Engineering Selectivity for Reduced Toxicity of Bacterial Kinase Inhibitors Using Structure-Guided Medicinal Chemistry

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ABSTRACT: Tuberculosis is a major global public health concern, and new drugs are needed to combat both the typical form and the increasingly common drug-resistant form of this disease. The essential tuberculosis kinase PknB is an attractive drug development target because of its central importance in several critical signaling cascades. A major hurdle in kinase inhibitor development is the reduction of toxicity due to nonspecific kinase activity in host cells. Here a novel class of PknB inhibitors was developed from hit aminopyrimidine 1 (GW779439X), which was originally designed for human CDK4 but failed to progress clinically because of high toxicity and low specificity. Replacing the pyrazolopyridazine headgroup of the original hit with substituted pyridine or phenyl headgroups resulted in a reduction of Cdk activity and a 3-fold improvement in specificity over the human kinome while maintaining PknB activity. This also resulted in improved microbiological activity and reduced toxicity in THP-1 cells and zebrafish.

KEYWORDS: Mycobacterium tuberculosis, kinase inhibitors, PknB, antibiotics

Tuberculosis is the deadliest single-agent infectious disease on Earth, killing approximately 1.5 million people annually and resulting in latent tuberculosis infection in one-third of the world population.1 The emergence of multidrug-resistant strains has worsened this global health crisis.2−4 Furthermore, the incidence of novel infectious respiratory diseases such as COVID-19 can potentially exacerbate the tuberculosis pandemic through coinfection5,6 and disruption of global economics and healthcare.7 Major challenges with current tuberculosis treatments include a prolonged duration of drug regimen and burdensome side effects, both of which limit patient adherence, especially in areas of economic instability and limited medical care.8 Drug development for tuberculosis is stagnant, with only one new drug approved in the last 50 years9 despite evidence of strain resistance.10 Coinfection of tuberculosis with emerging respiratory diseases like SARS-CoV2 creates complications such as more severe side effects and the potential for drug interactions.5,6 This further highlights the dire need for new tuberculosis therapeutics.

Mycobacterium tuberculosis contains 11 serine/threonine (S/T) protein kinases, three of which, PknA, PknB, and PknD, are essential for survival in broth and in infected host cells.11,12 PknB is a member of the penicillin binding and S/T associated (PASTA) kinase family and is necessary for signal transduction in M. tuberculosis to trigger growth and cell wall remodel-
expected to beneficially reduce potential resistance but also potentially introduce undesirable side effects due to non-specific kinase inhibition. Thus, designing specific inhibitors is a critical part of kinase drug development projects, as many compounds fail to progress to later development because of specificity and toxicity concerns.

Herein we present the development of a novel class of aminopyrimidine PknB inhibitors with anti-mycobacterial activity that were designed to balance effectiveness with reduced human kinase activity and toxicity. In previous work, we screened the Published Kinase Inhibitor Set (PKIS) libraries using in silico methods and found aminopyrimidine 1 (GW779439X), which was predicted to bind tuberculosis PknB (Figure 1).18

In related work, we found that 1 showed biochemical inhibition (200 nM < IC₅₀ < 2000 nM) of the methicillin-resistant Staphylococcus aureus (MRSA) PASTA kinase Stk1 (homologue of Mtb PknB) and antimicrobial activity against MRSA by increasing β-lactam susceptibility 2–512-fold.17 Subsequently, we identified 1 as a biochemical inhibitor of tuberculosis PknB23 with a Kᵢ of 420 nM (Table 1). We obtained 29 structurally related compounds from our work on Stk1 and tested these against PknB. We found that only four compounds with minor changes at the trifluoromethyl position had any activity, and none were better than 1. Any modification of the methylpiperidine tail and extension of the pyrazolopyridazine head appeared to be detrimental to activity (Figure 2).

The docking models (Figure 1) and data from related compounds24–26 suggested that the aminopyrimidine core hydrogen-bonds with the hinge region of the kinase, while the piperidine functional group was predicted to engage in an electrostatic interaction with residue D104 and the pyrazolopyridazine head to make contact with the catalytic lysine (K40). Given the docking predictions and data on related compounds, we determined that the aminopyrimidine core and piperidine tail were likely necessary for activity; therefore, we focused our design on simplifying the pyrazolopyridazine headgroup to retain binding to K40 while also gaining specificity by binding the unique PknB back pocket, following our previous work with GSK690693.18

To streamline the synthetic process, we designed over 100 molecules in silico, docked them to PknB, and ranked them by binding free energy (values for synthesized compounds are shown in Table S1). We visualized the predicted poses in the structure and rejected molecules that had an axis of alignment perpendicular to or reversed from that of the predicted orientation of 1 (Figure 1) or had computed binding free energies lower than two standard deviations from the mean. We prioritized molecules for which the aminopyrimidine core aligned with a favorable pattern of hydrogen bonding to the PknB hinge and that made contacts in the back pocket of PknB (Figure 3A), as our previous work had shown that another inhibitor, GSK690693, made critical contacts in this unique region.18

Compound 1 was originally designed as a Cdk4 inhibitor but also has appreciable activity on Cdk2 and other human kinases.27,28 Since a major goal in the design of new PknB inhibitors was to enhance bacterial target specificity, we focused on engineering, assessing, and advancing new PknB inhibitors with less human kinase activity early in the development process. Cdkks are well-studied, and as a result, a deep trove of structural information has been described.29 We docked the proposed molecules in cyclin-bound conformationally active (Cdk2-Cyclin A (PDB entry 1FIN) and Cdk4-CyclinD3 (PDB entry 3G33)) and inactive Cdkks and

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**Figure 1.** Aminopyrimidine 1 docked in PknB (PDB entry 1O6Y) aligns the aminopyrimidine core along the hinge region with several potential hydrogen bonds. The pyrazolopyridazine headgroup makes electrostatic contacts with the catalytic lysine (K40), and the methylpiperidine makes electrostatic contacts with D102. The PknB binding pocket is shown in purple mesh, and residues are shown in purple cartoon or sticks with the hinge and back pocket (BP) labeled. Black dashes and numbers indicate atomic distances in angstroms.

**Figure 2.** Structure–activity summary for aminopyrimidine 1.

**Table 1.** Representative PknB and Cdk2 Enzyme Inhibition: SAR of Pyridine Headgroups

| compd | R₁ | Kᵢ (nM) | Kⱼ (nM) | IC₅₀ (μM) | IC₅₀ (μM) |
|-------|----|--------|--------|----------|----------|
| 1     | see Figure 2 | 420 | 27 | 1.5 | 1.4 |
| 4     | shown above | 470 | 61 | 1.7 | 9.3 |
| 5     | H | 580 | 78 | 2.1 | 4.6 |
| 6     | Cl | 290 | 48 | 1.1 | 7.2 |
| 7     | NHBe | 2300 | 2100 | 7.9 | 7.3 |
| 8     | NHMe | 2700 | 920 | 9.4 | 39 |
interactions. Red × symbols indicate the absence of hinge region (labeled "H") are shown with black arrows. BP = PknB back pocket. Predicted hydrogen bonds to the

Figure 3. Structures and docking of (left) 1, (center) 9, and (right) 19 in (A) PknB (PDB entry 1O6Y), (B) Cdk2 (PDB entry 1FIN), and (C) Cdk4 (PDB entry 3G33). Predicted hydrogen bonds to the hinge region (labeled "H") are shown with black arrows. BP = PknB back pocket. Red × symbols indicate the absence of hinge interactions.

prioritized molecules that were predicted to disorient the binding position in Cdns compared with 1 (Figure 3B,C) or had binding free energies worse than two standard deviations from the mean. The remaining molecules were ranked by synthetic feasibility. Substituted phenyl and pyridine functional groups in the headgroup region were prioritized using these criteria. As a result, 17 analogues were synthesized to assess binding position in Cdks compared with 

To test our hypothesis that in silico selection against Cdk binding would improve specificity, we evaluated the Cdk biochemical activity using the same ATP competitive assay with Cyclin A-activated Cdk2, which revealed up to 28-fold reduction in Cdk2 biochemical inhibition for the five tested pyridine inhibitors relative to 1 (Table 1). The largest fold change was with the m-methylamine derivative 8; however, this reduction came with a 6-fold loss of PknB activity relative to 1. Although this result was promising, a more severe loss of human kinase activity was desirable for the development of low-toxicity inhibitors.

We next determined whether the basic nitrogen in the headgroup was necessary for Cdk2 activity by developing a series of phenyl headgroups with changes at the meta and para positions (Table 2). We found that with the exception of p-

Table 2. Representative PknB and Cdk2 Enzyme Inhibition: SAR of Phenyl Headgroups

| compd | R1 | R2 | Kd (nM) | Ki (nM) | Ic50 (μM) |
|-------|----|----|---------|---------|-----------|
| 1     | see Figure 2 | 420 | 27 | 1.5 | 1.4 |
| 9     | H   | H  | 1300 | 1200 | 4.6 | >40 |
| 10    | H   | F  | >12000 | 3000 | >40 | 12 |
| 11    | H   | Cl | 2,400 | 2200 | 8.1 | >40 |
| 12    | H   | Me | 440 | 30 | 1.6 | >40 |
| 13    | H   | CN | 1500 | 700 | 5.1 | 13 |
| 14    | H   | NO2 | 1100 | 410 | 3.8 | 0.18 |
| 15    | F   | H  | 1800 | 710 | 6.1 | 24 |
| 16    | CI  | H  | 1200 | 340 | 4.3 | >40 |
| 17    | OMe | H  | 1900 | 660 | 6.6 | 10 |
| 18    | CN  | H  | 700 | 73 | 2.5 | >40 |
| 19    | NO2 | H  | 320 | 34 | 1.2 | >40 |
| 20    | shown above | 970 | 430 | 3.4 | >40 |

Scheme 1. Synthesis of Aminopyrimidine 4 via Formal [3 + 3] Cycloaddition of Guanidine 2 and Vinylogous Amide 3 whereas those with an extended amine at the meta position (compounds 7 and 8) did not. As a confirmatory orthogonal binding assay, direct binding affinity (Kd) was also determined using microscale thermophoresis, and the values determined for Ki followed the same pattern as the Ic50 and Ki values (Table 1).

We assessed the set of pyridine analogues for biochemical activity against PknB using an ATP competitive biochemical assay (Table 1). We found that several new compounds (4–6) had Ic50/Ki values against PknB comparable to those for 1, fluorophenyl analogue 10, these compounds generally maintained PknB activity comparable to that of the pyridine family and within 1 log unit of that of compound 1, although again none were greatly improved compared to 1. It is unclear why compound 10 lost activity; docking results revealed overlapping alignments between the p-fluoro (10), p-methyl (12), and m-fluoro (15) analogues with nearly identical predicted binding free energies (Table S1). However, the position places the fluoride perpendicular to and in near proximity of the critical E59–K40 bridge. Generally speaking, halogen substitutions on the phenyl ring seem to be detrimental, and meta substitutions seem to be most favored. It is possible that the electron-withdrawing effect due to the extreme electronegativity of fluoride is less favorable for
binding, and this combined with its size and position may prevent any appreciable binding.

Importantly, though, our biochemical screen with Cdk2 showed that all but one of these compounds (14) had reduced Cdk2 activity, with seven analogues (9, 11, 12, 16, and 18–20) having no activity up to the highest concentration tested (40 μM), representing a greater than 28-fold reduction in activity relative to compound 1 (Table 2). This suggested that removing the nitrogen of the pyridine ring could generally reduce Cdk activity while maintaining PknB activity.

In addition to Cdk2, we decided to test the general selectivity of several aminopyrimidines by screening for binding affinity with other centrally important human kinases. Aminopyrimidines 1, 6, 9, 16, and 19 were selected for their similar on-target PknB activity, and their binding affinities were assessed using the commercial DiscoverX (Eurofins) KDSCREEN kinase assay service. Briefly, the kinase inhibitors were titrated in 2-fold 12-point serial dilutions and tested against PknB, Cdk4, CyclinD3, GSK3β, mTOR, and the MAP kinase Pim1 (Table 3). We observed that 1 had low-nanomolar binding affinity across the five kinases sampled. The novel inhibitors 6, 9, 16, and 19 all displayed improved selectivity for on-target PknB, with 19 showing the most promising differential (48-fold higher affinity for PknB vs Cdk4, with >100-fold affinity for PknB vs GSK3β, mTOR, and Pim1). These results suggest that the novel aminopyrimidine PknB inhibitors might have significantly reduced overall human kinase activity.

Although improvements away from Cdk2 and selected essential human kinases were made, understanding the effects across the human kinome was considered critical for compound advancement. To evaluate the global specificity profile of a representative novel aminopyrimidine, the commercial KinomeSCAN panel from DiscoverX (Eurofins) was used. This panel consists of 453 human kinases (403 wild-type and 50 mutant) as well as three pathogen kinases, conveniently including M. tuberculosis PknB. Other researchers have submitted 1 to kinome profiling, albeit with a smaller sample of the kinome available at the time, and discovered that it is fairly nonspecific. Our results corroborate this finding for 1, with a selectivity score (S-score) of 0.31 at 1% of control (S(1)) (Figure 4), indicating 99% or greater binding to 123 of 403 nonmutant human kinases at 10 μM. Analogue 9 was assessed in the same panel since the phenyl class generally had the most significant loss of Cdk2 activity (Table 2) and improvements in losing other human kinase activity (Table 3). Compound 9 demonstrated improved global on-target specificity with a selectivity score (S(1)) of 0.084 (Table S1). Comparatively, 19 showed similar improvements in selectivity score (S(1) = 0.12) relative to 1 (Figure 4 and Table S1) but with improved activity toward PknB (Table 2).

Table 3. Selectivity of PknB Inhibitors against Several Essential Human Kinases

| compd | PknB (nM) | Cdk4 | GSK3β | mTOR | Pim1 |
|-------|-----------|------|--------|------|-----|
| 1     | 26        | 4.5  | 5.3    | 44   | 63  |
| 6     | 47        | 440  | 2600   | 690  | 2200|
| 9     | 430       | 1200 | 2800   | >10000 | 5200|
| 16    | 180       | 3000 | 9300   | >10000 | >10000|
| 19    | 19        | 910  | 3800   | 5800 | 2200|

Binding of 9 and 19 to several centrally important human kinases was either severely reduced or lost, including Cdk2 and Cdk4 (Table S2), which complements the early-stage ATP competitive biochemical results for Cdk2 (Table 2).

Microbiological entry and inhibition are difficult to predict but remain a crucial part of any antibiotic development project. Other groups have developed potent PknB inhibitors, but despite achieving biochemical activity (Ki) of less than 1 nM, the microbiological activity has not improved beyond the low micromolar range (~5 μM). To assess whether these inhibitors would have improved biological activity, 1 and analogues thereof were evaluated using WuXi AppTec’s services for testing against an attenuated M. tuberculosis strain, H37Ra (ATCC 25177). Compound 1 showed appreciable activity against M. tuberculosis (25 μM), and eight of the 17 novel inhibitors with PknB activities similar to that of 1 were also evaluated against this strain (Tables 4 and S1). The MICs determined for these compounds in this strain were also very similar to the results of experiments conducted in our laboratory using an auxotrophic tuberculosis strain (Table S1). Interestingly, biochemical inhibition did not appear to have a linear relationship with M. tuberculosis inhibition, suggesting that there may be differences in cellular accumulation for this class of compounds or other off-target effects. Since M. tuberculosis has 11 S/T kinases, it is possible that some inhibitors may bind those kinases and increase microbial lethality. Dual Mtb kinase inhibitors are known for PknB and PknA as well as PknD and PknG. The biochemical activity (PknB Ki = 2 nM) did correspond appropriately with the microbiological activity (~5 μM) for specific PknA/B inhibitors; however, a known Mtb-kinase-selective PknD/G inhibitor had far lower biochemical activity (IC50 of 10–50 μM for both) than microbiological activity (~2 μM). It is possible that the PknD/G inhibitor acts by a synergistic mechanism or engages in non-kinase off-target effects, but these kinases and interacting pathways are poorly understood and the subject of future research. Nonetheless, to predict whether it was likely that these compounds bound other kinases, docking studies were undertaken with available structures of PknA, PknE, PknG, and PknL. There are no known structures of PknD, but a predicted structure was modeled by threading the PknD sequence on a PknB structure and folding with the iTasser server. The docking results revealed that all of our inhibitors bound to all other kinases more poorly than they did to PknB, with an average range of 9–32% lower binding free energy (Table S1). Visualization of the poses also revealed no consensus pattern as is seen with PknB. This suggests that these compounds likely do not have off-target activity on these kinases.

Other groups have found that designing PknB inhibitors with reduced lipophilicity correspondingly improves the microbiological activity, indicating that this may impact cellular entry and/or efflux. To test whether our inhibitors follow the same pattern, we determined their LogD values at pH 7.4 and compared their relative Mtb activities and THP-1 cytotoxicities (Table 4). Pyridines 4 and 5 had the LogD values most similar to that of 1, and although their inhibition of PknB is similar to that of 1, they also showed 2–4-fold worse Mtb MIC. Compound 6 had an increased LogD (4.3 vs 3.7 for 1); however, we found that its MIC was also similar (25 μM for both 6 and 1). The phenyl inhibitors 12, 16, 19, and 20 were microbiologically active (~19 μM) and more PknB-specific relative to Cdk2 (IC50 > 40 μM for those shown in Table 4)
despite being more lipophilic than the pyridines. Specifically, we suspected that 20 would display worse microbiological performance than more biochemically active PknB inhibitors such as 19, but we discovered that despite showing a 3-fold loss in PknB activity relative to 19 ($K_i = 320 \text{ nM}$ for 19 vs $970 \text{ nM}$ for 20), compound 20 showed a 2-fold lower MIC than inhibitor 19 (Mtb MIC = 6 μM for 20 vs 19 μM for 19). Our findings with respect to LogD and MIC indicated a trend contrary to that described by others: the best inhibitors biologically had a higher LogD than poorer-performing inhibitors (Tables 4 and S1).

Compound 20 had the best MIC of all compounds tested, but its LogD was among the worst. Despite appreciable PknB activity and low toxicity, it was not chosen for extensive follow-up because of concerns that it may exhibit off target effects due to its high lipophilicity and may pose subsequent challenges for drug development. Since PknB is essential, knockouts are not viable, and constructs altering the expression level would be analogous to altering the dose of inhibitor. The PknB homologue of S. aureus, Stk1, is not essential, and our previous work showed that the microbiological effects of compound 1 on S. aureus are not seen in the Stk1 knockout, suggesting that at least compound 1 acts on S. aureus in a specific manner. To understand whether the effects of our inhibitors on Mtb MIC are due to PknB activity or nonspecific target activity, compounds with similar properties but that have lost biochemical activity are needed. To test the hypothesis that the free piperidine amine is needed for PknB activity, two available N-Boc-protected piperidine derivatives of compounds 9 and 17 were assessed for biochemical activity. We found that Boc protection of this amine completely abolished the biochemical activity (Table S1). These compounds were even more lipophilic, with a LogD increase of at least 0.6 points, but both had completely abolished microbiological activity (Table S1). Although it is not possible to definitively conclude that the change in lipophilicity or charge did not reduce cellular entry, our efforts to alter the methylpiperazine tail with a solubilizing group predictably led to a reduction in LogD but also at the exclusion of both biochemical and microbiological activity (data not shown), further supporting this trend. We continue to investigate other possible tail modifications that will preserve biochemical activity but improve physiochemical properties.

Toxicity is a major concern with drug development, and many inhibitors fail because of cellular or organismal toxicity. To test the hypothesis that increasing the specificity of these

Table 4. Enzyme Inhibition Relating to LogD, MIC, and Cytotoxicity

| compd | PknB $K_i$ (nM) | LogD $\Delta t_{4}$ | Mtb MIC (μM) | THP-1 CC50 (μM) | % D. rario survival @ 5 μM |
|-------|----------------|--------------------|-------------|----------------|--------------------------|
| 1     | 420            | 3.71               | 25          | <0.05          | 0                        |
| 4     | 470            | 3.53               | 100         | 1.06           | 56                       |
| 5     | 580            | 3.57               | 50          | 0.39           | 30                       |
| 6     | 290            | 4.3                | 25          | 1.14           | 89                       |
| 9     | 1300           | 4.8                | 25          | 4.10           | 96                       |
| 12    | 440            | >5.85              | 12          | 4.94           | 0                        |
| 16    | 1200           | >6                 | 12          | 5.28           | 57                       |
| 19    | 320            | 4.5                | 19          | 3.89           | 0                        |
| 20    | 970            | >5.7               | 6           | 3.04           | 100                      |

Figure 4. Treespot diagrams from the DiscoverX KinomeSCAN of Compounds 1 and 19 (structures are shown for reference). Inhibitors were tested at 10 μM.
inhibitors toward PknB and away from human Cdkss would reduce their toxicity, a screen was done in the human THP-1 macrophage cell line. This line was chosen since M. tuberculosis invades human macrophages and this cell line is commonly used to test for human cytotoxicity in drug development.40 The data showed that the novel aminopyrimidines had generally lower toxicity over the initial hit 1, with several analogues, including 16, having over a 2 log unit improvement in cytotoxicity (Tables 3 and S1). This does support the hypothesis that a reduction in human kinase activity leads to lower the toxicity. Future efforts will be pursued to further reduce THP-1 cytotoxicity relative to microbiological activity.

Cellular toxicity is a valuable high-throughput method for inhibitor screening early in the development process; however, animal models are often the definitive test for any drug lead. Although mouse models would be premature at this stage of development, a small medium-throughput animal model may help prioritize compounds to advance to later testing stages. Zebrafish (Danio rerio) have a kinome with a median catalytic domain identity of 75% to the human kinome, allowing it to be used as an accurate model for kinase inhibitor toxicity.59 Since the embryos develop rapidly, most kinases are also expressed and active, providing the largest possible pool to assess for side effects. The small size of these embryos and ease of testing make this a suitable model for early inhibitor assessment. Two-day-old embryos were bathed in buffer containing novel aminopyrimidines at three different concentrations for 5 days and checked daily for death or other detrimental morphological changes. Novel aminopyrimidines were generally less toxic than 1, with several inhibitors (6, 9, and 20) showing 5 day survival at 5 μM at or near 100% (Tables 4 and S1). The embryos also showed lower rates of death and a dose response to inhibitor concentration (Figure S1). Interestingly, while compound 19 showed improved kinase selectivity and THP-1 cytotoxicity relative to 1, the zebrafish toxicity was the same as that of 1. This result is not entirely surprising, as nitroaromatics can give rise to metabolites with severe toxicities.40 Gratifyingly, compound 20 with best microbiological activity (6 μM) showed >60-fold improvement in THP-1 cytotoxicity relative to 1 and 100% zebrafish survival at a dose of 5 μM. Collectively, these data suggest the importance of designing for selectivity and early assessment of toxicity in the drug development pipeline.

Overall, we identified a hit PknB inhibitor (1) from the PKIS libraries (~1000 compounds) that had appreciable promiscuity across the human kinome and considerable toxicity. We were able to successfully develop the first iteration of a novel class of PknB inhibitors using the aminopyrimidine scaffold from 1. Despite having high lipophilicity, these novel PknB inhibitors collectively showed improvements in microbiological activity, toxicity, and kinase specificity over the initial hit. Specifically, some of the phenyl-headgroup-based inhibitors, exemplified by 19, allowed for the best reductions in off-target effects while maintaining PknB/M. tuberculosis activity. We found that compounds with high lipophilicity maintained their microbiological activity and even reduced their cytotoxicity and toxicity toward zebrafish, suggesting that general conclusions on lipophilicity with regard to microbial efficacy and cellular and organismal toxicity are difficult to predict. Our continuing efforts on this class of PknB inhibitors will focus on increased selectivity and microbiological activity with further-reduced toxicity. This will continue to demonstrate the feasibility of developing PknB inhibitors as a potential new class of antimicrobial drugs targeting this essential bacterial kinase.

ASSOCIATED CONTENT
Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsmedchemlett.0c00580.
Methods and Figure S1 (PDF)
Table S1 (XLSX)
Table S2 (XLSX)
NMR spectra (PDF)

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N.W. conceived, managed, and planned the project, performed experiments, collected and analyzed data, assisted with obtaining funding and designing compounds, and wrote the manuscript. J.B.F. designed compounds, managed synthesis and validation, assisted with experimental outsourcing, collected and analyzed data, and wrote the manuscript. Z.Y. and G.Y. synthesized compounds and collected data. J.B. and
R.P. performed experiments and collected data. J.E.G. provided direction and management for synthesis and design and edited the manuscript. R.S. conceived, managed, and planned the project, obtained funding, and edited the manuscript.

Notes

The authors declare the following competing financial interest(s): N.W. and R.S. are inventors on U.S. Patent 9,540,369, Use of Kinase Inhibitors to Increase the Susceptibility of Gram+ Bacteria to beta-Lactam Antibiotics. N.W., J.B.F., J.E.G., and R.S. are inventors on U.S. Patent 62,972,349, Inhibitors of Bacterial PASTA Kinases.

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ABBREVIATIONS

Mt, Mycobacterium tuberculosis; PknB, protein kinase B; Cdk, cyclin-dependent kinase; PASTA, penicillin binding and S/T associated; Stk1, serine threonine kinase 1; PKIS, Published Kinase Inhibitor Set; Gsk3β, glycogen synthase kinase 3β; mTOR, mammalian target of rapamycin; Pim1, pro-viral integration site for Moloney murine leukemia virus 1

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