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Undecaprenyl Diphosphate Synthase Inhibitors: Antibacterial Drug Leads

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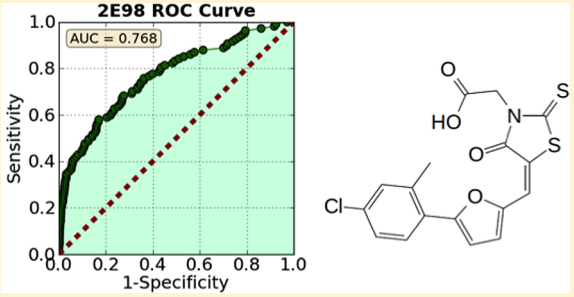
Supporting Information

ABSTRACT: There is a significant need for new antibiotics due to the rise in drug resistance. Drugs such as methicillin and vancomycin target bacterial cell wall biosynthesis, but methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) have now arisen and are of major concern. Inhibitors acting on new targets in cell wall biosynthesis are thus of particular interest since they might also restore sensitivity to existing drugs, and the *cis*-prenyl transferase undecaprenyl diphosphate synthase (UPPS), essential for lipid I, lipid II, and thus, peptidoglycan biosynthesis, is one such target. We used 12 UPPS crystal structures to validate virtual screening models and then assayed 100 virtual hits (from 450,000 compounds) against UPPS from *S. aureus* and *Escherichia coli*. The most promising inhibitors (IC_{50} ~2 μM, K_i ~300 nM) had activity against MRSA, *Listeria monocytogenes*, *Bacillus anthracis*, and a vancomycin-resistant *Enterococcus* sp. with MIC or IC_{50} values in the 0.25−4 μg/mL range. Moreover, one compound (**1**), a rhodanine with close structural similarity to the commercial diabetes drug epalrestat, exhibited good activity as well as a fractional inhibitory concentration index (FICI) of 0.1 with methicillin against the community-acquired MRSA USA300 strain, indicating strong synergism.



INTRODUCTION

The need for new antibiotics has arisen due to the widespread resistance to current drugs.[1] Despite this need, the antibiotic pipeline in the past few decades has been relatively dry in terms of new antibacterial classes when compared with progress against other diseases.[2] One strategy to fight bacterial resistance is to inhibit enzymes that are not the targets of current antibiotics but, instead, act in the same pathways as existing drugs since this might enable the restoration of drug sensitivity via combination therapy. Undecaprenyl diphosphate synthase (UPPS) is one such target. The undecaprenyl diphosphate product (UPP) is essential for bacterial cell growth because of its role in the formation of bacterial cell wall peptidoglycan,[1,3] Scheme 1, and it is not produced by humans.[2,4]

SmithKline Beecham screened their compound collection against UPPS but reported no chemically tractable low micromolar hits.[5] Novartis pursued tetramic and tetronic acids and dihydropyridin-2-ones, but noted issues associated with human serum albumin binding and a lack of in vivo activity.[6,7] Previously, we reported several potent UPPS inhibitors together with X-ray crystallographic (or modeled) binding modes for a variety of chemical classes including lipophilic bisphosphonates,[8] phthalic acids,[9] diketo acids,[10] anthranilic acids, benzoic acids,[11,12] aryl phosphonates, bis-amines, and bis-amidines.[12] The most promising of these compounds, a bis-amidine, was shown to have potent activity in biochemical assays, in cellular assays, and in a murine model of MRSA infection.[12]

Since UPPS must bind multiple substrates (IPP, FPP, or more elongated prenyl-PP intermediates) and many inhibitors are to some degree substrate mimics, it is common to observe numerous inhibitors simultaneously bound to UPPS, with up to 4 binding sites being occupied.[8] However, it is unclear whether inhibitory activity is due to binding to one specific site or to multiple sites. It has been shown that some inhibitors occupy only site 4, an allosteric site distant from the catalytic center, while others bind to site 1, the substrate binding site,[12] complicating docking studies and, regardless of the inhibitor-binding mode, the flexibility of UPPS creates challenges for

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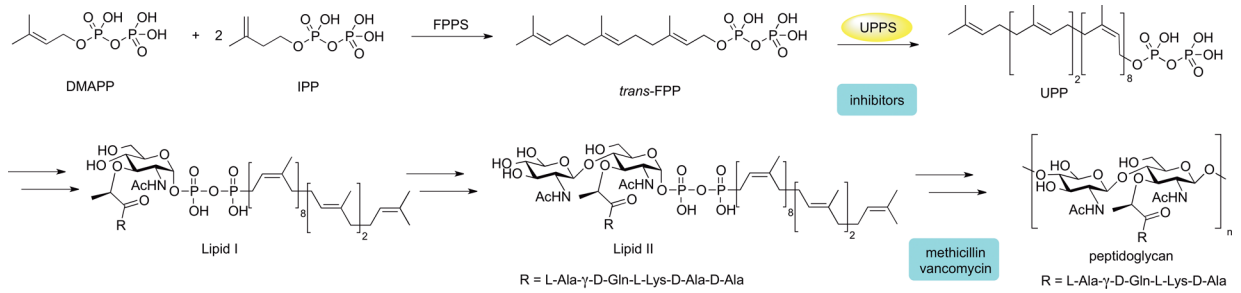
Scheme 1. Undecaprenyl Diphosphate Synthase Reaction and Relationship of UPP to Bacterial Cell Wall Biosynthesis



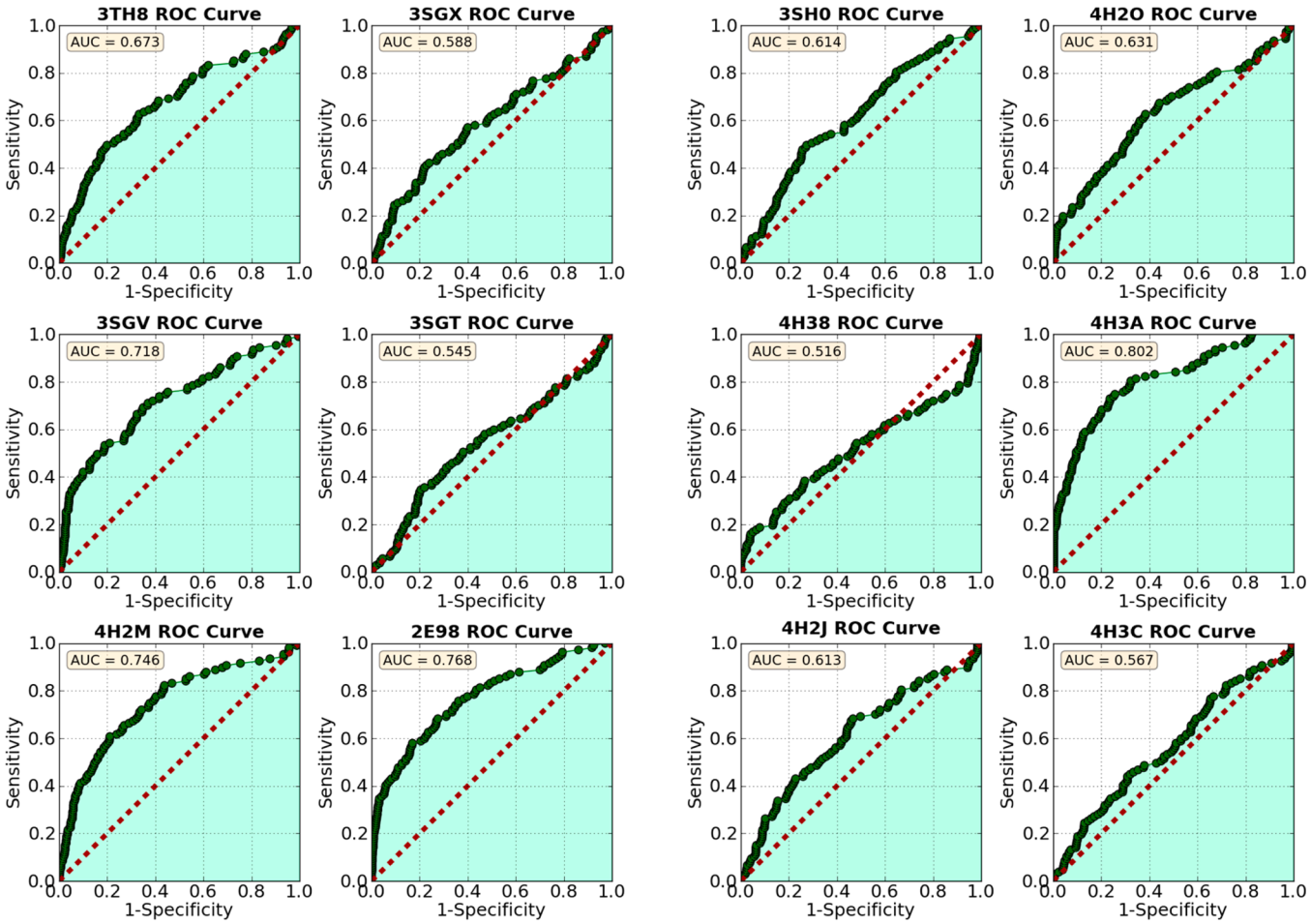


Figure 1. AUC/ROC curves for 12 EcUPPS crystal structures. 4H3A and 2E98 were chosen for further study.

virtual screening. Here, to help reduce these problems we employed the 12 crystallographic structures described in previous work[8,12] to select those that provided maximal enrichment in *retrospective* virtual screening studies. We then made *prospective* predictions using these structures, leading to novel UPPS inhibitors, some with promising antibacterial activity.

METHODS AND MATERIALS

Computational Aspects. Following the methods described in previous work,[12] we docked 112 known UPPS inhibitors having IC_{50} values <100 μM, together with 1000 decoys from the Schrödinger decoy collection (having an average molecular weight of 400 Da), to *Escherichia coli* UPPS (hereafter, EcUPPS). Docking was performed using the Glide[13−15] program, and compounds were ranked by their Glide XP score. The proteins were prepared by stripping water and ligand molecules, capping, and neutralizing any unsolved loops, followed by preparation with the Schrödinger protein preparation wizard using standard parameters.[16] After docking, compounds were ranked by their docking score, and then area under the curve (AUC) analyses were performed. Retrospective enrichment was quite good for 2/12 structures (PDB codes 2E98 and 4H3A), so we docked into these structures for the prospective studies (Figure 1). 2E98 is an EcUPPS X-ray structure containing four lipophilic bisphosphonates (BPH-629; IC_{50} ~300 nM), which bind to sites 1−4, one inhibitor to each site.[8] 4H3A is an EcUPPS structure containing a diketo acid inhibitor (BPH-1330) which has a 2 μM IC_{50}, and the inhibitor binds (in the solid state) only to site 4.[10,12] These structures thus have significant differences: only site 4 is occupied in 4H3A, while in 2E98, all four sites are occupied and the protein is in a "wide-open" conformation (Figure 2).

To find new inhibitors, we began with a library of ~450,000 commercially available compounds, the ChemBridge Experimental

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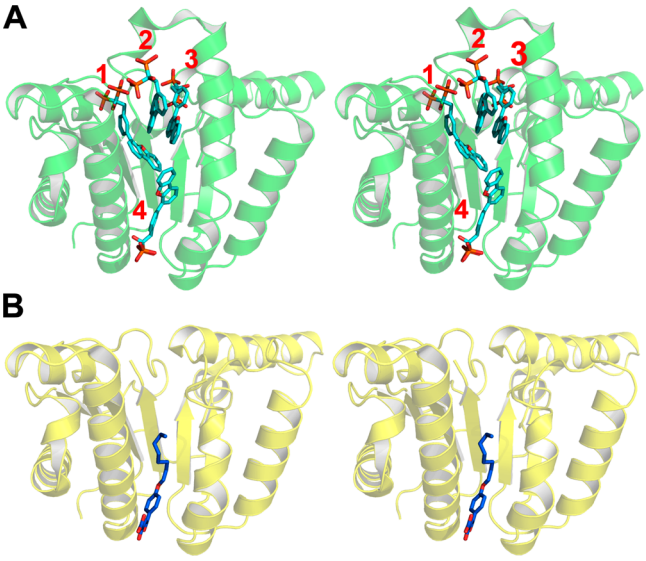

Figure 2. Stereo presentation of the X-ray structures chosen for further virtual screening from docking and ROC analysis. (A) 2E98 showing all four inhibitor binding sites. (B) 4H3A showing one inhibitor bound to site 4.

Library. The library was filtered to exclude compounds that had undesired, toxic or reactive functional groups; known promiscuous binders; MW >460 Da or MW <250 Da; more than 4 chiral centers; polar surface area (PSA) >150 Å^2 or PSA <50 Å^2; number of rotatable bonds >10; or clogP >5 or clogP ≤2. Salts were also removed. Next, the selected compounds (~100,000) were loaded into Schrödinger's virtual screening workflow, where they were prepared with Ligprep and then docked using the filtering procedure for efficiency and only retaining the top 20% of compounds in the two rapid, initial docking modules HTVS (top 20% retained) and SP (top 20% retained). Finally, Glide XP was used to assign a final docking score to each molecule. AUC analyses on active and decoy data sets were previously performed using the Glide XP module, however, this was impractical for the large filtered ChemBridge library. Therefore, we relied on HTVS and SP modules to provide early filtering before employing the more time intensive XP protocol.

We then extracted the ~400 top scoring compounds (docking score less than −7 kcal/mol). Binary Molprint2D fingerprints were generated using Canvas, and 40 clusters were generated using *K*-means clustering.[17−19] Of these 40 clusters, the top scoring compounds from each cluster were visually inspected and a representative was chosen from each cluster, resulting in a final list of 100 compounds. These were purchased from ChemBridge (ChemBridge Corporation, San Diego, CA) and then assayed for UPPS inhibition activity. Three out of the 100 compounds were UPPS inhibitors. Similarity searches based on these active compounds were then performed using PubChem and SciFinder, and additional compounds were obtained and tested.

■ ENZYME AND CELL GROWTH INHIBITION ASSAYS

Protein Expression and Purification. EcUPPS and SaUPPS were expressed and purified as described previously.[8] Molecular weights and purities were verified by mass spectrometry and SDS−PAGE, respectively.

UPPS Inhibition Screening. The UPPS inhibition assays were carried out as described previously.[8] Briefly, the condensation of FPP with IPP catalyzed by UPPS was monitored by using a continuous spectrophotometric assay[20] in 96-well plates with 200 μL reaction mixtures containing 400 μM 2-amino-6-mercapto-7-methylpurine ribonucleoside (MESG), 350 μM IPP, 35 μM FPP, 20 mM Tris·HCl buffer (pH 7.5), 0.01% v/v Triton X-100, and 1 mM $MgCl_2$. The IC_{50} values were obtained by fitting the inhibition data to a rectangular hyperbolic dose−response function using GraphPad PRISM 4.0 software (Graphpad Software, San Diego, CA). The IC_{50} values for the most active hits were verified using a radiometric assay[21] with 2.5 μM FPP, 25 μM [^{3}H]IPP, and 0.01% v/v Triton X-100.

Cell Strains. *Bacillus subtilis* subsp. *subtilis* (ATCC 6051), *Escherichia coli* (ATCC 29425), and *Saccharomyces cerevisiae* (ATCC 208352) were purchased from the American Type Culture Collection. *Bacillus subtilis* strain 168, *Bacillus anthracis* strain Sterne, *Listeria monocytogenes* strain 4b F2365, *Staphylococcus aureus* USA300 (methicillin-resistant), *E. coli* MC400, *Pseudomonas putida*, and *Enterococcus faecalis* U503 (vancomycin-resistant) were from our laboratory strain collection.[22]

***E. coli* ATCC 29425 Growth Inhibition Assay.** IC_{50} values for *E. coli* growth inhibition were determined by using a microbroth dilution method. A 16 h culture of *E. coli* was diluted 50-fold into fresh Luria−Bertani (LB) broth and grown to an OD_{600} of ~0.4. The culture was then diluted 500-fold into fresh LB medium, and 100 μL was inoculated into a 96-well flat bottom culture plate (Corning Inc., Corning, NY). The starting concentration of each compound was 0.3 mM, and this was 2-fold serially diluted. Plates were incubated for 3 h at 37 °C to midexponential phase. A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay (ATCC) was then carried out to obtain bacterial viability dose−response curves. Ten microliters of MTT reagent was added into each well, followed by incubation for 2−4 h until a purple precipitate was visible. Then, 100 μL of detergent reagent was added and plates were further incubated in the dark at 23 °C for 2 h. The absorbance was recorded at 570 nm. A nonlinear regression analysis was then carried out using Origin 6.1. For each inhibitor, two independent experiments were performed and the IC_{50} values found were averaged.

***B. subtilis* ATCC 6051 Growth Inhibition Assay.** A 16 h culture of *B. subtilis* was diluted 50-fold into fresh Luria−Bertani (LB) broth and incubated to an OD_{600} of ~0.4. The culture was then diluted 500-fold into fresh LB medium, and 100 μL was inoculated into a 96-well flat bottom culture plate (Corning Inc., Corning, NY). The starting concentration of each compound was 0.5 mM and was then serially diluted. Plates were incubated for 12−16 h at 37 °C. The absorbance was recorded at 570 nm. A nonlinear regression analysis was carried out on the data obtained using Origin 6.1. For each inhibitor, two independent experiments were performed and the IC_{50} values found were averaged.

***S. cerevisiae* Growth Inhibition Assay.** The protocol was the same as with the *B. subtilis* assay protocol except that YPD medium was used and the 96-well plate was incubated for 36 h instead of 12−16 h.

Evaluation of 1 and 4 Inhibitory Activity and Synergy. *B. subtilis* strain 168, *B. anthracis* strain Sterne, *E. coli* MC4100, and *P. putida* were grown to stationary phase in 10 mL of LB broth at 37 °C. *S. aureus* USA300 (methicillin-resistant), *E. faecalis* U503 (vancomycin-resistant), and *L. monocytogenes* strain 4b F2365 were grown to stationary phase in 10 mL of brain−heart infusion (BHI) medium at 37 °C. The cultures were adjusted to an OD_{600} of 0.016 in the designated medium before being added to 96-well microplates. Successive 2-fold dilutions of compounds **1** and **4** were added to the cultures (0.25−64 μg mL^{-1}). As a control, kanamycin (1−32 μg mL^{-1}) was added to samples of *E. coli*, *B. subtilis*, *B. anthracis*, *P. putida*,

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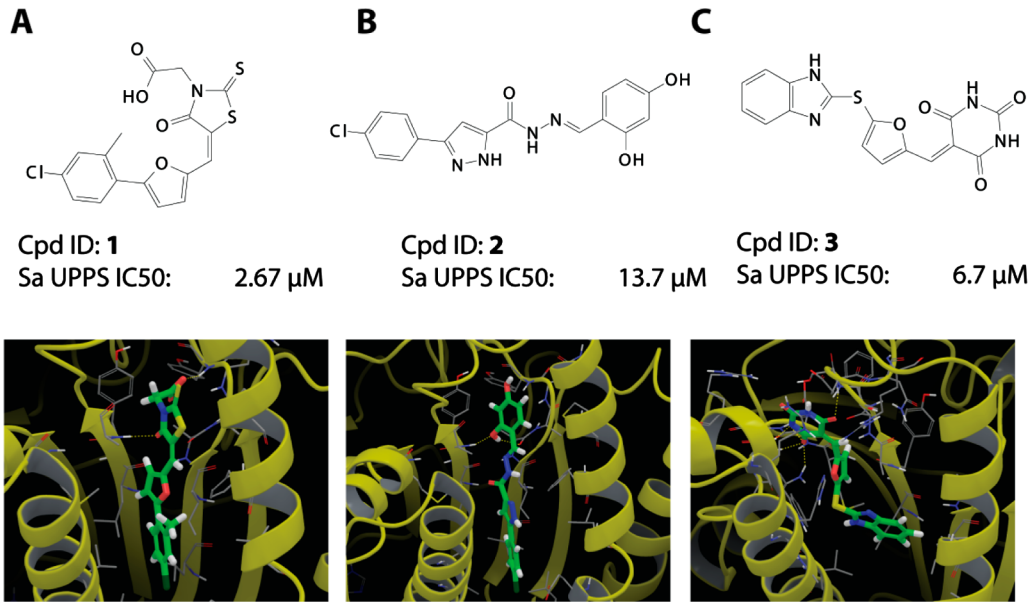

Figure 3. Three new classes of UPPS inhibitors discovered via virtual screening: (A) chemical structure and computed docking mode of compound **1**, a rhodanine derivative; (B) chemical structure and docking mode of compound **2**, a resorcinol derivative; (C) same for compound **3**, a barbiturate.

and *L. monocytogenes*. Gentamycin was used as a control for *S. aureus* and *E. faecalis* with dilutions from 1 to 64 μg mL^{-1}. As a negative control, an equal volume of DMSO lacking antibiotic was used. Plates were covered and incubated at 37 °C for 16 h with shaking. The minimum inhibitory concentration (MIC) that suppressed at least 99% of bacterial growth was established based on culture turbidity in the microbroth dilution assay. The assay was repeated in three replicates, and values were averaged, or a range was reported. Isobolograms were carried out as previously described.[10,23]

INHIBITOR CHARACTERIZATION

The purities of the key compounds investigated, obtained from ChemBridge (**1** and **4**), were determined by high-performance liquid chromatography and structures verified by NMR spectroscopy and high resolution mass spectrometry (Supporting Information Figures S2−S9) and were consistent with the structures provided by the vendor. Purities were >95% by HPLC.

RESULTS AND DISCUSSION

In previous work, we obtained moderate correlations between enzyme inhibition activity and docking scores within a congeneric series of UPPS inhibitors (lipophilic bisphosphonates)[8,24] using docking methods, so we first examined whether we could obtain similar correlations between docking scores and experimentally determined IC_{50} values for the 112 known actives. There was no significant correlation between docking scores and pIC_{50} values ($pIC_{50} = -\log IC_{50}$), Figure S1 in the Supporting Information. The wide variety of potential binding modes (sites 1, 2, 3, and 4[8,12]) and protein conformations would be expected to make it difficult to achieve a good correlation between a scoring function and the experimentally determined pIC_{50} values, in addition to the assumptions made in scoring functions that cause inaccuracy when compared to experimental affinities. Nevertheless, docking studies can provide enrichment of active compounds from large libraries, even though docking scores rarely correlate well with activity when structurally diverse compounds are involved. We thus next employed an area-under-the-curve (AUC) analysis, also known as the receiver-operating-characteristic (ROC), a method that has been shown to be useful in validating structure-based virtual screening protocols[25] and is a standard method for evaluating such protocols.[26]

We therefore tested 12 EcUPPS X-ray structures for their ability to separate actives (IC_{50} <100 μM) from decoys (presumed inactive compounds in the decoy library). Several EcUPPS X-ray structures showed a good separation of active from decoy compounds, with AUC values of ∼0.8. These structures also demonstrated early enrichment, as evidenced by the steep initial slope of the curve. This means that the best scores were given primarily to active compounds and suggests that, in screening a large compound library, the best scoring compounds would be enriched in UPPS inhibitors. We thus picked the two X-ray structures (PDB codes 2E98 and 4H3A) that provided significant early enrichment and a high AUC in the validation studies, for predictive studies. Using these two X-ray structures, we screened the ChemBridge EXPRESS-pick compound library (after filtering) and determined ∼400 hits with GlideXP scores less than −7 kcal/mol (lower energy is better). Since many highly ranked compounds were chemically very similar, we clustered the top scoring compounds and selected representatives from each cluster to ensure chemical diversity among the compounds to be tested.

Discovery of Novel UPPS Inhibitor Cores. The screening of the ChemBridge EXPRESS-pick compound library using the validated docking protocol resulted in the discovery of three new UPPS inhibitor classes: the 4-oxo-2-thioxo-1,3-thiazolidines, also known as rhodanines (e.g., compound **1**), dihydroxyphenyls (the resorcinol, compound **2**), and pyrimidinetrione (a barbiturate analogue, compound **3**). None of these have been previously reported to be UPPS inhibitors. All three compounds are predicted to bind in either site 1 or 3 of the 2E98 crystal structure (Figure 3), although X-ray crystallographic studies will be required to confirm this binding mode (and our attempts to obtain crystal structures of these systems have not been successful). In any case, the three new inhibitors discovered represent UPPS inhibitors with "drug-

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Table 1. 4-Oxo-2-thioxo-1,3-thiazolidines Investigated in UPPS and Bacterial Cell Growth Inhibition Assays[a]

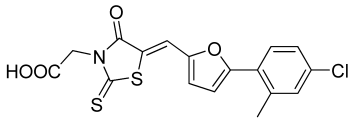
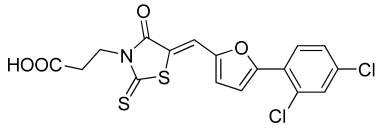
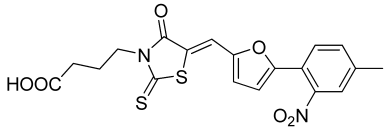
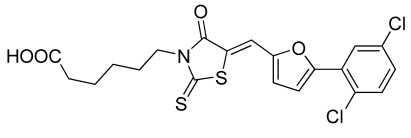
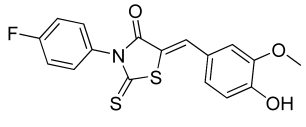
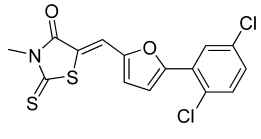
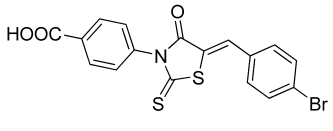
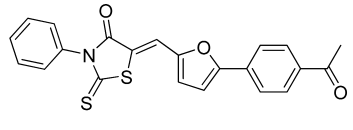
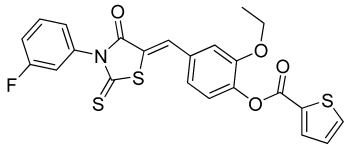
ID	Structure	Vendor #	EcUPPS IC_{50}	SaUPPS IC_{50}	*B. subtilis* IC_{50}	*E. coli* IC_{50}	*S. cerevisiae* IC_{50}
1		CB 7471392	260	2.7	2.9	>200	>200
4		CB 5523169	2.1	2.4	0.43	>200	180
5		L339644	>100	5.7	3.2	>200	>200
6		L339822	>100	3.8	5.6	>200	>200
7		CB 5674456	41	150	>200	>200	>200
8		CB 5143717	>200	>200	>200	>200	>200
9		CB 5280379	>200	170	>200	>200	>200
10		CB 5377413	85	32	>200	>200	>200
11		CB 6824270	>200	>200	>200	>200	>200

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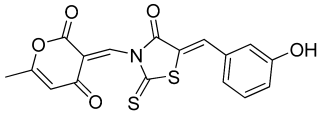
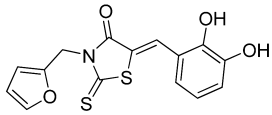
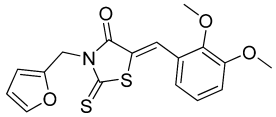
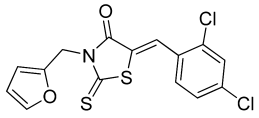
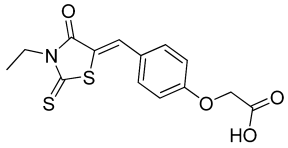
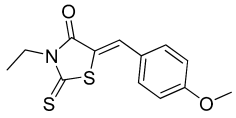
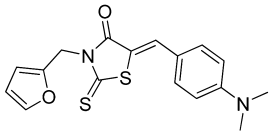
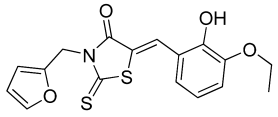
Table 1. continued

ID	Structure	Vendor #	EcUPPS IC_{50}	SaUPPS IC_{50}	*B. subtilis* IC_{50}	*E. coli* IC_{50}	*S. cerevisiae* IC_{50}
12		NCI 660017	>100	>100	>200	>200	>200
13		NCI 343985	>100	5.3	>200	>200	>200
14		NCI 337736	>100	>100	>200	>200	>200
15		NCI 320208	>100	>100	>200	>200	>200
16		NCI 90950	>100	>100	>200	>200	>200
17		NCI 36005	>100	>100	>200	>200	>200
18		NCI 320207	>100	>100	>200	>200	>200
19		NCI 318219	>100	>100	>200	>200	>200

[a]All concentrations are in μM.

like" physicochemical properties, passing the common drug-like filters as described in the Methods and Materials section. The most potent of the 3 compounds was the 4-oxo-2-thioxo-1,3-thiazolidine **1** (IC_{50} ~2.6 μM against *S. aureus* UPPS), which in an initial screen for bioactivity was also found to be active against *B. subtilis*, MIC (minimal inhibitory concentration) ~3 μg/mL (Table 1). For this reason, we chose to next investigate analogues based on the 4-oxo-2-thioxo-1,3-thiazolidine core.

Novel Core SAR. We next obtained 16 additional compounds from ChemBridge, from Sigma-Aldrich, and from the Drug Synthesis and Chemistry Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute (**4**–**19**, Table 1), containing the 4-oxo-2-thioxo-1,3-thiazolidine core and tested them for activity against SaUPPS and EcUPPS, as well as a preliminary activity screen against *B. subtilis*, *E. coli*, and *S. cerevisiae* (the latter as a general cytotoxicity control, since it

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Table 2. MIC Values for Two 4-Oxo-2-thioxo-1,3-thiazolidine Analogues, Compounds 1 and 4, Tested in Diverse Bacterial Cell Growth Inhibition Assays[a]

	Compound **1**	Compound **4**
	MIC (μg/mL)	MIC (μg/mL)
Bacillus subtilis	1	0.125
Bacillus anthracis	1-2	0.25
Staphylococcus aureus (MRSA)	8-64	4
Enterococcus faecalis (VRE)	1-8	4
Listeria monocytogenes	4	0.125
Pseudomonas putida	>64	>64
Escherichia coli	>64	>64

[a]The compounds were tested against a panel of both Gram-positive (top five) and Gram-negative (lower two) bacteria.

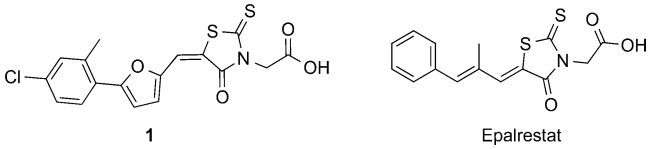
lacks UPPS). The alkyl carboxylic acid containing compounds with the 4-oxo-2-thioxo-1,3-thiazolidine core were active in assays against *B. subtilis*, and the most potent compound was **4** (an analogue of **1**). **4** was roughly equipotent against SaUPPS and EcUPPS with an IC_{50} of ∼2 μM. Additionally, **4** was active against *B. subtilis* with an MIC ∼0.43 μg/mL and was very weakly active (∼200 μg/mL) against *S. cerevisiae*, indicating that **4** was not generally cytotoxic. Since **1** and **4** showed significant activity in enzymatic and bacterial growth assays, we subsequently tested them against several pathogens. Both **1** and **4** gave MIC values in the high ng/mL to low μg/mL range against *B. anthracis* Sterne, MRSA, VRE, and *L. monocytogenes*, Table 2. This promising antibacterial activity suggested the potential utility of these UPPS inhibitors in synergizing with other cell wall agents but where significant resistance has emerged, such as with methicillin (MRSA) and vancomycin (VRE).

Synergistic Interactions. To investigate the possibility of synergistic interactions with known cell wall biosynthesis inhibitors, we determined the fractional inhibitory concentration index (FICI) values for three systems: MRSA, using **1** + methicillin; VRE, using **1** + vancomycin; and *B. anthracis*, using **1** + ampicillin. The FICI is defined as

$$\mathrm{FICI} = \mathrm{FIC(A)} + \mathrm{FIC(B)} = \mathrm{MIC(AB)/MIC(A)} + \mathrm{MIC(BA)/MIC(B)}$$

where FIC(A) and FIC(B) are the fractional inhibitory concentrations of drugs A and B, MIC(A) and MIC(B) are the MIC values of drugs A and B acting alone, and MIC(AB) and MIC(BA) are the MIC values of the most effective combination of drug A or B in the presence of drug B or A. Using this method, FICI values of <0.5 represent synergism, >0.5 and <1.0 represent additivity, >1 and <2 represent an indifferent effect, and >2 represents drug antagonism. Isobolograms are shown in Figure 4. As can be seen in Figure 4B, the FICI for **1** + methicillin in MRSA is 0.11, which indicates strong synergism during late stage growth. However, with both VRE (**1** + vancomycin) and *B. anthracis* (**1** + ampicillin) the FICI values are in the 1−2 range, which indicates an indifferent effect.

What is particularly interesting about the most active species investigated here (**1**) is that it has a structure that is very similar to that found in the drug epalrestat, an aldolase reductase



inhibitor[27] that is used to treat diabetic neuropathy, and is approved for clinical use in Japan, China, and India. This is encouraging because rhodanines as a class are known to often have activity in widely different assays, and indeed computer programs such as PAINS[28] categorize, e.g., **1**−**4** (as well as epalrestat) as possible "pan assay interference compounds". This can mean that the compounds cause false positives in assays, or that they may be multitarget inhibitors. In some cases multitargeting may be undesirable; however, in the context of anti-infective development, multitargeting is expected to increase efficacy as well as decrease the possibility of resistance development,[29] both very desirable features.

CONCLUSIONS

The results described herein are of interest for several reasons. First, we carried out an in silico screen of ∼100 known UPPS inhibitors and 1000 decoys using 12 reported UPPS X-ray structures. The two X-ray structures providing the best enrichment in an AUC-ROC analysis were then used to screen

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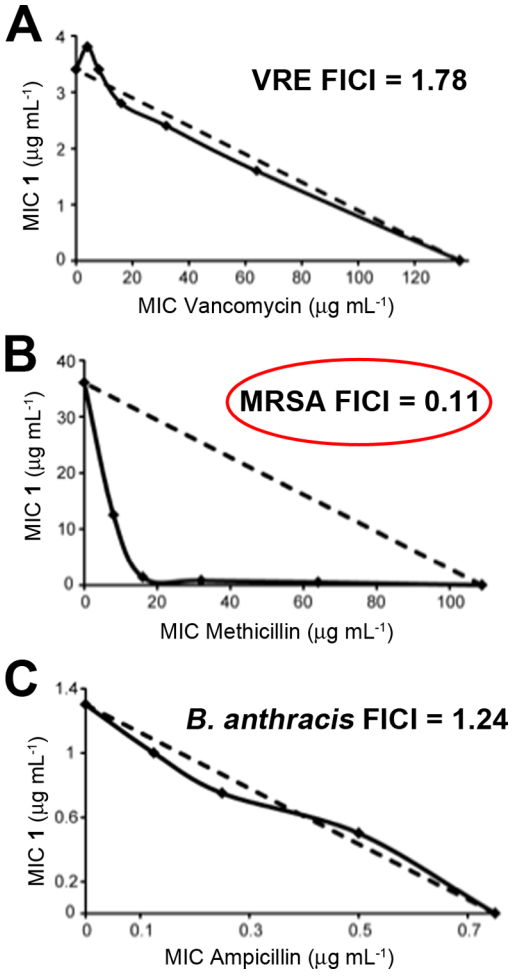

Figure 4. In vitro synergy assays. Isobolograms for growth inhibition of VRE, MRSA, and *B. anthracis* strain Sterne. (A) **1** and vancomycin inhibition of *E. faecalis* U503 (vancomycin-resistant, VRE). FICI = 1.78. (B) **1** and methicillin inhibition of *S. aureus* (USA300). FICI = 0.11. (C) **1** and ampicillin inhibition of *B. anthracis* strain Sterne. FICI =1.24.

a subset of ~100,000 compounds selected for drug-like activity from an initial ChemBridge library of ~450,000 compounds. We then tested the ~100 in silico hits in vitro against SaUPPS and EcUPPS, leading to several μM UPPS inhibitors (as deduced from both PPi release and radioactive assays). The most potent lead was **1**, which is structurally quite similar to epalrestat, in clinical use to treat diabetic neuropathy. **1** (and its analogue **4**) inhibited the growth of Gram positives; they did not inhibit the growth of Gram negatives (important with *E. coli* in the context of maintaining commensal microflora), and they had no activity against *S. cerevisiae*. Activity against *B. anthracis*, a vancomycin-resistant *Enterococcus* spp., and *Listeria monocytogenes* was good, in the 0.125–4 μg/mL range, and there was very strong synergy (FICI = 0.11) with methicillin and **1** in a MRSA strain of *S. aureus*, suggesting that **1** could be a promising lead (in combination therapies) for treating staph infections.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Correlation of pIC_{50} with docking scores analysis, as well as ^1H NMR, HPLC analysis, mass spectra, and high-resolution mass spectra of compounds **1** and **4**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

MRSA, methicillin-resistant *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococci*; UPPS, undecaprenyl diphosphate synthase; MIC, minimal inhibitory concentration; FICI, fractional inhibitory concentration index; UPP, undecaprenyl diphosphate; IPP, isopentyl diphosphate; FPP, farnesyl diphosphate; BPH, bisphosphonate; ROC, receiver-operating-characteristic; AUC, area under the curve; LB, Luria–Bertani; PAINS, pan assay interference compounds

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