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REVIEW

Protein kinase PknB as a promising target for the development of antibacterial drugs toward *Staphylococcus aureus*

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Abstract: Antimicrobial resistance is one of this century's most serious global public health threats. Uncontrolled use of antibiotics has led to extensively drug-resistant bacterial strains which are non-susceptible to almost all currently known antimicrobial drugs. *Staphylococcus aureus* is the pathogen of most significant concern in the clinic worldwide because it colonized approximately 30% of the human population. Therefore, the search for new antistaphylococcal drugs is an urgent need of mankind. In prokaryotic cells, a number of cellular processes are regulated by serine/threonine protein kinases. In particular, the protein kinase PknB is a key component of the signaling network of bacterial cells, which can be considered as a promising molecular target for the search for new antibacterial drugs. In this review we focus on the structure, functions and mechanisms of PknB activity regulation. We also summarized recent research results of its inhibitors search.

Keywords: antimicrobial resistance; antibacterial drugs; *Staphylococcus aureus*; protein kinase PknB; inhibition.

Introduction

Antibiotic resistance is one of the biggest challenges in modern medicine. Uncontrolled use of antibiotics has led to the emergence of multidrug and extensively drug-resistant bacterial strains which are non-susceptible to almost all currently known antimicrobial drugs. Unfortunately, only a few novel antibacterial drugs have been developed in recent decades.

Approximately 30% of the human population is colonized by *Staphylococcus aureus* [1]. *S. aureus* is an opportunistic pathogen which is normally found on the skin and mucous membranes of the upper respiratory tract. *S. aureus* is a causative pathogen for a number of human diseases ranging from minor skin infections to life-threatening bacteremia, sepsis, and meningitis [2]. Unfortunately, the treatment of staphylococcal infections is complicated due to the ability of *S. aureus* to produce antibiotic-neutralizing enzymes [3]. Today, methicillin- (MRSA) and vancomycin-resistant (VRSA) *S. aureus* strains are very widespread in the world and become serious medical and public problem. For example, in 2019, more than 1 million people died from infections caused by antibiotic-resistant *S. aureus* [5]. Therefore, the search of novel antistaphylococcal agents with unexploited mechanisms of action is of urgent need.

The serine/threonine protein kinase PknB is involved in a number of important signaling pathways of *S. aureus*, such as cell wall metabolism, antibiotic susceptibility, and virulence regulation [6]. Taking into account that protein kinase PknB is a key component of the bacterial cell signaling network involved in a number of important biological processes, this enzyme can be considered as a promising molecular target for the search of novel inhibitors as antibacterial agents [7]. In this review we analyzed the current data on the structure, mechanisms of PknB activity regulation and functions, and also summarized the results of inhibitors search.

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Structure of *S. aureus* PknB

Protein phosphorylation is one of the key regulatory mechanisms in bacteria that controls major metabolic pathways, cell wall biosynthesis, production of various toxins, expression of virulence factors, biofilm formation, antibiotic resistance, etc. Usually, extracellular signals are transformed into cellular responses by regulating the activity of the corresponding proteins depending on the phosphorylation of specific amino acid residues sites containing serine, threonine or tyrosine [8].

S. aureus encodes an eukaryotic-type serine/threonine protein kinase, PknB (Stk1), which plays an important role in cell wall metabolism, virulence, and drug resistance. Depending on the presence of arginine amino acid residue, which precedes the conserved catalytic aspartic acid, serine/threonine protein kinases can be classified into RD and non-RD kinases. PknB belongs to RD family kinase and contains N-terminal intracellular kinase domain, hydrophobic transmembrane domain, three extracellular PASTA domains (for penicillin-binding protein and serine/threonine kinase-associated domains) and an Ig-like domain at the C-terminus. PASTA domains consist of ~65 amino acids and are considered to be sensory motives for the binding of β-lactam compounds as well as cell wall fragments (e.g., peptidoglycans) [9]. PknB is of particular importance for the survival and pathogenesis of *S. aureus*, since this protein kinase regulates purine biosynthesis, autolysis, and other central metabolic processes of the bacterium and is involved in the development of antibiotic resistance [7].

Interestingly, deletion of *pknb* leads to β-lactam antibiotics susceptibility of MRSA, indicating that PknB may be a potential target for combination therapy [10, 11].

Regulation of PknB activity in *S. aureus*

The regulation of cellular processes by the protein kinase PknB and its corresponding phosphatase Stp is widespread in bacteria, in particular in *S. aureus* [12].

The protein kinase PknB possesses six physiological phosphorylation sites (Ser159, Thr161, Ser162, Thr164, Thr166, and Thr172) which are required for the regulation of catalytic activity. This means that differential phosphorylation of the activation loop regulates the kinase activity of PknB. Interestingly, it was found that the replacement of Thr172 in the GT/S motif with Ala or Asp leads to the formation of non-functional form of this protein kinase, indicating that the phosphorylation of Thr172 is crucial for PknB activation. The protein kinase PknB uses a cis-autophosphorylation mechanism to transfer phosphate from ATP to Thr172. Other serines/threonines located in the activation loop are phosphorylated by the *trans*-autophosphorylation mechanism. If Thr172 is not phosphorylated, phosphorylation of other residues in the activation loop does not affect the kinase activity of PknB, which confirms that phosphorylation of Thr172 is the first step for PknB activation [9].

A model of activation of the protein kinase PknB was proposed. In the inactive state, the unphosphorylated activation loop is folded into the catalytic cleft, mimicking a substrate, which prevents substrate binding. Under external stimulation, ATP binding triggers *cis*-autophosphorylation of Thr172, changing the conformation of the activation loop, which leads to PknB activation. Subsequent interaction with other PknB molecule induces trans-autophosphorylation of other residues in the activation loop to support optimal kinase activity. The mutation of Thr172 results in a complete loss of kinase activity, regardless of the phosphorylation state of other residues in the activation loop. The related phosphatase Stp dephosphorylates protein kinase PknB, restoring the inactive, unphosphorylated kinase [9].

Therefore, the phosphorylation of Thr172 of the GT/S motif in the activation loop is necessary for *S. aureus* PknB activation.

Taking into account high sequence variability in the activation loop of bacterial serine/threonine protein kinases, the understanding of PknB autophosphorylation mechanisms has practical implications for the rational design of selective and effective PknB inhibitors to combat antibiotic-resistant *S. aureus* [9].

Involvement of PknB in the formation of *S. aureus* virulence

Bacteria adapt to different environments to survive in changing conditions, and, accordingly, they have a number of protein kinases involved in signal recognition and transduction [13]. The most known signaling cascades in bacteria are two-component systems, which generally consist of a membrane histidine kinase that senses an extracellular signal, autophosphorylates histidine residue, and transfers the phosphate group to an aspartate residue of downstream regulator or transcription factor [14]. Serine/threonine phosphorylation, which is the main mechanism of cellular function regulation in eukaryotes, was identified in bacteria much later and quickly became an object of great interest due to its involvement in virulence [15]. Serine/threonine kinases were described in a number of bacteria and regulate a wide range of bacterial functions, including glycolysis, protein translation, sporulation, and in pathogenic bacteria, virulence and antibiotic resistance [16].

The serine/threonine protein kinase PknB and phosphatase Stp were characterized in *S. aureus*, a bacterium which adapts well to different conditions and quickly develops resistance to many antibiotics. Deletion and overexpression strains of PknB and Stp were used to study the functions of corresponding enzymes in experiments *in vitro* and *in vivo* [11].

A recent study has demonstrated that serine/threonine phosphorylation is involved in *S. aureus* metabolism regulation which allows bacteria grow on different substrates. A detailed metabolic model of this metabolic adaptation was constructed and quantitatively validated with an independent experimental transcriptomic dataset

from *S. aureus* strain. According to this hypothesis PknB and its close association with the glmR regulator and the cdaA operon supports a stable glucose flux, improved synthesis of aromatic amino acids, nucleotides and peptidoglycan. Also, PknB is important for cell wall homeostasis by activating central virulence regulators (AgrABCD, ArlS, SaeRS, SarA, and MgrA), which affects virulence and antibiotic resistance [7].

Therefore, taking into account the role of PknB in virulence, this protein kinase can be considered as a potential therapeutic target for the treatment of *S. aureus* infections.

Role of PknB in the regulation of *S. aureus* growth and antibiotic resistance

Despite the fact that *S. aureus* is a usual member of the microbiota in healthy persons, it can cause a wide range of diseases. In addition to skin infections, *S. aureus* causes life-threatening invasive disorders such as bacteremia, endocarditis, and osteomyelitis [17]. The development of bacterial resistance to a number of antibiotics, such as penicillin, methicillin, and vancomycin, makes these infections quite difficult to treat [18]. Most antibiotics are effective toward metabolically active bacteria. Thus, bacteria with reduced energy consumption, which leads to metabolic dormancy, have increased resistance to antibiotics [11].

Signal transduction through protein phosphorylation is the main regulatory mechanism that provides the rapid response to environmental changes [19]. In *S. aureus*, a eukaryotic-type serine/threonine kinase PknB and related phosphatase Stp can regulate basic cellular processes, allowing bacteria to quickly respond to environmental changes and regulate the dormancy [22].

Recent studies have shown that the serine/threonine kinase PknB and phosphatase Stp regulate antibiotic resistance in *S. aureus* persistent strains. Recently, the authors Huemer et al. found that conditions mimicking acid stress experienced by *S. aureus* in host tissues significantly changed the serine and threonine phosphoproteome, and increased threonine phosphorylation in the activation loop of PknB. It was shown that the deletion of *stp* increases the subpopulation of dormant bacteria in response to stress. Additional analyses demonstrated that the Δ*stp* mutant has lower levels of intracellular ATP, reduced protein translation and increased antibiotic tolerance. Using phosphoproteomic approaches authors identified the targets of Ser/Thr phosporylation regulating bacterial growth and metabolism such as ribosomal proteins including elongation factor EF-G [12]. It may be concluded that the process of serine/threonine phosphorylation regulates bacterial quiescence and antibiotic tolerance despite full susceptibility of *S. aureus* to antibiotics. Therefore, inhibition of PknB and Stp can be promising therapeutic strategy to prevent *S. aureus* persister formation.

Involvement of PknB in *S. aureus* cell wall synthesis

The search for new antibiotics targeting the pathways of cell wall biosynthesis is the most promising strategy to combat antibiotic resistance [23].

The bacterial cell envelope is essential for bacterial survival under environmental stresses and contributes to virulence and antibiotic resistance. The cell wall of Gram-positive bacteria consists of a multilayered network of cross-linked peptidoglycan (PGN). PGN is composed of repeating disaccharide chains including *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc). The lactoyl group of MurNAc is substituted by a peptide stem (L-Ala-D-isoGlu-L-Lys-D-Ala-D-Ala). The polysaccharide chains of staphylococcal PGN are strongly cross-linked by interpeptide bridges of five glycyl residues protruding from the *L*-lysine of the stem peptides. The overall synthesis of PGNs can be divided into three main steps: (1) formation of a nucleotide sugar-linked precursor UDP-MurNAc pentapeptide (Park's nucleotide), (2) transfer of the soluble PGN precursor to the lipid carrier undecaprenyl diphosphate (formation of lipid I) and attachment of UDP-GlcNAc, resulting in the formation of lipid II, and (3) transport of this subunit across the cytoplasmic membrane and cross-bridge formation [24].

Several studies have shown that phosphorylation plays an important role in cell wall metabolism [25, 26]. Particularly, it was shown that the serine/threonine protein kinase PknB and the related phosphatase Stp in *S. aureus* are involved in the signaling during cell wall synthesis. It was found that pentaglycine-lipid II interacts with extracellular PASTA domains of the PknB which in turn leads to the activation of protein kinase activity [22]. Indeed, the deletion of *pknb* and *stp* causes cell division defects in *S. aureus*, leading to the formation of multiple and incomplete septa, changes in cell size and cell wall thickness [6]. In addition, *pknb* and *pknb*/*stp* deletion strains are more sensitive to antibiotics that act through the cell wall, such as tunicamycin and fosfomycin [27]. In addition, Stp phosphatase contributes to vancomycin sensitivity and virulence [28]. PknB cross-talks with two-component systems are implicated in cell wall metabolism by phosphorylating the response regulators VraTSR8, WalRK9, and GraSR24, affecting the expression of cell wall stimulators and hydrolases, as well as cell wall charge [29, 30]. PknB homologs also regulate cell wall synthesis and cell division in *Streptococcus*, *Bacillus*, and *Streptomyces* [31-33].

In many Gram-positive bacteria, including *S. aureus*, the transpeptidase enzyme sortase A (SrtA) attaches surface proteins to the cell wall and plays an important role in bacterial pathogenesis. In *S. aureus*, SrtA is phosphorylated by PknB and by the low molecular weight phosphodonor acetyl phosphate (AcP) *in vitro*. Both PknB- and AcP-mediated phosphorylation inhibit the enzymatic activity of SrtA *in vitro*. Also, it was demonstrated that the deletion of the *stp* gene encoding the serine/threonine phosphatase Stp

resulted in an increase in the level of SrtA phosphorylation [34].

Based on these findings, PknB and/or Stp inhibitors can be promising antibacterial agents for the treatment of *S. aureus* infections.

S. aureus PknB inhibitors

Arylsulfonamides as adjuvants of bactericidal activity of β-lactams.

β-Lactams are widely used to treat infections caused by *Staphylococcus aureus*. But, modification and expression of penicillin-binding proteins (PBPs), drug inactivation by synthesis of *β*-lactamases, decreasing drug effectiveness due to biofilm formation, and expression of an efflux pumps all caused the development of *S. aureus* resistance to *β*-lactam antibiotics. The synthesis of *β*-lactamases and modification of PBPs led to penicillin resistance. With the emergence of MRSA strains, the resistance to methicillin was also reported. Then MRSA infections were treated with cephalosporin and carbapenems, but resistant strains have also emerged during these antibiotic therapies.

S. aureus is one of the priority resistant pathogen microorganisms that require the development of novel antibiotics and innovative preventive approaches [35].

There is a growing interest in the use of adjuvants to restore MRSA susceptibility to *β*-lactams. Adjuvants are auxiliary compounds for antibiotics that enhance their effect, for example, by inhibiting bacterial resistance mechanisms [36].

The use of eukaryotic serine/threonine kinase inhibitors demonstrates potent antibiotic efficacy against various types of bacteria, including MRSA, enterococci, mycobacteria, and gram-negative bacteria. Previously, it was reported that inhibitors targeting bacterial protein kinases enhance the antibacterial activity of *β*-lactam antibiotics [7]. Inhibiting PknB with small molecule inhibitors improves the efficacy of *β*-lactam antibiotics such as nafcillin and imipenem. Genetic deletion of PknB increases the susceptibility of *S. aureus* to *β*-lactams. However, no changes were found in the sensitivity to vancomycin.

The screening of a small library of drugs for inhibitory activity toward PknB revealed four arylsulfonamides that were active at a concentration of 2 μM. The general chemical structure of arylsulfonamides is presented in Figure 1. Staurosporine, a pan-kinase inhibitor, was also active. These inhibitors are adjuvants of the bactericidal activity of *β*-lactam antibiotics and can inhibit bacterial growth by 50% at a concentration of 13 μM. None of the four sulfonamides were toxic in animal models. In an *in vitro* growth assay, none of the MRSA strains were inhibited by the presence of 4 mg/L^{-1} nafcillin. On the contrary, at the same concentration of nafcillin and in the presence of 13 μM sulfonamide (or staurosporine), bacterial growth was inhibited by 50%. Thus, PknB inhibitors are adjuvants of the bactericidal activity of *β*-lactams [37].

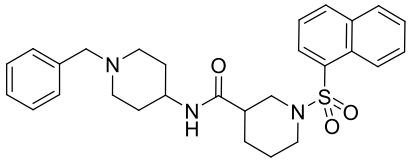

Figure 1. Chemical structure of arylsulfonamides.

Pyrazolo[3,4-d]pyrimidines

A recent study revealed the powerful antibacterial potential of pyrazolo[3,4-d]pyrimidines, which are promising protein kinase inhibitors possessing antiproliferative properties [38]. In particular, six compounds were identified that showed bacteriostatic activity against gram-positive *S. aureus* and gram-negative *E. coli* [39]. The pyrazolo[3,4-d]pyrimidine compounds **1-6**, presented in Figure 2, were tested for their antibacterial activity against *S. aureus* and *E. coli*. These studies showed that all investigated compounds have significant antibacterial activity against *S. aureus* and *E. coli*. Compound **3** at a concentration of 200 μg/mL was able to almost completely inhibit the growth of *S. aureus*. Compounds **1** and **4** did not show growth inhibition alone at 12.5 μg/mL or 25 μg/mL, but in combination with sublethal doses of ampicillin (0.02 μg/mL), a significant bacterial growth inhibition was observed within 14 hours. Compound **4** showed a moderate increase of kanamycin activity (0.2 μg/mL) in *S. aureus* [12, 39].

Triarylimidazole derivatives

Triarylimidazole derivatives inhibit *S. aureus* PknB and enhance activity of *β*-lactam antibiotics. For example, it was established that compound **7** (Figure 2) at a concentration of 7 mg/L^{-1} decreased the MIC value of oxacillin from 256 mg/L^{-1} to 4 mg/L^{-1} for MRSA252 strain; from 16 mg/L^{-1} to 4 mg/L^{-1} for MRSA NRS123 strain; and from 32 mg/L^{-1} to 0.5 mg/L^{-1} for MRSA NRS70 strain [37].

GW779439X and its pyrazolopyridazine derivatives

The compound GW779439X (compound **8**, Figure 2) was identified as a novel inhibitor of *S. aureus* PknB. The inhibitor GW779439X enhanced the effect of *β*-lactams, in particular, MRSA-active ceftaroline, against several MSSA and MRSA strains. GW779439X at the concentration of 5 μM reduced the MIC of ceftaroline by 2-fold for MRSA USA 300-LAC strain. After addition of GW779439X 2-fold decrease was also observed for meropenem, 8-fold for nafcillin, and 16-fold for oxacillin, while the MIC for vancomycin did not change [37].

Seven derivatives of GW779439X were synthesized and structure-activity relationships were studied. It was found that the presence and orientation of the methylpiperazine is important for inhibitory activity toward *S. aureus* PknB (Figure 3). Particularly, the presence of a positive charge on *p-N*-methylpiperazine is crucial for inhibitory activity [40]. Therefore, pyrazolopyridazine scaffold is promising for the development of *S. aureus* PknB inhibitors as antibiotic adjuvants.

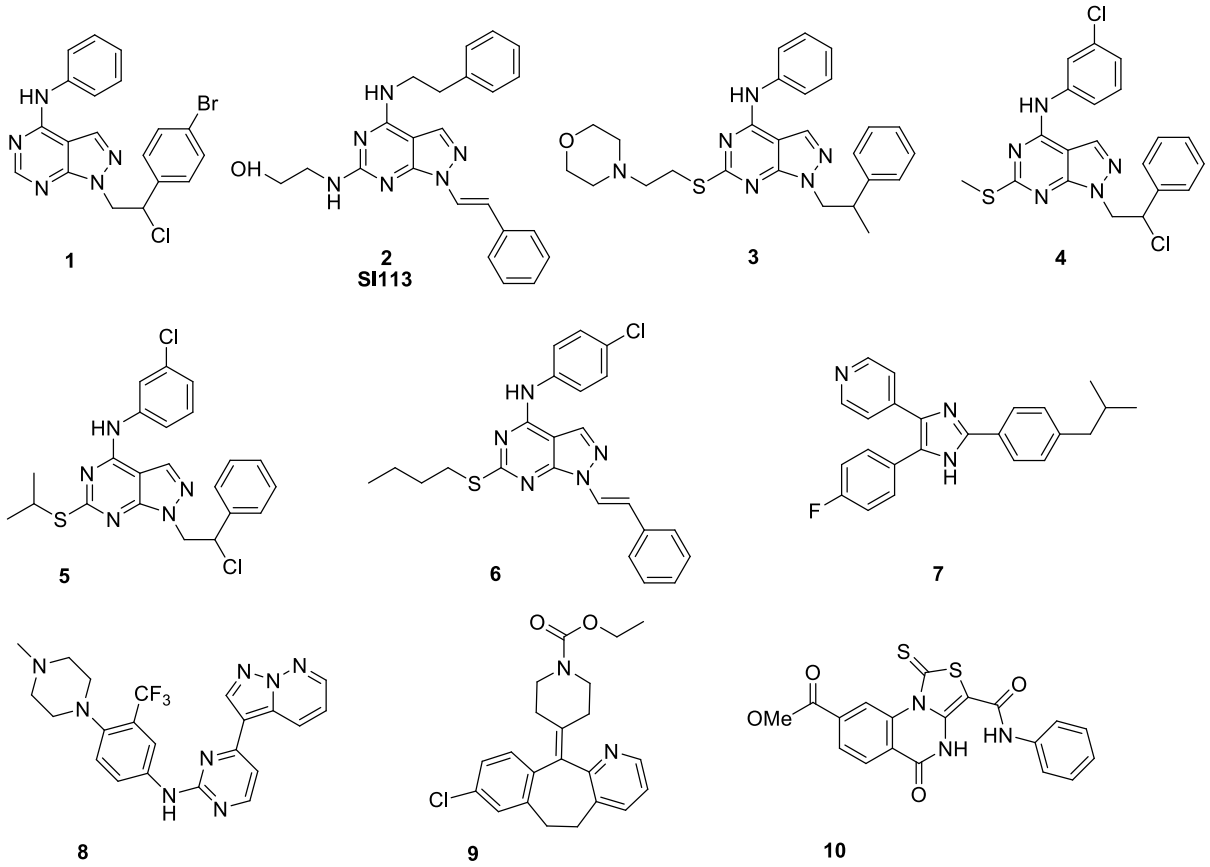

Figure 2. Chemical structure of PknB inhibitors: pyrazolo[3,4-d]pyrimidine derivatives (**1-6**), 4-[5-(4-Fluoro-phenyl)-2-(4-isobuthyl-phenyl)-1H-imidazol-4-yl]-pyridine (**7**), GW779439X (**8**), loratadine (**9**), Inh2-B1 (**10**).

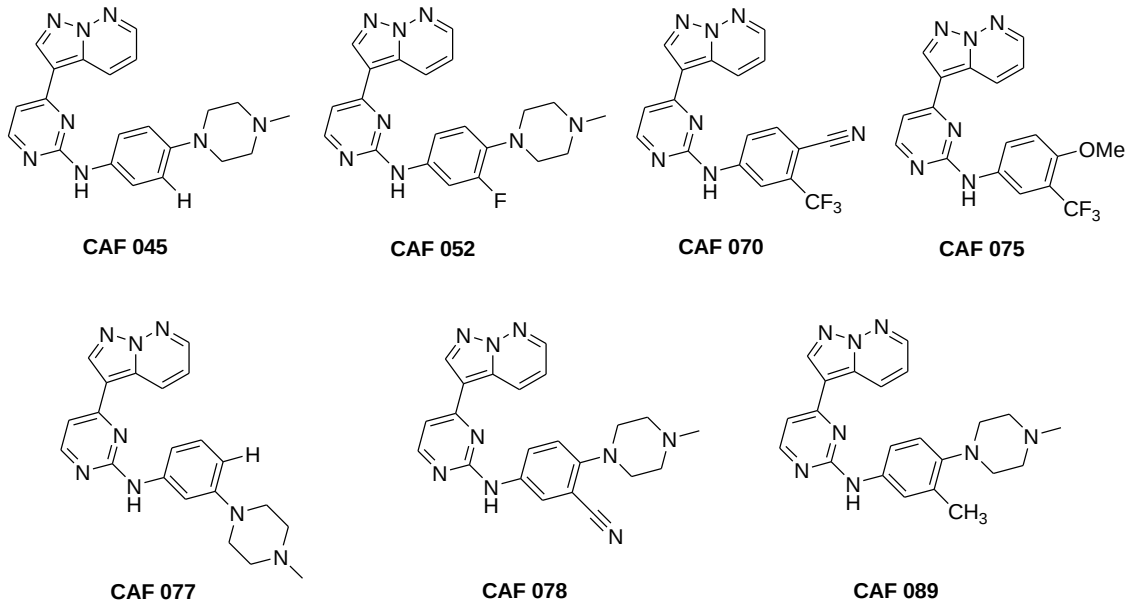

Figure 3. Structures of pyrazolopyridazine compounds.

Loratadine

The non-toxic antihistamine loratadine (compound **9**, Figure 2) was identified as an inhibitor of *S. aureus* PknB, which inhibits biofilm formation and enhances the activity of *β*-lactam antibiotics and vancomycin. During comparison of the activity of loratadine with a structurally similar compound, desloratadine, was revealed that the presence of ethyl carboxyester in the structure of loratadine is crucial for PknB inhibitory activity and consequently for antibacterial activity in both biofilm inhibition assay and the antibiotic potency assay, since desloratadine was not active [41].

Quinazoline-based inhibitors.

The small molecule compound methyl 5-oxo-3-(phenylcarbamoyl)-1-thioxo-4,5-dihydro[1,3]thiazolo[3,4-a]quinazoline-8-carboxylate (Inh2-B1) was identified as ATP-competitive PknB inhibitor. Although Inh2-B1 (compound **10**, Figure 2) is a relatively weak inhibitor (IC_{50} = 50 μM) of PknB autophosphorylation, it, in combination with *β*-lactam, protected mice from the lethal effects of MRSA. Separately, *β*-lactam and Inh2-B1 were ineffective. However, only Inh2-B1 inhibited biofilm formation [37].

The quinazoline core is typical for many eukaryotic kinase inhibitors. Several aminoquinazoline-based inhibitors (mitoxantrone, VI12112) have been previously described as inhibitors of the mycobacterial PknB. Quinazoline inhibitor AX20017 blocks the activity of PknG, a PASTA-free mycobacterial kinase that promotes bacterial survival inside human macrophages. AX20017 restores macrophage-mediated killing but does not inhibit the growth of extracellular bacteria [42].

The compound Inh2-B1 acts as so-called antibiotic resistance breaker by inhibiting the kinase activity of *S. aureus* PknB, which contributes significantly to antibiotic resistance by modulating the function of cell wall biosynthesis mechanisms. In high concentrations, neither Inh2-B1, nor ceftriaxone, or cefotaxime can inhibit bacterial growth by themselves. Therefore, Inh2-B1 is effective when used in combination therapy with low-efficient cephalosporins [12].

Inh2-B1 is not cytotoxic and does not have apoptotic effects on human cells. The last property is very important, since a lot of protein kinase inhibitors are successfully used to treat tumors due to their ability to induce apoptosis in human cells. Inh2-B1 downregulates cell wall hydrolase genes and disrupt MRSA biofilm formation which makes this inhibitor a therapeutically important agent to overcome antibiotic resistance [6].

Therefore, serine/threonine protein kinase PknB is a valuable molecular target for the development of novel antibacterial compounds toward *Staphylococcus aureus*. Several classes of inhibitors have been already reported, but none of them demonstrate high activity. Therefore, the search of novel classes and optimization of known PknB inhibitors is of great interest.

Notes

The authors declare no conflict of interest.

Author contributions. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

References

1. Laux, C.; Peschel, A.; Krismer, B. *Staphylococcus aureus* colonization of the human nose and interaction with other microbiome members. *Microbiology Spectrum* **2019**, *7*, 723-730.
2. Pidwill, G.R.; Gibson, J.F.; Cole, J.; Renshaw, S.A.; Foster, S.J. The role of macrophages in *Staphylococcus aureus* infection. *Frontiers in immunology* **2021**, *11*, 3506.
3. Ahmad-Mansour, N.; Loubet, P.; Pouget, C.; Dunyach-Remy, C.; Sotto, A.; Lavigne, J.P.; Molle, V. *Staphylococcus aureus* toxins: An update on their pathogenic properties and potential treatments. *Toxins* **2021**, *13*, 677.
4. Raineri, E. J.; Altulea, D.; van Dijl, J. M. Staphylococcal trafficking and infection - from 'nose to gut' and back. *FEMS Microbiology Rev.* **2022**, *46*, fuab041.
5. Ikuta, K.S.; Swetschinski, L.R.; Aguilar, G.R.; Sharara, F.; Mestrovic, T.; Gray, A.P.; Dhingra, S. Global mortality associated with 33 bacterial pathogens in 2019: a systematic analysis for the Global Burden of Disease Study 2019. *The Lancet* **2022**, *400*, 2221-2248.
6. Kant, S.; Asthana, S.; Missiakas, D.; Pancholi, V. A novel STK11-targeted small-molecule as an "antibiotic resistance breaker" against multidrug-resistant *Staphylococcus aureus*. *Scientific reports* **2017**, *7*, 1-19.
7. Liang, C.; Rios-Miguel, A.B.; Jarick, M.; Neurgaonkar, P.; Girard, M.; François, P.; Dandekar, T. *Staphylococcus aureus* transcriptome data and metabolic modelling investigate the interplay of Ser/Thr kinase PknB, its phosphatase Stp, the glmR/yvcK regulon and the cdaA Operon for metabolic adaptation. *Microorganisms* **2021**, *9*, 2148.
8. Bonne Køhler, J.; Jers, C.; Senissar, M.; Shi, L.; Derouiche, A.; Mijakovic, I. Importance of protein Ser/Thr/Tyr phosphorylation for bacterial pathogenesis. *FEBS letters* **2021**, *594*, 2339-2369.
9. Zheng, W.; Cai, X.; Li, S.; Li, Z. Autophosphorylation mechanism of the Ser/Thr kinase Stk1 from *Staphylococcus aureus*. *Frontiers in Microbiology* **2018**, *9*, 758.
10. Tamber, S.; Schwartzman, J.; Cheung, A. L. Role of PknB kinase in antibiotic resistance and virulence in community-acquired methicillin-resistant *Staphylococcus aureus* strain USA300. *Infection and immunity* **2010**, *78*, 3637-3646.
11. King, A.; Blackledge, M.S. Evaluation of small molecule kinase inhibitors as novel antimicrobial and antibiofilm agents. *Chemical biology & drug design* **2021**, *98*, 1038-1064.
12. Huemer, M.; Shambat, S.M.; Pereira, S.; Van Gestel, L.; Bergada-Pijuan, J.; Gómez-Mejia, A.; Zinkernagel, A.S. Ser/Thr phospho-regulation by PknB and Stp mediates bacterial quiescence and antibiotic persistence in *Staphylococcus aureus*. *BioRxiv* **2021**, 1-35.
13. Mo, R.; Liu, Y.; Chen, Y.; Mao, Y.; Gao, B. Evolutionary principles of bacterial signaling capacity and complexity. *Mbio* **2022**, *13*, e00764-22.
14. Zschiedrich, C.P.; Keidel, V.; Szurmant, H. Molecular mechanisms of two-component signal transduction. *Journal of molecular biology* **2016**, *428*, 3752-3775.
15. Dworkin, J. Ser/Thr phosphorylation as a regulatory mechanism in bacteria. *Current opinion in microbiology* **2015**, *24*, 47-52.
16. Janczarek, M.; Vinardell, J.M.; Lipa, P.; Karaś, M. Hanks-type serine/threonine protein kinases and phosphatases in bacteria: roles in signaling and adaptation to various environments. *International journal of molecular sciences* **2018**, *19*, 2872.
17. Guo, Y.; Song, G.; Sun, M.; Wang, J.; Wang, Y. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. *Frontiers in cellular and infection microbiology* **2020**, *10*, 107.

18. Mukherjee, R.; Priyadarshini, A.; Pandey, R.P.; Raj, V.S. Antimicrobial resistance in *Staphylococcus aureus*. *Insights into drug resistance in Staphylococcus aureus* **2021**, 85.
19. Kyriakis, J.M. In the beginning, there was protein phosphorylation. *Journal of Biological Chemistry* **2014**, *289*, 9460-9462.
20. Stock, A.M.; Robinson, V.L.; Goudreau, P.N. Two-component signal transduction. *Annual review of biochemistry* **2000**, *69*, 183-215.
21. Bakal, C.J.; Davies, J E. No longer an exclusive club: eukaryotic signalling domains in bacteria. *Trends in cell biology* **2000**, *10*, 32-38.
22. Hardt, P.; Engels, I.; Rausch, M.; Gajdiss, M.; Ulm, H.; Sass, P.; Grein, F. The cell wall precursor lipid II acts as a molecular signal for the Ser/Thr kinase PknB of *Staphylococcus aureus*. *International Journal of Medical Microbiology* **2017**, *307*, 1-10.
23. Schneider, T.; Sahl, H.G. An oldie but a goodie-cell wall biosynthesis as antibiotic target pathway. *International Journal of Medical Microbiology* **2010**, *300*, 161-169.
24. Scheffers, D. J.; Pinho, M. G. Bacterial cell wall synthesis: new insights from localization studies. *Microbiology and molecular biology reviews* **2005**, *69*, 585-607.
25. Canova, M.J.; Baronian, G.; Brelle, S.; Cohen-Gonsaud, M.; Bischoff, M.; Molle, V. A novel mode of regulation of the *Staphylococcus aureus* Vancomycin-resistance-associated response regulator VraR mediated by Stk1 protein phosphorylation. *Biochemical and biophysical research communications* **2014**, *447*, 165-171.
26. Liebeke, M.; Meyer, H.; Donat, S.; Ohlsen, K.; Lalk, M. A metabolomic view of *Staphylococcus aureus* and its ser/thr kinase and phosphatase deletion mutants: involvement in cell wall biosynthesis. *Chemistry & biology* **2010**, *17*, 820-830.
27. Donat, S.; Streker, K.; Schirmeister, T.; Rakette, S.; Stehle, T.; Liebeke, M.; Ohlsen, K. Transcriptome and functional analysis of the eukaryotic-type serine/threonine kinase PknB in *Staphylococcus aureus*. *Journal of bacteriology* **2009**, *191*, 4056-4069.
28. Cameron, D.R.; Ward, D.V.; Kostoulias, X.; Howden, B.P.; Moellering Jr.R.C.; Eliopoulos, G.M.; Peleg, A.Y. Serine/threonine phosphatase Stp1 contributes to reduced susceptibility to vancomycin and virulence in *Staphylococcus aureus*. *The Journal of infectious diseases* **2012**, *205*, 1677-1687.
29. Jarick, M.; Bertsche, U.; Stahl, M.; Schultz, D.; Methling, K.; Lalk, M.; Ohlsen, K. The serine/threonine kinase Stk and the phosphatase Stp regulate cell wall synthesis in Staphylococcus aureus. *Scientific reports* **2018**, *8*, 13693.
30. Fridman, M.; Williams, G.D.; Muzamal, U.; Hunter, H.; Siu, K.M.; Golemi-Kotra, D. Two unique phosphorylation-driven signaling pathways crosstalk in *Staphylococcus aureus* to modulate the cell-wall charge: Stk11/Stp1 meets GraSR. *Biochemistry* **2013**, *52*, 7975-7986.
31. Fleurie, A.; Manuse, S.; Zhao, C.; Campo, N.; Cluzel, C.; Lavergne, J.P.; Grangeasse, C. Interplay of the serine/threonine-kinase Stk1P and the paralogs DivIVA and GpsB in pneumococcal cell elongation and division. *PLoS genetics* **2014**, *10*, e1004275.
32. Pompeo, F.; Foulquier, E.; Galinier, A. Impact of serine/threonine protein kinases on the regulation of sporulation in *Bacillus subtilis*. *Frontiers in microbiology* **2016**, *7*, 568.
33. Ladwig, N.; Franz-Wachtel, M.; Hezel, F.; Soufi, B.; Macek, B.; Wohlleben, W.; Muth, G. Control of morphological differentiation of *Streptomyces coelicolor* A3 (2) by phosphorylation of MreC and PBP2. *PloS one* **2015**, *10*, e0125425.
34. Chen, F.; Di, H.; Wang, Y.; Peng, C.; Chen, R.; Pan, H.; Lan, L. The enzyme activity of sortase A is regulated by phosphorylation in *Staphylococcus aureus*. *Virulence* **2023**, *14*, 2171641.
35. Kumar, A.; Kaushal, M. Progression of β-Lactam Resistance in *Staphylococcus aureus*. *Insights Into Drug Resistance in Staphylococcus aureus* **2021**, 147-158.
36. Melander, R.J.; Melander, C. The challenge of overcoming antibiotic resistance: an adjuvant approach?.*ACS infectious diseases* **2017**, *3*, 559-563.
37. Fisher, J.F.; Mobashery, S. β-Lactams against the Fortress of the Gram-Positive *Staphylococcus aureus* Bacterium. *Chemical reviews* **2020**, *121*, 3412-3463.
38. Abbruzzese, C.; Matteoni, S.; Persico, M.; Ascione, B.; Schenone, S.; Musumeci, F.; Paggi, M.G. The small molecule SI113 hinders epithelial-to-mesenchymal transition and subverts cytoskeletal organization in human cancer cells. *Journal of Cellular Physiology* **2019**, *234*, 22529-22542.
39. Greco, C.; Catania, R.; Balacco, D.L.; Taresco, V.; Musumeci, F.; Alexander, C.; Schenone, S. Synthesis and antibacterial evaluation of new pyrazolo [3, 4-d] pyrimidines kinase inhibitors. *Molecules* **2020**, *25*, 53543.
40. Schaenzer, A.J.; Wlodarchak, N.; Drewry, D.H.; Zuercher, W.J.; Rose, W.E.; Ferrer, C.A.; Striker, R. GW779439X and its pyrazolopyridazine derivatives inhibit the serine/threonine kinase Stk1 and act as antibiotic adjuvants against β-lactam-resistant *Staphylococcus aureus*. *ACS infectious diseases* **2018**, *4*, 1508-1518.
41. Cutrona, N.; Gillard, K.; Ulrich, R.; Seemann, M.; Miller, H.B.; Blackledge, M.S. From antihistamine to anti-infective: loratadine inhibition of regulatory PASTA kinases in Staphylococci reduces biofilm formation and potentiates β-lactam antibiotics and vancomycin in resistant strains of *Staphylococcus aureus*. *ACS infectious diseases* **2019**, *5*, 1397-1410.
42. Scherr, N.; Honnappa, S.; Kunz, G.; Mueller, P.; Jayachandran, R.; Winkler, F.; Steinmetz, M.O. Structural basis for the specific inhibition of protein kinase G, a virulence factor of *Mycobacterium tuberculosis*. *Proceedings of the National Academy of Sciences* **2007**, *104*, 12151-12156.

Протеїнкіназа PknB як перспективна мішень для розробки антибактеріальних препаратів проти Staphylococcus aureus

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Резюме: Резистентність до протимікробних препаратів є однією з найсерйозніших глобальних загроз громадському здоров'ю нашого століття. Неконтрольоване застосування антибіотиків призвело до широкого розповсюдження штамів бактерій, які несприйнятливі майже до всіх відомих на сьогодні антимікробних препаратів. Одним з патогенів, що викликає найбільше занепокоєння в клінічній практиці, є Staphylococcus aureus, оскільки він колонізував приблизно 30% людської популяції. Тому пошук нових протистафілококових препаратів є невідкладною потребою людства. У прокаріотичних клітинах низка клітинних процесів регулюється серин/треоніновими протеїнкіназами. Зокрема, протеїнкіназа PknB є ключовим компонентом сигнальної мережі бактеріальних клітин, що дає змогу розглядати цей фермент як перспективну молекулярну мішень для пошуку нових антибактеріальних препаратів. У цьому огляді ми проаналізували сучасні дані щодо структури та механізму регуляції протеїнкінази PknB, а також результати досліджень стосовно пошуку її інгібіторів.

Ключові слова: протимікробна стійкість; антибактеріальні препарати; золотистий стафілокок *Staphylococcus aureus*; протеїнкіназа PknB; інгібування.