorath, *J. Med. Chem.,* **2010,** *53,* 8461–8467.
19. K. J. Simmons, I. Chopra, and C. W. G. Fishwick, *Nat. Rev. Microbiol.*, **2010**, *8*, 501–510.
20. J. J. Irwin, T. Sterling, M. M. Mysinger, E. S. Bolstad, and R. G. Coleman, *J. Chem. Inf. Model.,* **2012**. *52,* 1757–1768.
21. H. Barreteau, A. Bouhss, M. Fourgeaud, J.-L. Mainardi, T. Touzé, F. Gérard, D. Blanot, M. Arthur, and D. Mengin-Lecreulx, *J. Bacteriol.*, **2009**, *191*, 3657–3664.
22. J. H. Miller, *Experiments in molecular genetics*, Cold Spring Harbor Laboratory Press, **1972**.
23. J. Sambrook, E. F. Fritsch, and T. Maniatis, *Molecular cloning: a laboratory manual,* 2nd edn., Cold Spring Harbor Laboratory Press, **1989**.
24. U. K. Laemmli and M. Favre, *J. Mol. Biol.*, **1973**, *80*, 575–599.
25. M. M. Bradford, *Anal. Biochem.*, **1976**, *72*, 248–254.

26. P. A. Lanzetta, L. J. Alvarez, P. S. Reinach, and O. A. Candia, *Anal. Biochem.*, **1979**, *100*, 95–97.
27. S. Dementin, A. Bouhss, G. Auger, C. Parquet, D. Mengin-Lecreulx, O. Dideberg, J. van Heijenoort, and D. Blanot, *Eur. J. Biochem.*, **2001**, *268*, 5800–5807.
28. J. Boström, J. R. Greenwood, and J. Gottfries, *J. Mol. Graph. Model.*, **2003**, *21*, 449–462.
29. J. A. Grant, M. A. Gallardo, and B. T. Pickup, *J. Comput. Chem.*, **1996**, *17*, 1653–1666.
30. D. Patin, J. Bostock, D. Blanot, D. Mengin-Lecreulx, and I. Chopra, *J. Bacteriol.*, **2009**, *191*, 7430–7435.
31. M. S. Anderson, S. S. Eveland, H. R. Onishi, and D. L. Pompliano, *Biochemistry*, **1996**, *35*, 16264–16269.
32. B. K. Shoichet, *J. Med. Chem*, **2006**, *49*, 7274–7277.
33. M. McGann, *J. Chem. Inf. Model.*, **2011**, *5*1, 578–596.

Povzetek

MurF je esencialen bakterijski encim, ki sodeluje pri zadnji znotrajcelični stopnji biosinteze peptidoglikana in je kot tak obetavna tarča za razvoj novih protibakterijskih učinkovin. V tem prispevku poročamo o uspešni ekspresiji, izolaciji in biokemijski karakterizaciji encima MurF iz pomembnega patogenega seva *Streptococcus pneumoniae*. Poleg tega smo tudi uspešno uporabili virtualno rešetanje na osnovi liganda in odkrili novo spojino zadetek z zaviralno aktivnostjo v mikromolarnem območju na encima MurF iz *S. pneumoniae* in *Escherichia coli*.