Design, Synthesis, and Biological Evaluation of Densely Substituted Dihydropyrido[2,3-c]pyrazoles via a Taurine-Catalyzed Green Multicomponent Approach

Ghanshyam Mali, Badrodin A. Shaikh, Shivani Garg, Akhilesh Kumar, Sudipta Bhattacharyya, Rohan D. Erande, and Asha V. Chate*

ABSTRACT: An efficient taurine-catalyzed green multicomponent approach has been described for the first time to synthesize densely substituted therapeutic core dihydropyrano[2,3-c]pyrazoles. Applications of the developed synthetic strategies and technologies revealed the synthesis of a series of newly designed 1,4-dihydropyrano[2,3-c]pyrazoles containing isonicotinamide, spirooxindole, and indole moieties. Detailed in silico analysis of the synthesized analogues revealed their potential to bind wild-type and antibiotic-resistant variants of dihydrofolate reductase, a principal drug target enzyme for emerging antibiotic-resistant pathogenic Staphylococcus aureus strains. Hence, the synthesized dihydropyrano[2,3-c]pyrazole derivatives presented herein hold immense promise to develop future antistaphylococcal therapeutic agents.

INTRODUCTION

Pyra[2,3-c]pyrazole is one of the most important classes of bioactive heterocycles among pyranopyrazoles possessing a unique 4H-pyran ring fused with pyrazole, which are well known for their medicinal importance and applications in myriad agrochemicals and pharmaceutical ingredients.1 In view of the pharmaceutical ingredients, several drug candidates containing the dihydropyrano[2,3-c]pyrazole skeleton display an extremely wide range of biological activities such as antibacterial,1 antimicrobial,3 antianaphylactic,4 antiallergenic,5 antiproliferative,6 antitumor,7 cytotoxic,8 mutagenic,9 sex pheromonal,10 anti-inflammatory,11 hypoglycaemic,12 analgesic,13 molluscicidal,14 anticoagulant,15 spasmolytic,16 diuretic,17 human Chk1 kinase inhibitory,18 and UV absorber19 properties and different mounting phases for their clinical trials. Their fungicidal, bactericidal, and herbicidal properties facilitate their significant role in agrochemical research.8 They are found to be novel selective PPARγ agonists with partial binding properties and candidates for the treatment of type II diabetes. In line, a broad scale of anticancer activity was also reported by Adibi et al.19 and Kamel independently.20 In vitro anticancer activity against human liver carcinoma (HepG2), human mouth carcinoma (KB), human colon adenocarcinoma (SW48), and human lung carcinoma (A549) and reports against human gastric cancer (NUGC), human colon cancer (DLD1), human liver cancer (HA22T and HEPG2), nasopharyngeal carcinoma (HONE1), and human breast cancer (MCF) show the importance of pyranopyrazoles and its synthetic demand.21,22 The broad-scale pharmaceutical applications of pyra[2,3-c]pyrazole including its appearance in numerous biologically important scaffolds manifest its significant demand worldwide (Figure 1). Moreover, it opened a new door for organic synthesis, which led to several elegant methodologies for its rapid construction. Among all the
Motivated with the recent report on developing the multicomponent reaction\(^7\) and our ongoing research interest, we describe here full details of our effort to overcome drawbacks from the previous report and further expansions of this method. In this manuscript, for the first time, we utilized taurine as a catalyst for a green multicomponent approach for the synthesis of a densely substituted 1,4-dihydropyran-2,3-c-pyrazole framework. Considering its wide range of medicinal uses, we performed in silico molecular docking of synthesized dihydropyran-2,3-c-pyrazole derivatives with staphylococcal wild-type and antibiotic (trimethoprim)-resistant variants of dihydrofolate reductase (DHFR) enzymes. Intriguingly, the specific high-affinity binding of synthesized derivatives was observed at trimethoprim binding sites. Moreover, the binding of the synthesized ligands to staphylococcal DHFR was found to be unaffected by trimethoprim resistance-causing mutations. Overall, the biological outcome of the synthesized dihydropyran-2,3-c-pyrazole derivatives presented in this work holds immense promise to develop future antistaphylococcal therapeutic agents.

## RESULTS AND DISCUSSION

Encouraged by our earlier report on the taurine-catalyzed multicomponent reaction for the synthesis of spirooxindole, dihydroquinazolinones, and novel 1,2-(dihydroquinazolin-3-(4H)isonicotinamides,\(^8\) we became interested in examining similar conditions on the multicomponent reaction of benzaldehyde 1, malononitrile 2, hydrazine hydrate 3, and ethyl acetooacetate 4, mimicking the reported methods to check the yield and selectivity toward the formation of dihydropyran-2,3-c-pyrazoles. Next, we screened protic and aprotic solvents on the reaction. Obviously, protic solvents were better for the reaction, especially when applied, because they stabilize the carbocation intermediate, a polar protic solvent, such as ethanol, methanol and water, have a permanent dipole, which means that the delta negative (partial negative charge) on the molecule will have dipole-dipole interactions with the carbocation, stabilizing it, and this protic solvent can interact electrostatically with the nucleophile, thereby stabilizing it. This reduces the reactivity of the nucleophile, which favors a reaction. To our delight, the reaction underwent smoothly with the catalytic amount of taurine in CH\(_3\)CN at 80 °C for 3 h that afforded 1,4-dihydropyran-2,3-c-pyrazole 5 selectively in 44% yield (see entry 1, Table 1). To further establish the optimal conditions for better comparison of the reactions and selectivity in product formation, screening of different catalysts and solvents and temperature variations were performed. Notably, the results obtained with changing the solvents from CH\(_3\)CN such as toluene, EtOH, MeOH, and a H\(_2\)O/EtOH mixture furnished the required products but in marginal yields (entry 2–6, Table 1). Interestingly, the taurine-catalyzed multicomponent reaction in water medium delivered the required product in the highest yield of 92%. Moreover, screening of disparate catalysts such as p-TSA, AcOH, Cu(OTf)\(_2\), and \(\beta\)-cyclodextrin (\(\beta\)-CD) by keeping water as a green solvent did not increase the yield. The best results obtained were of the taurine-catalyzed water-mediated reaction at 80 °C that gave 92% yield with excellent selectivity (see entry 7, Table 1). It is worth mentioning that there is not even a trace amount of formation of another isomer 5a’ observed; instead, the reaction...
proceeded selectively that led to 1,4-dihydropyrano[2,3-c]pyrazole 5a as the only product. Taurine (2-amino-ethanesulfonic acid) is a \( \beta \)-amino acid, a kind of sulfur-containing amino acid which does not participate in the biosynthesis of protein but considered as a conditionally semi-essential amino acid for mammals. In addition to this, it improves water solubility and is capable of forming H-bonds, while its strong inductive effect can be utilized to tune pK\(_a\) values of adjacent or remote amino groups. The experiment was also carried out without any catalyst, where all the components were heated in H\(_2\)O at 80\(^\circ\)C for 24 h that resulted in no product formation; hence, the requirement of the catalyst is indispensable in the reaction (Table 1, entry 12).

On the basis of these observations, it can be concluded that water favors the formation of product 5a. It was found that 10–15 mL of water was sufficient for the formation of the final product. To substantiate the abovementioned experimental observation, magnificently high yields, structural determination, and selectivity, the reaction was further expanded to synthesize a number of novel analogues (5\( - \)7).

Table 1. Green Multicomponent Approach for the Synthesis of 1,4-Dihydropyrano[2,3-c]pyrazole\(^a\)

| entry | catalyst (10 mol %) | solvent\(^b\) | temp. (°C) | time (h) | yield\(^c\) 5a (%) |
|-------|---------------------|---------------|------------|----------|-------------------|
| 1     | taurine             | CH\(_3\)CN     | 80         | 3        | 44                |
| 2     | taurine             | toluene       | 120        | 5        | 61                |
| 3     | taurine             | MeOH          | 80         | 2        | 79                |
| 4     | taurine             | EtOH          | 80         | 3        | 84                |
| 5     | taurine             | EtOH/H\(_2\)O (9:1) | 80–100   | 12       | 68                |
| 6     | taurine             | EtOH/H\(_2\)O (1:9) | 80–100   | 2        | 74                |
| 7     | taurine             | H\(_2\)O      | 80         | 2        | 92                |
| 8     | p-TSA               | H\(_2\)O      | 80         | 24       | 30                |
| 9     | CH\(_3\)COOH\(^d\) | H\(_2\)O      | 80         | 24       | 72                |
| 10    | Cu(OTf)\(_2\)       | H\(_2\)O      | 80         | 3.5      | 80                |
| 11    | \( \beta \)-CD     | H\(_2\)O      | 80         | 2        | 76                |
| 12    | H\(_2\)O            | H\(_2\)O      | 80         | 24       | 0                 |

\(^a\) Reaction conditions: stoichiometric ratio (1.0 mol) of all the reactants. \(^b\) Solvent 10–15 mL. \(^c\) Isolated yields. \(^d\) Glacial acetic acid added at 1 mL per 1.0 mol reactant.

Having optimized reaction conditions in hand, we next focused on exploring the substrate scope and checking the generality of the reaction; simple hydrazine hydrate 3a was planned to be replaced with isoniazid 3b (Scheme 3). Isoniazid is also known as isonicotinic acid hydrazide (INH), used as an antibiotic for the treatment of tuberculosis. The remarkable biological properties of isoniazid prompted us to synthesize a new class of nicotinamide-based pyranopyrazoles. To our delight, one-pot four-component reactions of ethyl acetoacetate 4a, malononitrile 2a, isoniazid 3b, and assorted aldehydes (1a–1k) under optimized conditions resulted in the newly designed products 6a–6k in 72–97% yield (Scheme 3). The developed strategy was well-tolerated to both the directing groups on their benzene ring. Aldehydes 1b–1f containing electron-deactivating groups (4-F, 4-Cl, 4-Br, 4-NO\(_2\), and 2,6-dichloro derivatives of benzaldehyde) and aldehydes 1g–1k...
containing electron-activating groups (4-OH, 4-OH-3-OMe, 2,4,6-trimethoxy derivatives of benzaldehyde, 1H-indole-3-carbaldehyde, and 1-methoxy-2-naphthaldehyde) afforded the corresponding products 6a–6k in excellent yields (72–97%; Scheme 3). The structure of all the newly synthesized nicotinamide 3b-based 1,4-dihydropyrano[2,3-c]pyrazoles 6a–6k was unambiguously established using spectroscopic analysis techniques (1H and 13C NMR, IR, and mass spectrometry).

Inspired by the results obtained, we turned our attention to the development of innovative spiro-analogues of 1,4-dihydropyrano[2,3-c]pyrazole 7a (Scheme 4). Thus, we decided to replace the aldehyde moiety with bioinspired group isatin 1m and anticipated that it would lead to novel spiro 1,4-dihydropyrano[2,3-c]pyrazole framework 7a in respect to the change in hydrazine 3a. Isatin 1m is a well-known natural product found in plants and humans, which is also a building block in the synthesis of numerous biologically active compounds and their spiro-congeners possessing antiviral, anti-HIV, antitumor, and antitubercular properties. Interestingly, taurine-catalyzed water-mediated one-pot four-component reactions of ethyl acetoacetate 4a, isatin 1m, malononitrile 2a, and hydrazine hydrate 3a under optimized conditions furnished a well-designed novel fused spirooxindole 1,4-dihydropyrano[2,3-c]pyrazole 7a with 79% yield. The structure of the synthesized novel architecture 7a was confirmed from its spectroscopic data (1H and 13C NMR, IR, and HRMS).

Recycling of the Catalyst. The recyclability of the taurine catalyst was studied for the synthesis of 1,4-dihydropyrano[2,3-c]pyrazoles (6a) under the optimized conditions. After completion of the reaction, the catalyst was separated from the reaction mixture by simple filtration, and the filtrate was cooled at 5 °C; the white shiny taurine reappeared and was then filtered directly, dried, and applied for repeated reactions for the same transformation under the same reaction conditions. Accordingly, the catalyst can be reused for a minimum of three times with little deactivation, still being an essential aspect of green chemistry (Figure 2).

Plausible Mechanism. A plausible mechanism for the abovementioned taurine-catalyzed green multicomponent reaction of ethyl acetoacetate 4a, hydrazine hydrate 3b, benzaldehyde 1a, and malononitrile 2a is depicted in Scheme 5. Naturally occurring β-amino sulfonic acid called taurine is acidic in nature (pKₐ = 1.5), compared to carboxylic acid, and exists in its zwitterion form in water. Taurine plays an important role as a bifunctional donor−acceptor reagent, in which the carbonyl electrophile site gets activated by taurine and then attacked by the negatively activated group in the nucleophile.33,34 Accordingly, the reaction was presumably triggered by taurine-activated ethyl acetoacetate 4a attacked by an activated hydrazine 3b moiety to construct the pyrazolone skeleton 4c. Subsequently, the Knoevenagel condensation occurs between taurine-activated benzaldehyde 1a and taurine-enolized malononitrile 2a, leading to the Knoevenagel adduct 2b. Next, pyrazolone 4c tautomerizes to form pyrazolol 4d, which gives Michael-type addition with the so-formed Knoevenagel product 2b to furnish condensed intermediate 4e. Finally, keto−enol tautomerization of intermediate 4e followed by its intramolecular attack on the nitrile moiety to generate pyrane derivative 4f and subsequent imine−enamine tautomerization delivered the targeted product 1,4-dihydropyrano[2,3-c]pyrazoles 6a.

Biological evaluation of synthesized dihydropyrano[2,3-c]pyrazole derivatives and structure activity analysis against staphylococcal drug target proteins:

Scheme 3. Synthesis of a Series of Novel 1,4-Dihydropyrano[2,3-c]pyrazoles

Scheme 4. Synthesis of a Series of Novel Spirooxindole 1,4-Dihydropyrano[2,3-c]pyrazoles

Figure 2. Reuse and recovery of taurine and their effect on yields.
Considering the wide range of biological applications of 1,4-dihydropyrano[2,3-c]pyrazoles, newly designed and synthesized densely substituted analogues were further evaluated for their plausible antibacterial potential through in silico molecular docking analysis against emerging antibiotic-resistant *Staphylococcus aureus* drug targets. *S. aureus*, an opportunistic human pathogen, is the causative agent of a wide range of diseases, from relatively benign skin infections to potentially fatal systemic disorders.35,36 The global emergence of community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains along with recently identified vancomycin-resistant strains (VRSA) leaves limitedly few antibiotics to treat this superbug.37 This situation got further worse as MRSA strains have also been found to acquire point mutations in the chromosomal dihydrofolate reductase gene to become trimethoprim-resistant.38 The bacterial dihydrofolate reductase (DHFR) enzyme is one of the most widely used drug-designing targets, as the inhibition of the enzyme can block several essential biochemical pathways including biosynthesis of purines, thymidylate, glycine, methionine, pantothenic acid, and bacterial N-formyl methionyltRNA.39−41

Moreover, the active site structural difference of human and bacterial DHFR orthologues (Figure 1c) makes bacterial DHFR an ideal target for antibacterial drug-designing research. In the present work, we evaluated the plausible antistaphylococcal role of synthesized dihydropyrano[2,3-c]pyrazole derivatives through their potential to bind the staphylococcal DHFR enzyme. Intriguingly, preliminary molecular docking analysis clearly indicates strong binding of a couple of synthesized dihydropyrano[2,3-c]pyrazole derivatives at the active site of wild-type and trimethoprim-resistant variants of staphylococcal DHFR.

In order to assess the plausible antibacterial potential of synthesized novel dihydropyrano[2,3-c]pyrazole derivatives, a ligand-based drug-screening approach was taken into consideration using the drug target library of MRSA. The preliminary in silico experiments identified three potential drug target enzymes (Supporting Information Table S1), among which DHFR is one of the crucial enzymes, which has been targeted before and recently suffers from emergence of drug-resistant mutations. Initial reverse docking experiments shows DHFR as one of the best targets of synthesized novel dihydropyrano[2,3-c]pyrazole derivatives in terms of free energy of binding. Accordingly, the three-dimensional coordinates of *S. aureus* wild-type DHFR (PDB ID: 2w9g) and its trimethoprim-resistant variant, S1DHFR [(Phe98Tyr + Gly43Ala) PDB ID: 2w9s], were used for targeted docking experiments with all synthesized dihydropyrano[2,3-c]pyrazole derivatives. Among all tested dihydropyrano[2,3-c]pyrazole derivatives, 6a and 6e exhibited an excellent mode of binding as indicated by their free energy of binding (Table 2 and Supporting Information Table S1).

Moreover, both of these molecules manifested an almost equal ability to bind both wild-type DHFR and S1 DHFR (Table 2). Molecular interaction analysis shows that a wide

![Scheme 5. Plausible Mechanism for the Formation of 1,4-Dihydropyrano[2,3-c]pyrazoles](image)

### Table 2. In Silico Molecular Docking-Based Binding Energy Calculation of Dihydropyrano[2,3-c]pyrazole Derivatives against Staphylococcal Drug Target Enzyme DHFR and Its Trimethoprim-Resistant Variant S1DHFR

| docked dihydropyrano[2,3-c]pyrazole derivatives binding energy (Kcal/mol) | compound | S. aureus wild-type DHFR (PDB ID: 2w9g) | S. aureus S1 DHFR (PDB ID: 2w9s) |
|------------------------------------------------|----------|---------------------------------|---------------------------------|
| 6a                                             | −8.8     | −7.5                            |
| 6e                                             | −8.8     | −8.7                            |

Considering the wide range of biological applications of 1,4-dihydropyrano[2,3-c]pyrazoles, newly designed and synthesized densely substituted analogues were further evaluated for their plausible antibacterial potential through in silico molecular docking analysis against emerging antibiotic-resistant *Staphylococcus aureus* drug targets. *S. aureus*, an opportunistic human pathogen, is the causative agent of a wide range of diseases, from relatively benign skin infections to potentially fatal systemic disorders.35,36 The global emergence of community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains along with recently identified vancomycin-resistant strains (VRSA) leaves limitedly few antibiotics to treat this superbug.37 This situation got further worse as MRSA strains have also been found to acquire point mutations in the chromosomal dihydrofolate reductase gene to become trimethoprim-resistant.38 The bacterial dihydrofolate reductase (DHFR) enzyme is one of the most widely used drug-designing targets, as the inhibition of the enzyme can block several essential biochemical pathways including biosynthesis of purines, thymidylate, glycine, methionine, pantothenic acid, and bacterial N-formyl methionyltRNA.39−41

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Moreover, both of these molecules manifested an almost equal ability to bind both wild-type DHFR and S1 DHFR (Table 2). Molecular interaction analysis shows that a wide
Figure 3. Potential role of dihydropyrano[2,3-c]pyrazole derivatives as the plausible inhibitors of staphylococcal dihydrofolate reductase (DHFR). [a] Molecular docking of a dihydropyrano[2,3-c]pyrazole derivative (6e shown as Ligand) with *S. aureus* wild-type DHFR. The left panel shows the position of the docked ligand at the DHFR active site. The middle panel shows the zoomed-in view of the ligand interactions with the DHFR active site amino acid residues in the 3D space. The right panel shows 2D representation of the array of ligand–protein interactions. Hydrogen bond formation is indicated by the green dotted line, whereas hydrophobic interactions are indicated by the spiked arcs. [b] The docked dihydropyrano[2,3-c]pyrazole derivative (6e) can act as a trimethoprim (TMP) resistance-resistant competitive inhibitor. The left panel shows the relative position of the docked ligand to trimethoprim and NADPH bound to the active site of *S. aureus* wild-type DHFR (PDB ID: 2w9g). The positions of the active site guarding “Met20 loop” and two amino acid mutation hotspot residues responsible for trimethoprim resistance are also indicated. The middle panel shows 2D representation of the trimethoprim interaction with the active site of *S. aureus* wild-type DHFR. Hydrogen bond formation is indicated by the green dotted line, whereas hydrophobic interactions are indicated by the spiked arcs. The right panel shows the superimposition of dihydropyrano[2,3-c]pyrazole derivative (6e)-docked conformations of *S. aureus* wild-type DHFR (PDB ID: 2w9g) and the trimethoprim-resistant DHFR variant, S1DHFR (PDB ID: 2w9s). The same ligand binds equally well with little self-conformational alterations at the active site of wild-type DHFR and S1DHFR. The relative positions of amino acid mutations responsible for trimethoprim resistance and the Met20 loop have been indicated. [c] Difference in staphylococcal and human DHFR in terms of nonconservation of active site amino acid residues (indicated by a blue star for staphylococcal DHFR active site amino acids). The difference of the active site amino acid residues between staphylococcal and human DHFR counterparts and the indispensability of staphylococcal DHFR justify its potential as the antistaphylococcal drug target enzyme.
array of hydrophobic and salt bridge interactions stabilize 6e at the trimethoprim drug binding site of staphylococcal wild-type DHFR (Figure 3a all panels and Figure 3b left panel). This may indicate that just like trimethoprim, 6e may competitively replace the DHFR substrate, dihydrofolate.42,43 However, trimethoprim docking of 6e causes steric hindrance with the nicotinamide moiety of bound NADPH (Figure 2b right panel). Steric hindrance due to 6e ligand binding with NADPH at the active site of S1DHFR may abolish the need for an NADPH-mediated positive cooperative effect for inhibitor binding as observed for trimethoprim binding.43 Importantly the loss of NADPH-mediated binding synergy with incoming trimethoprim is found to play a pivotal role in the decreased trimethoprim binding potency of S1DHFR.37 Furthermore, the docked 6e at the wild-type (Figure 3a right panel) DHFR active site lacks the Leu5 main chain peptide carbonyl oxygen-mediated hydrogen bonding of the N7 atom of bound trimethoprim (Figure 3b middle panel). The lack of Leu5 carbonyl oxygen-mediated hydrogen bonding with docked 6e may render it unresponsive toward Phe98Tyr mutation, which was found to decrease trimethoprim binding to DHFR by engaging the Leu5 carbonyl oxygen to form a hydrogen bond with the O6 atom of Tyr98.35 Altogether, the unique interaction pattern exorted by one of the synthesized dihydropyrano[2,3-c]pyrazole derivatives (6e), in contrast to trimethoprim at the active site of antistaphylococcal drug target enzyme DHFR, may clearly suggest its ability to withstand the molecular perturbations brought in by trimethoprim-resistant DHFR mutations. These findings further justify the almost equal ability of 6e to bind wild-type and trimethoprim-resistant S1DHFR (Figure 3b right panel and Table 2).

**CONCLUSIONS**

Here, we have reported the taurine-catalyzed green multi-component approach for the first time to synthesize densely substituted therapeutic core dihydropyrano[2,3-c]pyrazoles. In line, we have designed and synthesized a series of their synthetic congeners including several novel architectures containing isonicotinamide, spiroindole, and indole moieties and evaluated them for biological application. Detailed in silico-based structure activity analysis of the docked dihydropyrano[2,3-c]pyrazole derivatives with the staphylococcal drug target enzyme, DHFR, may divulge their potential role as future drug resistance-resistant therapeutic leads against multiple-antibiotic-resistant *S. aureus*. The developed protocol is robust and could be applied in various organic transformations to achieve complexity of the targeted architecture in a one-pot manner. The effort toward the construction of such a bioactive heterocycle and obtaining its single-crystal data is ongoing and would be disclosed in due course.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04773.

Experimental procedures, full characterization for all new compounds 5–7, and in silico evaluation of all compounds (PDF)

**AUTHOR INFORMATION**

**Corresponding Author**
Asa V. Chate – Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431004, India; Email: chateasha2@gmail.com

**Authors**
Ghanshyam Mali – Department of Chemistry, Indian Institute of Technology Jodhpur, Jodhpur 342037, India
Badrodin A. Shaikh – Department of Chemistry, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad 431004, India
Shivani Garg – Department of Bioscience and Bioengineering, Indian Institute of Technology Jodhpur, Jodhpur 342037, India
Akhilesh Kumar – Department of Chemistry, Indian Institute of Technology Kanpur, Kanpur 208016, India
Sudipta Bhattacharyya – Department of Bioscience and Bioengineering, Indian Institute of Technology Jodhpur, Jodhpur 342037, India
Rohan D. Erande – Department of Chemistry, Indian Institute of Technology Jodhpur, Jodhpur 342037, India;

**Author Contributions**
A.V.C. and B.A.S. performed the experiments and characterized data; S.B. and S.G. performed the biological evaluation and docking studies; A.K. worked to get the crystal data; A.V.C. and B.A.S. performed the experiments and characterized data; S.B. and S.G. performed the biological evaluation and docking studies; A.K. worked to get the crystal data; R.D.E. and G.M. coordinated the project and worked on writing the manuscript and the Supporting Information, and finally, all authors have given approval to the final version of the manuscript and the Supporting Information.

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**ABBREVIATIONS**

β-CD, β-cyclodextrin; DHFR, dihydrofolate reductase; INH, isonicotinic acid hydrazide; VRSA, vancomycin-resistant strains; CA-MRSA, community-associated methicillin-resistant *S. aureus*

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