Pyrazolidine-3,5-diones and 5-Hydroxy-1*H*-pyrazol-3(2*H*)-ones, Inhibitors of UDP-*N*-acetylenolpyruvyl Glucosamine Reductase

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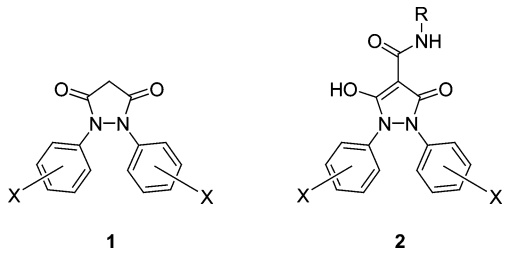
A series of pyrazolidine-3,5-dione and 5-hydroxy-1*H*-pyrazol-3(2*H*)-one inhibitors of *Escherichia coli* UDP-*N*-acetylenolpyruvyl glucosamine reductase (MurB) has been prepared. The 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones show low micromolar IC_{50} values versus *E. coli* MurB and submicromolar minimal inhibitory concentrations (MIC) against *Staphylococcus aureus* GC 1131, *Enterococcus faecalis* GC 2242, *Streptococcus pneumoniae* GC 1894, and *E. coli* GC 4560 imp, a strain with increased outer membrane permeability. None of these compounds show antimicrobial activity against *Candida albicans*, a marker of eukaryotic toxicity. Moreover, these compounds inhibit peptidoglycan biosynthesis, as assessed by measuring the amount of soluble peptidoglycan produced by *Streptococcus epidermidis* upon incubation with compounds. A partial least squares projection to latent structures analysis shows that improving MurB potency and MIC values correlate with increasing lipophilicity of the C-4 substituent of the 5-hydroxy-1*H*-pyrazol-3(2*H*)-one core. Docking studies using FLO and PharmDock produced several binding orientations for these molecules in the MurB active site.

Introduction

The majority of efforts to develop new antibacterial therapies have focused on synthesizing new derivatives of known compound classes (fluoroquinolones, tetracyclines, oxazolidinones). While these new derivatives are clinically effective, new strategies to treat bacterial infection are necessary due to the rapid emergence of bacterial resistance. Therapeutic failure of clinically used antibiotics against methycillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecalis* (VRE), and penicillin-resistant *Streptococcus pneumoniae* (PRSP) have created an urgent need for development of new structural classes of antibiotics with new mechanisms of action.[1]

Since the intact cell wall is critical for bacterial survival, we decided to target the cytoplasmic enzymes involved in the early steps of bacterial cell wall biosynthesis. Specifically we focused on targeting MurB[a] (UDP-*N*-acetylenolpyruvyl glucosamine reductase),[2] the enzyme that catalyzes the stereospecific reduction of enolpyruvyl uridine diphosphate *N*-acetylglucosamine (UDP-EP-GluNAc) to uridine diphosphate *N*-acetylmuramic acid (UDP-MurNAc). This enzyme is only found in prokaryotes,[3] has been shown to be essential for bacterial cell wall biosynthesis, and has been the subject of biochemical[4,5] and structural studies in *Escherichia coli* and *S. aureus*.[6] Thus MurB represents an attractive target for the development of new antibacterial therapeutics. To date, there have been few disclosures of small molecule MurB inhibitors with antibacterial properties.[7−11]

Chart 1



1 **2**

While doing a moderate-throughput screen of an in-house collection of molecules with antibacterial activity against *S. aureus* and no activity against a yeast strain, *Candida albicans*, we identified examples of pyrazolidine-3,5-diones and 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones (**1** and **2**, Chart 1) that inhibit *E. coli* MurB and peptidoglycan biosynthesis and possess antibacterial activity against Gram-positive bacteria.[12] The following paper describes the synthesis and structure−activity relationship development of these two classes of MurB inhibitors.

Chemistry

Preparations of the antibacterial pyrazolidine-3,5-diones and 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones **1** and **2** are outlined in Schemes 1 and 2, respectively. For pyrazolidine-3,5-diones **1**, anilines **3**−**6** were dimerized to the corresponding diazo compounds **7**−**10** via oxidation with MnO_2.[13] Reduction to the corresponding hydrazines **11**−**15** was accomplished via the action of Zn dust and NH_4Cl in aqueous acetone.[14] Pyrazolidine-3,5-diones **16**−**20** (Scheme 1) were prepared via heating with diethyl malonate in EtOH and distilling the reaction solution to dryness (oil bath temperature ∼200 °C).

Pyrazolidine-3,5-diones **16**−**20** were treated with isocyanates in the presence of Et_3N in toluene[15] to yield 4-amido compounds **21**−**39** and **41**−**47** (Scheme 2). The corresponding 4-keto derivatives **48**−**57** were prepared from **17** using the above conditions, except that acid chlorides were used in place of isocyanates.[16]

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[a] Abbreviations: MurB, UDP-*N*-acetylenolpyruvyl glucosamine reductase; UDP-EP-GluNAc, enolpyruvyl uridine diphosphate *N*-acetylglucosamine; UDP-MurNAc, uridine diphosphate *N*-acetylmuramic acid; MIC, minimal inhibitory concentrations; SPG, soluble peptidoglycan; PLS, partial least squares projection to latent structures.

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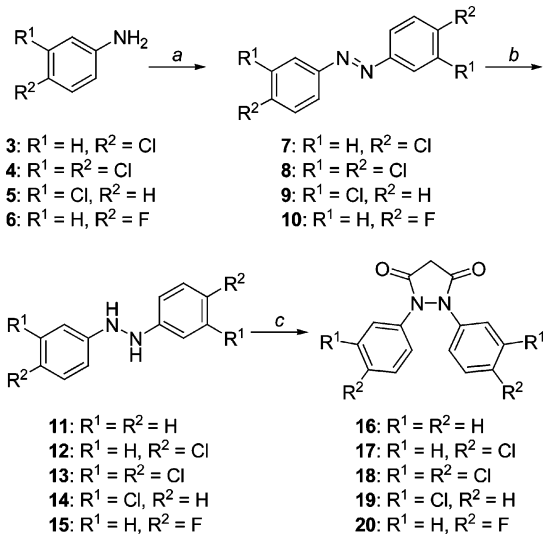
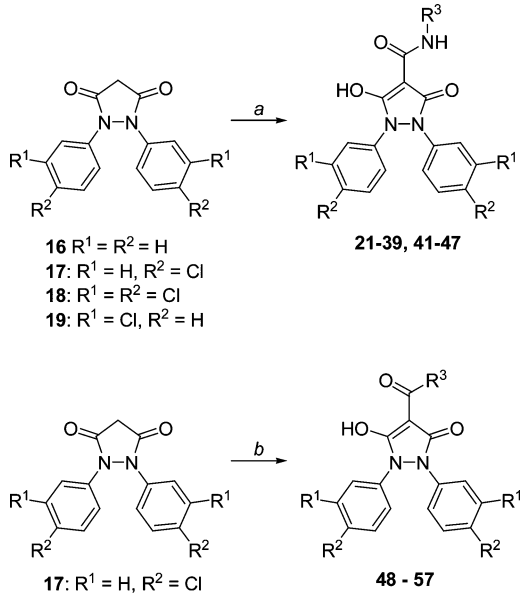
Scheme 1. Preparation of Pyrazolidine-3,5-diones **16**−**20**[a]



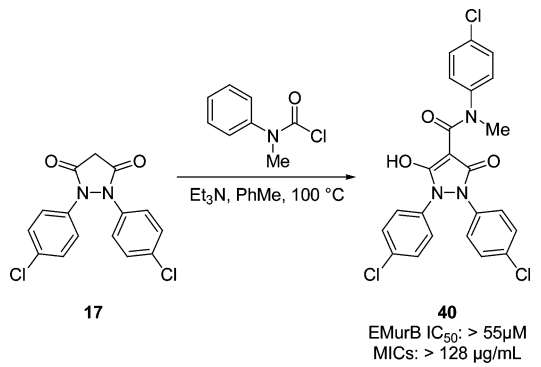
[a] Reagents: (a) MnO_2, PhMe, 110 °C; (b) $Zn_{(dust)}$, NH_4Cl, acetone, H_2O, 23 °C; (c) NaOEt, diethyl malonate, EtOH, 50−200 °C (dryness).

Scheme 2. Preparation of 5-Hydroxy-1*H*-pyrazol-3(2*H*)-ones **21**−**39**, **41**−**47**, and **48**−**57**[a]



[a] Reagents: (a) R^3NCO, Et_3N, PhMe, 0−100 °C; (b) R^3COCl, Et_3N, PhMe, 0−100 0 °C.

Methyl amide **40** was prepared by using *N*-methyl-*N*-phenylcarbamoyl chloride in place of an isocyanate, as shown in eq 1.



(1)

Biology

E. coli MurB was purified according to published procedures.[2,17] The inhibition of MurB activity was analyzed by incubating an enzyme/inhibitor mixture with enolpyruvyl UDP-*N*-acetyl glucosamine and NADPH. MurB activity was measured by monitoring the amount of NADPH oxidation (reduction in the absorbance at 340 nm using a SpectroMax 250-plate reader). Minimal inhibitory concentrations (MIC) against *S. aureus* GC 1131; *E. faecalis* GC 2242; *S. pneumoniae* GC 1894; the *E. coli* GC 4560 imp strain, a strain with increased outer membrane permeability;[12] and a yeast strain, *C. albicans*, were measured using the microbroth dilution method. None of the 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones showed activity against *C. albicans*. The inhibition of peptidoglycan biosynthesis was determined by measuring the amount of soluble peptidoglycan (SPG) produced by a *Streptococcus epidermidis* upon incubation with compounds under study.

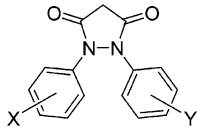
Results and Discussion

Four out of the five pyrazolidine-3,5-diones prepared, **16**, **17**, **19**, **20**, display weak MurB inhibition (IC_{50} > 68 μM) (Table 1). However, the bis((3,4-Cl)Ph) analogue **18** shows moderately potent MurB inhibition with an IC_{50} = 25 μM. Moreover, **18** has moderate antimicrobial activity against *S. aureus* GC 1131, *E. faecalis* GC 2242, *S. pneumoniae* GC 1894, and the *E. coli* GC 4560 imp strain (respective MICs: 8, 8, 8, and 32 μg/mL). Both **18** and **19** were tested for SPG inhibitory activity, and both show weak inhibition (**18**, SPG IC_{50} = 41 μM; **19**, SPG IC_{50} = 56 μM).

The 4-substitued 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones exhibited more potent MurB inhibition and improved MIC values (Table 2). The phenyl carboxamide **21** possesses a MurB IC_{50} of 9.8 μM and an excellent MIC profile. Of special note is that **21** shows MIC activity against the *E. coli* imp strain (MIC = 4 μg/mL) and submicromolar SPG inhibition (IC_{50} = 0.78 μM). Substitution of the carboxamide phenyl ring affects MurB inhibition, MICs, and SPG inhibition. Electron-withdrawing/lipophilic moieties such as chlorine (**22**, **25**, **26**) give compounds with comparable MurB IC_{50} values to that of **21** or compounds that are more potent. MIC values for these analogues are generally comparable to that of **21** except that only **22** shows similar MIC activity in the *E. coli* imp strain (MIC = 2 μg/mL). The high value for **22** in the SPG assay is not understood at this time.

Other electron-withdrawing substituents (CF_3, **23**, **24**; CN, **28**, CO_2Et, **30**) exhibit similar MurB IC_{50}s compared to **21**. The MIC profile of these compounds is also similar, but they do not show comparable antimicrobial activity against the *E. coli* imp strain. All of these analogues possess SPG inhibitory activity with IC_{50} values in the micromolar range. Polar electron-withdrawing substituents (CO_2H, **31**; $C(O)NH(CH_2)_3NMe_2$, **33**) give inactive compounds. Electron-donating substituents (OMe, **27**; OH, **32**) provide compounds that are slightly less active against MurB and a panel of microorganisms than **21**. Extension of the phenyl carboxamide **21** to a phenethyl carboxamide **37** generates a compound with similar MurB inhibition but comparatively weaker antibacterial activity against *S. aureus* and *E. coli* imp. The corresponding benzyl carboxamides (**34**−**36**) have similar profiles in that they show good MurB inhibition but comparatively weaker MICs against *S. aureus* and *E. coli* imp. Compounds **34** and **35** also show comparable SPG inhibition activity to the corresponding phenyl analogues. Methyl substitution of the benzyl carbon (**38** and **39**) is tolerated, but these compounds do not offer improvements in MurB potency

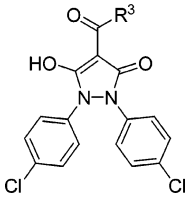
Table 1. *E. coli* MurB Inhibition, Minimal Inhibition Concentrations (MIC), and Soluble Peptidoglycan Biosynthesis Inhibition of Pyrazolidine-3,5-diones



compd	X	Y	MurB IC_{50} (μM)[a]	MIC (μg/mL)[b] *S. aureus* GC 1131	*E. faecalis* GC 2242	*S. pneumo* GC 1894	*E. coli* imp	inhibn of SPG IC_{50} (μM)[c]
16	4-H	4-H	>100	–	–	–	–	–
17	4-Cl	4-Cl	71.6	8	8	8	16	
18	3,4-Cl	3,4-Cl	25.1	8	8	4	32	41.0
19	3-Cl	3-Cl	68.5	16	16	8	200	56.0
20	4-F	4-F	>87.0	128	128	64	64	

[a] Average of three runs. Standard deviation ±5%. [b] Average of two runs. Standard deviation ±5%. [c] Average of three runs. Standard deviation ±6%.

Table 2. *E. coli* MurB Inhibition, Minimal Inhibition Concentrations (MIC), and Soluble Peptidoglycan Biosynthesis Inhibition of 1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazole-4-carboxamides



compd	R^3	MurB IC_{50} (μM)[a]	MIC (μg/mL)[b] *S. aureus* GC 1131	*E. faecalis* GC 2242	*S. pneumo* GC 1894	*E. coli* imp	inhibn of SPG IC_{50} (μM)[c]
21	Ph	9.8	1	<0.12	<0.12	4	0.78
22	(4-Cl)Ph	13.7	1	0.50	<0.12	2	42.2
23	(3-CF_3)Ph	5.5	1	0.25	<0.12	16	4.7
24	(4-CF_3)Ph	5.9	16	0.25	>128	16	8.3
25	(3-Cl)Ph	5.9	0.5	<0.12	<0.12	32	7.0
26	(2-Cl)Ph	12.2	1	0.25	<0.12	8	
27	(4-OMe)Ph	16	8	1	0.5	>128	
28	(4-CN)Ph	13.1	2	0.25	<0.12	>128	6.9
29	cyclohexyl	8.1	1	0.25	0.25	8	<0.87
30	(4-CO_2Et)Ph	17.6	8	0.5	0.25	>128	11.1
31	(4-CO_2H)Ph	>52	64	128	64	64	
32	(4-OH)Ph	21.9	8	8	4	4	2.6
33	(4-CONH$(CH_2)_3NMe_2$)Ph	>44	8	8	4	4	>44.0
34	(3,4-Cl)PhCH_2	<6.2	26.1	0.41	0.2	>105	7.5
35	(2,4-Cl)PhCH_2	<6.2	8	0.25	0.25	>128	8.8
36	(2-Cl)PhCH_2	<6.2	6.1	0.76	<0.10	>100	
37	PhCH_2CH_2	9.6	11.7	0.37	<0.10	>100	
38	(4-Br)PhCH(Me)	5.6	3.4	0.21	0.21	>109	
39	naphthylCH(Me)	<6.2	26	0.4	0.2	>103	

[a] Average of three runs. Standard deviation ±5%. [b] Average of two runs. Standard deviation ±5%. [c] Average of three runs. Standard deviation ±6%.

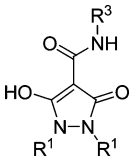
or antimicrobial activity when compared to corresponding phenyl analogues.

That the internal hydrogen bond between the amide N−H and the 5-hydroxy-1*H*-pyrazol-3(2*H*)-one carbonyl may be important for activity is shown in N−Me amide analogue **40** (eq 1). This compound is devoid of MurB inhibitory activity (MurB IC_{50} > 55 μM) as well as antibacterial activity (MIC > 128 μg/mL). The rigidification of the pyrazolidinedione-4-carboxamide structure provided by this internal hydrogen bond could lock these amides into a bioactive conformation and is the probable cause of this effect.

The effects of varying the N(1) and N(2) substituents of the 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones can be seen in Table 3. Compounds **41**−**43** all possess unsubstituted Ph groups at R^1 and all of these compounds exhibit weak MurB activity (IC_{50} = 42−54 μM) and only moderate MIC values. An improvement in MurB inhibition and MICs is seen in compounds **44** and **45**, which have (3-Cl)Ph groups at R^1. These analogues show MurB IC_{50}s and antibacterial activity against the Gram-positive strains that are similar to the corresponding compounds where R^1 is (4-Cl)Ph (see **22** and **24**). The R^1 = (3,4-Cl) analogues **46** and **47** show similar MurB inhibition to **44** and **45** but weaker *S. aureus* antibacterial activity.

The corresponding ketone analogues of several compounds in Tables 2 and 3 show good MurB inhibition and MICs (Table 4). Analogues with electron-withdrawing groups on the phenyl ketones [**48**, (4-Cl)Ph; **49**, (3,4-Cl)Ph; **52**, (4-OCF_3)Ph; and **55**, (4-CF_3)Ph] possess good MurB inhibition (IC_{50} = 5.4−15.0 μM) and good antibacterial activity against the Gram-positive bacteria tested (MIC = 0.39−12.7 μg/mL). These analogues also have moderate MICs against the *E. coli* imp strain (MIC = 16.0−49 μg/mL). Analogues with electron-donating groups on the phenyl ketones [**50**, (4-OMe)Ph; **51**, (4-O-*n*-Bu)Ph] exhibit similar MurB inhibition and MICs [IC_{50} = 4.5−10.0 μM; MIC = 0.39−11.3 μg/mL). The thiophene ketone **54** (IC_{50} = 5.8 μM) shows

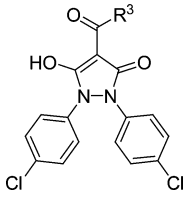
Table 3. *E. coli* MurB Inhibition, Minimal Inhibition Concentrations (MIC), and Soluble Peptidoglycan Biosynthesis Inhibition of 5-Hydroxy-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazole-4-carboxamides



compd	R^1	R^3	MurB IC_{50} (μM)[a]	MIC (μg/ mL)[b] *S. aureus* GC 1131	*E. faecalis* GC 2242	*S. pneumo* GC 1894	*E. coli* imp	inhibn of SPG IC_{50} (μM)[c]
41	Ph	(4-Cl)Ph	54	32	1.0	0.5	>128	
42	Ph	(4-Br)Ph	42	16	1.0	1.0	>128	
43	Ph	Ph	54	64	8.0	16.0	>128	
44	(3-Cl)Ph	(4-Cl)Ph	8.6	0.5	0.25	<0.12	>128	3
45	(3-Cl)Ph	(4-CF_3)Ph	8.7	0.5	<0.12	<0.12	64	2.2
46	(3,4-Cl)Ph	(4-Me)Ph	4.8	16	0.25	<0.12	>128	4
47	(3,4-Cl)Ph	(3,4-Cl)Ph	4.8	64	0.5	0.5	>128	

[a] Average of three runs. Standard deviation ± 5%. [b] Average of two runs. Standard deviation ± 5%. [c] Average of three runs. Standard deviation ± 6%.

Table 4. *E. coli* MurB Inhibition, Minimal Inhibition Concentrations (MIC), and Soluble Peptidoglycan Biosynthesis Inhibition of 4-Acetyl-1,2-bis(4-chlorophenyl)-5-hydroxy-1*H*-pyrazol-3(2*H*)-ones



compd	R^3	MurB IC_{50} (μM)[a]	MIC (μg/mL)[b] *S. aureus* GC 1131	*E. faecalis* GC 2242	*S. pneumo* GC 1894	*E. coli* imp	inhibn of SPG IC_{50} (μM)[c]
48	(4-Cl)Ph	11.0	5.7	1.40	0.72	23.0	
49	(3,4-Cl)Ph	13.0	6.2	0.77	0.39	49.0	
50	(4-OMe)Ph	10.0	11.3	5.7	2.8	23.0	
51	(4-On-Bu)Ph	4.5	1.6	0.39	0.39	>100	7.3
52	(4-OCF_3)Ph	5.4	12.7	0.79	0.79	25.4	2.0
53	(4-Ph)Ph	6.1	3.1	0.39	0.3	>100	4.9
54	thiophene	5.8	5.4	1.3	0.67	21.6	1.0
55	(4-CF_3)Ph	15.0	8.0	2.0	0.50	16.0	
56	(4-OMe)PhCH_2	40.0	11.7	1.4	0.73	>94	
57	cyclohexyl	24.0	1.3	0.34	0.17	5.4	

[a] Average of three runs. Standard deviation ±5%. [b] Average of two runs. Standard deviation ±5%. [c] Average of three runs. Standard deviation ±6%.

good MurB inhibitory activity while the cyclohexyl ketone **57** (IC_{50} = 24.0 μM) is less potent.

In line with what we have previously reported,[12] all of the test compounds **21**−**57** showed no antimicrobial activity against *S. pneumoniae* in the presence of 4% bovine serum albumin (BSA) (MIC > 128 μg/mL). This effect of serum albumin reducing MIC values is presumably due to the high protein-binding properties of these compounds, leaving them unavailable to interact with the bacterial target.[18] This BSA effect prevents us from testing the 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones for efficacy in animal models.

While not normally expected for antibacterial agents, correlation of the 5-hydroxy-1*H*-pyrazol-3(2*H*)-one physical properties, MurB inhibition, MIC activity, as well as SPG inhibitory activity is possible using partial least squares projection to latent structures (PLS) analysis[19,20] using Simca-P[21] (Figure 1). A four-component PLS model is generated where 75% of the calculated physical properties data (R^2X = 0.75) and 50% of the MurB, MIC, and SPG data (R^2Y = 0.5) is used in the model. As MurB inhibition increases (moving from the origin into the NE quadrant of the figure), the MICs against the Gram-positive bacteria, *S. aureus*, *E. faecalis*, and *S. pneumoniae*, also improve. The calculated physicochemical properties that correlate with this increasing activity are increasing R5_clogP, decreasing R5_HBA (hydrogen-bond-acceptor count), and decreasing R5_PSA. *E. coli* imp MICs improve less strongly than MICs against Gram-positive bacteria, but the activity is modulated by the same calculated properties. Increasing SPG inhibition (moving from the origin into the SE quadrant of the figure) is not as closely correlated to the MurB inhibition and/MIC activity as SPG inhibition is located in a different quadrant that the other biological activities. Decreasing R5_HBD (hydrogen-bond-donor count), R5_MW, and R5_cMR appear to be the variables that correlate best with increasing SPG inhibition.

A computational chemistry effort was undertaken to ascertain the binding mode of 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones in the MurB active site. All attempts to obtain well-defined density for a member of the 5-hydroxy-1*H*-pyrazol-3(2*H*)-one series were unsuccessful.[22] Diffuse density is identified that radiates from above the flavine ring of the FAD cofactor. Docking studies were carried out using FLO[23] and PharmDock[24] against the available structure of the S229A MurB mutant/substrate complex.[25] Each docking algorithm produces three families of orientations (Figure 2). The first coincides with the substrate-binding site and places the 4-substituent above the flavine ring, and the 1,3-dicarbonyl group overlays the diphosphate of the

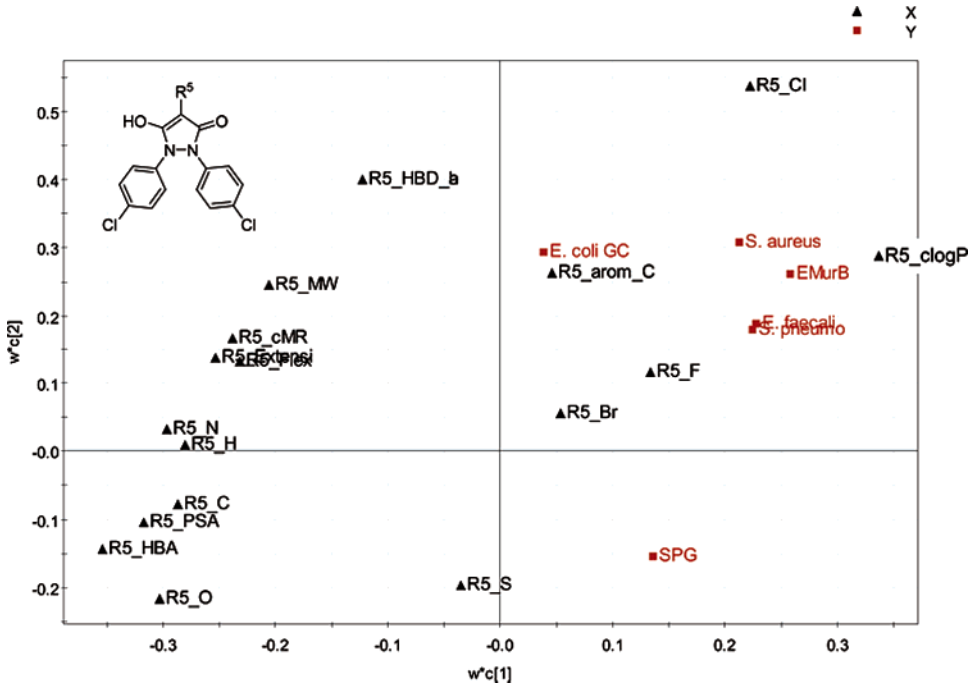


Figure 1. PLS loadings plot (w*c[1] vs w*c[2]) of 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones **16**−**38** and **47**−**56**: calculated physiochemical properties (**X**-matrix), negative log (*E. coli* MurB IC_{50}, MIC Data and SPG Inhibitory Data) (**Y**-Matrix) ($A = 4$; $R^2X = 0.75$; $R^2Y = 0.5$; $Q^2(\text{cum}) = 0.35$).

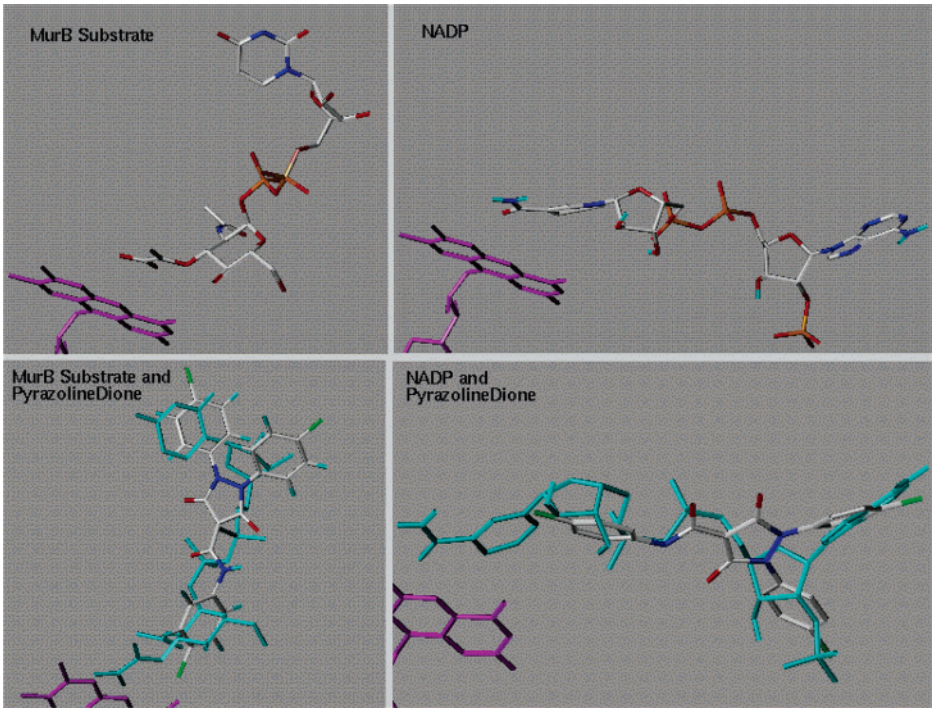


Figure 2. The top panel shows the orientation of the MurB substrate in the crystal structure and a proposed orientation of NADP in the perpendicular site. The bottom panel shows how 5-hydroxy-1*H*-pyrazol-3(2*H*)-one docks into the two sites. A third orientation was found in which the NADP-overlapped orientation is rotated 180°.

substrate. The second and third families of orientations extend the molecule into the perpendicular site. Preliminary studies, to be published elsewhere, suggest that NADPH, which is required to recycle FADH after reduction of the substrate, may occupy the site. In the crystal structure of the substrate, the enol ether is situated directly above and parallel to the flavine ring. The orientation of NADP described in this study places the nicotinamide ring in the same position, from which the reduced ring can transfer a hydrogen to FAD. One orientation places the 4-substituent overlapping the first orientation above the flavine ring. The other related orientation places the compound in the same position, but rotated 180° so that the diaryl pyrazole ring system lies above FAD. All three orientations dock with similar scores, and we believe the poor resolution obtained by X-ray may be due to the presence of a mixture of these orientations.

In all three orientations, there is an intramolecular bond formed between the amide N−H and the 5-hydroxy-1*H*-pyrazol-3(2*H*)-one's carbonyl, which is consistent with the loss of activity of the *N*-methyl analogue. The orientation that mimics the substrate shares some of its key interactions near the diphosphate group with the pyrazolidinedione carbonyls forming

hydrogen bonds with TYR190 and GLN288. In both of the orientations that lie in the putative NADPH binding pocket, hydrogen bonds exist between the carbonyl groups and TYR190 and ASN233. It is interesting to note that TYR190 bisects and is therefore available for hydrogen bonds with both general orientations. In all three orientations, hydrogen-bond-donating residues exist that can interact with small substituents at the 4-position of the bis-aryl rings of the series.

Conclusion

In summary, we have identified a series of 5-hydroxy-1*H*-pyrazol-3(2*H*)-ones as novel potent inhibitors of *E. coli* MurB with antibacterial activity against *S. aureus* GC 1131, *E. faecalis* GC 2242, *S. pneumonia* GC 1894, and the *E. coli* GC 4560 imp strain. Inhibition of the cell wall synthetic enzyme MurB by these compounds affects peptidoglycan biosynthesis and leads to the inhibition of cell growth.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover Mel-temp apparatus and are uncorrected. The proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 300 MHz on a Bruker DPX-300 spectrometer using tetramethylsilane (δ 0.0) as an internal standard. Combustion analyses were obtained using a Perkin-Elmer Series II 2400 CHNS/O analyzer. Mass spectra were obtained using a Micromass platform electrospray ionization quadrapole mass spectrometer. Flash chromatography was performed using EM Science 230−400 mess silica gel. Thin-layer chromatography (TLC) was performed in Analtech silica gel GHLF 250 μm prescored plates. Analytical LC/MS was performed using an HP Agilent 1100 LC/MS with an Agilent 1100 diode array detector (Agilent MS Column, Waters Xterra MS C18 30 mm × 2.1 mm i.d., 3.5 μm; flow rate, 1.00 mL/min; run time, 5.00 min; gradient elution, 0 min 90% water, 10% acetonitrile; 3 min 10% water, 90% acetonitrile; column temperature, 50.0 °C; UV signals, 215, 254 nm. MS parameters: mass range, 100−1000, Fragmentor 140, Gain EMV 1.0). Preparative RP-HPLC was performed using a Gilson HPLC [Phenomenex LUNA C_{18} column, 60 mm × 21.20 mm i.d., 5 μm particle size, eluted with an acetonitrile/water gradient (containing 0.2% Et_3N)].

Bis(4-chlorophenyl)diazene (7). To a mechanically stirred suspension of 4-chloroaniline (25.51 g, 200 mmol) **3** in toluene (700 mL) was added manganese(IV) dioxide (86.9 g, 1000 mmol). The mixture was refluxed for 8 h with water removal (Dean−Stark) and then filtered through Celite. The filter cake was washed with several volumes of toluene, and the filtrate was evaporated to give **7** as a dark orange solid (9.6 g, 38 mmol). An additional quantity of **7** was obtained by exhaustive extraction of the Celite cake with toluene and CH_2Cl_2 (2.7 g, 10.8 mmol). The total yield of **7** was 12.3 g (49.0 mmol, a 49% yield). The material was used in the next step without further purification: mp 180−183 °C; MS (APCI+) *m*/*z* 251 $(M + H)^+$. Anal. ($C_{12}H_8Cl_2N_2$) C, H, N.

Bis(3,4-dichlorophenyl)diazene (8). The title compound was prepared according to the procedure of compound **7** using 3,4-dichloroaniline **4** in place of 4-chloroaniline: yield 89%; MS (APCI+) *m*/*z* 321 $(M + H)^+$.

Bis(3-chlorophenyl)diazene (9). The title compound was prepared according to the procedure of compound **5** using 3-chloroaniline **5** in place of 4-chloroaniline: yield 88%; MS (APCI+) *m*/*z* 321 $(M + H)^+$.

Bis(4-fluorophenyl)diazene (10). The title compound was prepared according to the procedure of compound **7** using 3-chloroaniline **6** in place of 4-chloroaniline: yield 79%; MS (APCI+) *m*/*z* 219 $(M + H)^+$.

***N,N′*-Bis(4-chlorophenyl)hydrazine (12).** A suspension of 5.64 g (22.5 mmol) of bis(4-chlorophenyl)diazene **7** (5.64 g, 22.46 mmol) and 115 mL of acetone was treated with saturated aqueous ammonium chloride. Zinc dust (11.3 g, 172.3 mmol) was added, the suspension was stirred at room temperature for 5 h and filtered through Celite. The cake was washed with several portions of acetone until a clear filtrate was obtained. The combined filtrates were concentrated to about half the original volume, and the residue was diluted with 200 mL of ice/water to precipitate the product. The precipitate was stirred under nitrogen for 1 h and then concentrated to remove residual acetone. The tan solid was collected, washed with water, and dried in vacuo over P_2O_5 to give 5.49 g (21.9 mmol, a 97% yield) of **12** as a pale yellow solid: mp 122−124 °C; MS (EI) *m*/*z* 252 $(M + H)^+$. Anal. ($C_{12}H_{10}Cl_2N_2$) C, H, N.

***N,N′*-Bis(3,4-dichlorophenyl)hydrazine (13).** The title compound was prepared according to the procedure of compound **12** using bis(3,4-dichlorophenyl)diazene **8** in place of bis(4-chlorophenyl)diazene: yield 94%. Anal. ($C_{12}H_8Cl_4N_2$) C, H, N.

***N,N′*-Bis(3-chlorophenyl)hydrazine (14).** The title compound was prepared according to the procedure of compound **12** using bis(3-chlorophenyl)diazene **9** in place of bis(4-chlorophenyl)diazene: yield 85%; MS (EI) *m*/*z* 252 $(M + H)^+$.

***N,N′*-Bis(4-fluorophenyl)hydrazine (15).** The title compound was prepared according to the procedure of compound **12** using bis(4-fluorophenyl)diazene **10** in place of bis(4-chlorophenyl)-diazene: yield 95%; MS (APCI+) *m*/*z* 221 $(M + H)^+$. Anal. ($C_{12}H_{10}F_2N_2$) C, H, N.

1,2-Diphenylpyrazolidine-3,5-dione (16). To a solution of sodium metal (574 mg, 23.9 mmol) in anhydrous ethanol (25 mL) under nitrogen was added 4.2 g (22.8 mmol) of 1,2-bisphenylhydrazine **11**. The suspension was heated at 50 °C to achieve complete solution. Diethyl malonate (3.5 mL, 3.67 g, 23.1 mmol) was added, and after stirring for 15 min, the mixture was placed in an oil bath preheated at 50 °C. The flask was fitted with a takeoff adapter and the temperature was slowly raised to 150 °C (over approximately 90 min) to distill off the volatiles. The dry residue was heated at 200 °C for an additional 3 h, cooled, and partitioned between water (200 mL) and diethyl ether (100 mL). The aqueous layer was again extracted with diethyl ether and then acidified in the cold with 2 N HCl. The precipitate was extracted with CH_2Cl_2. The extracts were washed with brine, dried over anhydrous $MgSO_4$, and evaporated to dryness to provide a tan-colored solid. Recrystallization from ethanol provided **16** (3.0 g, 11.9 mmol, a 52% yield) as a yellow solid: mp 178−180 °C; MS (ES−) *m*/*z* 251 $(M - H)^-$. Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

1,2-Bis(4-chlorophenyl)pyrazolidine-3,5-dione (17). The title compound was prepared according to the procedure of compound **16** using *N,N′*-bis(4-chlorophenyl)hydrazine **12** in place of 1,2-bisphenylhydrazine: yield 47%; mp 194−197 °C; ^{1}H NMR (DMSO-d_6) δ 7.42 (m, 4H), 7.32 (m, 4H), 3.78 (brs, 2H); MS (ES−) *m*/*z* 319.1 $(M - H)^-$. Anal. ($C_{15}H_{10}Cl_2N_2O_2 \cdot 0.2H_2O$) C, H, N.

1,2-Bis(3,4-dichlorophenyl)pyrazolidine-3,5-dione (18). The title compound was prepared according to the procedure of compound **16** using *N,N′*-bis(3,4-dichlorophenyl)hydrazine **13** in place of 1,2-bisphenylhydrazine: yield 25%; mp 206 °C dec; MS (ES−) *m*/*z* 387 $(M - H)^-$. Anal. ($C_{15}H_8Cl_4N_2O_2$) C, H, N.

1,2-Bis(3-chlorophenyl)pyrazolidine-3,5-dione (19). The title compound was prepared according to the procedure of compound **16** using *N,N′*-bis(3,4-dichlorophenyl)hydrazine **14** in place of 1,2-bisphenylhydrazine: yield 30%; mp 145−147 °C; MS (APCI+) *m*/*z* 321 $(M + H)^+$. Anal. ($C_{15}H_{10}Cl_2N_2O_2$) C, H, N.

1,2-Bis(4-fluorophenyl)pyrazolidine-3,5-dione (20). The title compound was prepared according to the procedure of compound **16** using *N,N′*-bis(4-fluorophenyl)hydrazine **15** in place of 1,2-bisphenylhydrazine: mp 150−152 °C; ^{1}H NMR (DMSO-d_6) δ 7.37 (m, 4H), 7.21 (m, 4H), 3.35 (brs, 2H); MS (APCI+) *m*/*z* 289 $(M + H)^+$. Anal. ($C_{15}H_{10}F_2N_2O_2$) C, H, N.

1,2-Bis(4-chlorophenyl)-3,5-dioxo-*N*-phenylpyrazolidine-4-carboxamide (21). To a 0 °C suspension of 200 mg (0.62 mmol) of 1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **17** and 20 mL of toluene under nitrogen was added 958 μL (6.88 mmol) of Et_3N followed by 819 mg (6.88 mmol) of phenyl isocyanate. After stirring at room temperature for 15 min, the mixture was heated at 100 °C for 12 h. After cooling to room

temperature, the reaction mixture was diluted with ethyl acetate, washed with 1 N hydrochloric acid and brine/water (1:1, v/v), dried over anhydrous Na_2SO_4, and evaporated to dryness. The residue was redissolved in dichloromethane and evaporated to provide a brown foam. Trituration with hexane containing a small amount of Et_2O precipitated a solid which was collected and dried in vacuo to provide 170 mg (0.38 mmol, 61% yield) of **21** as a light brown solid: mp 200−203 °C; MS (ES+) *m/z* 440 $(M + H)^+$. Anal. ($C_{22}H_{15}Cl_2N_3O_3$) C, H, N.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (4-Chlorophenyl)amide (22). The title compound was prepared according to the procedure of compound **21** using 4-chlorophenyl isocyanate in place of phenyl isocyanate: yield 65%; mp 215−218 °C; ^{1}H NMR (DMSO-d_6) δ 10.55 (s, 1H), 7.57 (d, $J = 6.3$ Hz, 2H), 7.32 (m, 8H), 7.27 (d, $J = 6.3$ Hz, 2H), OH proton not visible; MS (APCI+) *m/z* 474 $(M + H)^+$. Anal. ($C_{22}H_{14}Cl_3N_3O_3$) C, H, N.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (3-Trifluoromethylphenyl)amide (23). The title compound was prepared according to the procedure of compound **21** using 3-trifluoromethylphenyl isocyanate in place of phenyl isocyanate: yield 51%; mp 148.5−151.5 °C; MS (APCI+) *m/z* 508 $(M + H)^+$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (4-Trifluoromethylphenyl)amide (24). The title compound was prepared according to the procedure of compound **21** using 4-trifluoromethylphenyl isocyanate in place of phenyl isocyanate: yield 66%; mp 208−210 °C; ^{1}H NMR (DMSO-d_6) δ 10.80 (s, 1H), 7.74 (d, $J = 6.3$ Hz, 2H), 7.58 (d, $J = 6.3$ Hz, 2H), 7.33 (m, 7H), OH proton not visible; MS (EI+) *m/z* 508 $(M + H)^+$. Anal. ($C_{23}H_{14}Cl_2F_3N_3O_3$) C, H, N.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (3-Chlorophenyl)amide (25). The title compound was prepared according to the procedure of compound **21** using 3-chlorophenyl isocyanate in place of phenyl isocyanate: yield 85%; mp 143−145 °C; ^{1}H NMR (DMSO-d_6) δ 10.62 (s, 1H), 7.38−7.30 (m, 9H), 7.27−7.18 (m, 2H), 6.96 (m, 1H), OH proton not visible; MS (ESI+) *m/z* 474 $(M + H)^+$. Anal. ($C_{22}H_{14}Cl_3N_3O_3$) C, H, N.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (2-Chlorophenyl)amide (26). The title compound was prepared according to the procedure of compound **21** using 2-chlorophenyl isocyanate in place of phenyl isocyanate: yield 33%; mp 226−230 °C; MS (ESI+) *m/z* 474 $(M + H)^+$. Anal. ($C_{22}H_{14}Cl_3N_3O_3$) C, H, N.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (4-Methoxyphenyl)amide (27). The title compound was prepared according to the procedure of compound **21** using 4-methoxyphenyl isocyanate in place of phenyl isocyanate: yield 66%; mp 187−190 °C; ^{1}H NMR (DMSO-d_6) δ 10.30 (s, 1H), 7.44 (d, $J = 6.6$ Hz, 2H), 7.33 (m, 8H), 6.84 (d, $J = 6.6$ Hz, 2H), 3.71 (s, 3H), OH proton not visible; MS (APCI+) *m/z* 470 $(M + H)^+$. Anal. ($C_{23}H_{17}Cl_2N_3O_4$) C, H, N.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (4-Cyanophenyl)amide (28). The title compound was prepared according to the procedure of compound **21** using 4-cyanophenyl isocyanate in place of phenyl isocyanate: yield 66%; mp 250−253 °C; MS (APCI+) *m/z* 465 $(M + H)^+$. Anal. ($C_{23}H_{14}Cl_2N_4O_3 \cdot 0.25H_2O$) C, H, N.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid Cyclohexylamide (29). The title compound was prepared according to the procedure of compound **21** using cyclohexyl isocyanate in place of phenyl isocyanate: yield 32%; mp 144−149 °C; MS (APCI+) *m/z* 465 $(M + H)^+$. Anal. ($C_{22}H_{21}Cl_2N_3O_3$) C, H, N.

4-[1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carbonyl]aminobenzoic Acid Ethyl Ester (30). The title compound was prepared according to the procedure of compound **21** using ethyl 4-isocyanatobenzoate in place of phenyl isocyanate: yield 85%; mp 214−215 °C; MS (APCI−) *m/z* 511 $(M - H)^-$. Anal. ($C_{25}H_{19}Cl_2N_3O_5$) C, H, N.

4-[1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carbonyl]aminobenzoic Acid (31). To a suspension of 170 mg (0.33 mmol) of 4-[1,2-bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carbonyl]aminobenzoic acid ethyl ester **30** and 10 mL of EtOH under nitrogen was added 660 μL (0.66 mmol) of 1 N NaOH, and the mixture was refluxed for 12 h. The mixture was acidified to pH 4 with 1 N HCl, and the EtOH removed in vacuo. The residue was diluted with H_2O, and the precipitate was collected, washed with H_2O, and dried in vacuo at 40 °C to provide 120 mg (0.25 mmol, a 76% yield) of **31** as a white solid: mp >260 °C; MS (APCI−) *m/z* 482 $(M - H)^-$. Anal. ($C_{23}H_{15}Cl_2N_3O_5 \cdot 0.20H_2O$) C, H, N.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (4-Hydroxyphenyl)amide (32). To a −78 °C solution of 200 mg (0.43 mmol) of 1,2-bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic acid (4-methoxyphenyl)amide **27** in 4 mL of CH_2Cl_2 was added 1.3 mL (1.3 mmol) of a 1 M solution of BBr_3/CH_2Cl_2, and the mixture was allowed to warm to 0 °C. After stirring for 3 h at 0 °C, the reaction was quenched by the addition of aqueous NH_4OH, and the resulting mixture was stirred at room temperature overnight. After extraction with EtOAc and drying over $MgSO_4$, the solvent was evaporated in vacuo. Flash chromatography on SiO_2, eluting with 5% to 10% MeOH in CH_2Cl_2, gave **31** as an amorphous pale brown solid: MS (APCI−) *m/z* 455 $(M - H)^-$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid [4-(3-(Dimethylamino)propylcarbamoyl)-phenyl]amide (33). To a 23 °C solution of 250 mg (0.52 mmol) of 4-[1,2-bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carbonyl]aminobenzoic acid **31** and 3 mL of DMF (0.097 g, 0.20 mmol) was added 230 μL (1.32 mmol) of *i*-Pr_2NEt, followed by 85 mg (0.63 mmol) of 1-hydroxybenzotriazole hydrate and 120 mg (0.63 mmol) of 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride. After stirring at 23 °C for 30 min, 70 mg (0.67 mmol) of diethanolamine was added. After stirring at 23 °C for an additional 26 h, the solvent was removed in vacuo, and the residue diluted with EtOAc and 2 N HCl. The organic layer was washed with brine/H_2O (1:1, v/v), dried over $MgSO_4$, and evaporated to dryness. Flash chromatography on SiO_2, eluting with 1% to 5% MeOH in CH_2Cl_2, gave **33** as an amorphous solid: mp 212−220 °C; MS (ESI−) *m/z* 569 $(M - H)^-$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid 3,4-Dichlorobenzylamide (34). The title compound was prepared according to the procedure of compound **21** using 3,4-dichlorobenzyl isocyanate in place of phenyl isocyanate with the following differences: (1) parallel synthesis format was used to prepare this compound, (2) analytical an LC/MS as described above (Chemistry) was used for reaction monitoring, and (3) compound purification was performed using preparative RP-HPLC as described above (Chemistry): HPLC 100.0%; MS (ESI−) *m/z* 520 $(M - H)^-$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid 2,4-Dichlorobenzylamide (35). The title compound was prepared according to the procedure of compound **34** using 2,4-dichlorobenzyl isocyanate in place of 3,4-dichlorobenzyl isocyanate: HPLC 100.0%; ^{1}H NMR (DMSO-d_6) δ 8.80 (s, 1H), 7.59 (d, $J = 2.0$ Hz, 1H), 7.41 (dd, $J = 2.0$, 6.3 Hz, 1H), 7.35−7.31 (m, 9H), 4.45 (s, 2H), OH proton not visible; MS (ESI−) *m/z* 520 $(M - H)^-$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid 2-Chlorobenzylamide (36). The title compound was prepared according to the procedure of compound **34** using 2-chlorobenzyl isocyanate in place of 3,4-dichlorobenzyl isocyanate: HPLC 100.0%; MS (ESI+) *m/z* 488 $(M + H)^+$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid Phenethylamide (37). The title compound was prepared according to the procedure of compound **34** using phenethyl isocyanate in place of 3,4-dichlorobenzyl isocyanate.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid [1-(4-Bromophenyl)ethyl]amide (38). The title compound was prepared according to the procedure of

compound **34** using 1-(4-bromophenyl)ethyl isocyanate in place of 3,4-dichlorobenzyl isocyanate.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (1-Naphthalen-2-ylethyl)amide (39). The title compound was prepared according to the procedure of compound **34** using 2-(1-isocyanatoethyl)naphthalene in place of 3,4-dichlorobenzyl isocyanate.

1,2-Bis(4-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid Methylphenylamide (40). The title compound was prepared according to the procedure of compound **21** using *N*-methyl-*N*-phenylcarbamoyl chloride in place of phenyl isocyanate: mp 181−183 °C; ^{1}H NMR (DMSO-d_6) δ 7.50−6.90 (m, 13H), 3.21 (s, 3H); MS (ESI+) *m*/*z* 454 (M + H)$^+$. Anal. ($C_{23}H_{17}Cl_2N_3O_3$) C, H, N.

5-Hydroxy-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (4-Chloro-phenyl)amide (41). The title compound was prepared according to the procedure of compound **21** using 5-hydroxy-1,2-diphenyl-1,2-dihydropyrazol-3-one **16** in place of 1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydro-pyrazol-3-one **17** and 4-chlorophenyl isocyanate in place of phenyl isocyanate: mp 166.5−168 °C; HPLC 100.0%; MS (ESI−) *m*/*z* 404 (M − H)$^-$.

5-Hydroxy-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (4-Bromophenyl)amide (42). The title compound was prepared according to the procedure of compound **21** using 5-hydroxy-1,2-diphenyl-1,2-dihydropyrazol-3-one **16** in place of 1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **17** and 4-bromophenyl isocyanate in place of phenyl isocyanate: mp 156−160 °C; yield 53%; MS (APCI+) *m*/*z* 450 (M + H)$^+$. Anal. ($C_{22}H_{16}BrN_3O_3$) C, H, N.

5-Hydroxy-3-oxo-1,2-diphenyl-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid Phenylamide (43). The title compound was prepared according to the procedure of compound **21** using 5-hydroxy-1,2-diphenyl-1,2-dihydropyrazol-3-one **16** in place of 1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **17**: mp 171−175 °C; yield 47%; MS (APCI+) *m*/*z* 389 (M + H)$^+$. Anal. ($C_{22}H_{17}N_3O_3$) C, H, N.

1,2-Bis(3-chlorophenyl)-3,5-dioxopyrazolidine-4-carboxylic Acid (4-Chlorophenyl)amide (44). The title compound was prepared according to the procedure of compound **21** using 1,2-bis(3-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **17** in place of 1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **17**: mp 189−192 °C; yield 70%; MS (APCI+) *m*/*z* 474 (M + H)$^+$. Anal. ($C_{22}H_{14}Cl_3N_3O_3$) C, H, N.

1,2-Bis(3-chlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (4-Trifluoromethylphenyl)amide (45). The title compound was prepared according to the procedure of compound **21** using 1,2-bis(3-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **19** in place of 1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **17** and 4-trifluoromethylphenyl isocyanate in place of phenyl isocyanate: mp 151−156 °C; yield 37%; MS (APCI+) *m*/*z* 508 (M + H)$^+$. Anal. ($C_{23}H_{14}Cl_2F_3N_3O_3$·0.2$H_2O$) C, H, N.

1,2-Bis(3,4-dichlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid *p*-Tolylamide (46). The title compound was prepared according to the procedure of compound **21** using 1,2-bis(3,4-dichlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **18** in place of 1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **17** and 4-methylphenyl isocyanate in place of phenyl isocyanate: mp 185−187 °C; yield 70%; ^{1}H NMR (DMSO-d_6) δ 10.24 (s, 1H), 7.69 (d, J = 2.1 Hz, 2H), 7.52 (d, J = 6.6 Hz, 2H), 7.42 (d, J = 6.3 Hz, 2H), 7.20 (dd, J = 2.1, 6.6 Hz, 2H), 7.04 (d, J = 6.3 Hz, 2H), 2.23 (s, 3H), OH proton not visible; MS (APCI+) *m*/*z* 522 (M + H)$^+$. Anal. ($C_{23}H_{15}Cl_4N_3O_3$) C, H, N.

1,2-Bis(3,4-dichlorophenyl)-5-hydroxy-3-oxo-2,3-dihydro-1*H*-pyrazole-4-carboxylic Acid (3,4-Dichlorophenyl)amide (47). The title compound was prepared according to the procedure of compound **21** using 1,2-bis(3,4-dichlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **18** in place of 1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one **17** and 3,4-dichlorophenyl isocyanate in place of phenyl isocyanate: mp 160−163 °C; yield 35%; MS (APCI+) *m*/*z* 576 (M + H)$^+$. Anal. ($C_{22}H_{11}Cl_6N_3O_3$) C, H, N.

4-(4-Chlorobenzoyl)-1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one (48). The title compound was prepared according to the procedure of compound **34** using 4-chlorobenzoyl chloride in place of 3,4-dichlorobenzyl isocyanate: HPLC 100.0%; MS (ESI−) *m*/*z* 457 (M − H)$^-$.

1,2-Bis(4-chlorophenyl)-4-(3,4-dichlorobenzoyl)-5-hydroxy-1,2-dihydropyrazol-3-one (49). The title compound was prepared according to the procedure of compound **34** using 3,4-dichlorobenzoyl chloride in place of 3,4-dichlorobenzyl isocyanate: HPLC 100.0%; MS (ESI−) *m*/*z* 491 (M − H)$^-$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-4-(4-methoxybenzoyl)-1,2-dihydropyrazol-3-one (50). The title compound was prepared according to the procedure of compound **34** using 4-methoxybenzoyl chloride in place of 3,4-dichlorobenzyl isocyanate.

4-(4-Butoxybenzoyl)-1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one (51). The title compound was prepared according to the procedure of compound **34** using 4-*n*-butoxybenzoyl chloride in place of 3,4-dichlorobenzyl isocyanate: HPLC 98.0%; MS (ESI−) *m*/*z* 497 (M + H)$^+$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-4-(4-trifluoromethoxybenzoyl)-1,2-dihydropyrazol-3-one (52). The title compound was prepared according to the procedure of compound **34** using 4-trifluoromethyoxybenzoyl chloride in place of 3,4-dichlorobenzyl isocyanate: HPLC 100.0%; MS (ESI−) *m*/*z* 507 (M − H)$^-$.

4-(Biphenylyl-4-carbonyl)-1,2-bis(4-chlorophenyl)-5-hydroxy-1,2-dihydropyrazol-3-one (53). The title compound was prepared according to the procedure of compound **34** using 4-biphenylcarbonyl chloride in place of 3,4-dichlorobenzyl isocyanate: HPLC 80.0%; MS (ESI−) *m*/*z* 499 (M − H)$^-$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-4-(thiophene-2-carbonyl)-1,2-dihydropyrazol-3-one (54). The title compound was prepared according to the procedure of compound **34** using 2-thiophenecarbonyl chloride in place of 3,4-dichlorobenzyl isocyanate.

1,2-Bis(4-chlorophenyl)-5-hydroxy-4-(4-trifluoromethylbenzoyl)-1,2-dihydropyrazol-3-one (55). The title compound was prepared according to the procedure of compound **34** using 4-trifluoromethylbenzoyl chloride in place of 3,4-dichlorobenzyl isocyanate: HPLC 100.0%; MS (ESI−) *m*/*z* 491 (M − H)$^-$.

1,2-Bis(4-chlorophenyl)-5-hydroxy-4-[2-(4-methoxyphenyl)-acetyl]-1,2-dihydropyrazol-3-one (56). The title compound was prepared according to the procedure of compound **34** using 4-methoxyphenylacetyl chloride in place of 3,4-dichlorobenzyl isocyanate: HPLC 88.0%; MS (ESI+) *m*/*z* 469 (M + H)$^+$.

1,2-Bis(4-chlorophenyl)-4-cyclohexanecarbonyl-5-hydroxy-1,2-dihydropyrazol-3-one (57). The title compound was prepared according to the procedure of compound **34** using cyclohexanecarbonyl chloride in place of 3,4-dichlorobenzyl isocyanate: HPLC 100.0%; MS (ESI−) *m*/*z* 429 (M − H)$^-$.

Biology.[12] **MurB Purification.** The purification of *E. coli* MurB followed the method of Benson[2] and Dhalla[17] with some modifications. *E. coli* BL21 (λDE3) cells harboring the expression plasmid were grown in Luria-Bertani broth containing kanamycin (50 μg/mL) at 37 °C. The expression of *E. coli* MurB was induced by the addition of IPTG to a final concentration of 1 mM. After incubation at 37 °C for 2 h, the cells were harvested; resuspended in 25 mM Tris-HCl, pH 8.0, 5 mM DTT (buffer A); and lysed by two passages through a French press, at approximately 15 000 psi. The lysate was centrifuged at 100 000*g* for 30 min at 4 °C. Ammonium sulfate was added to the supernatant to a final concentration of 40%. Precipitated material was removed by centrifugation and the supernatant was loaded onto a Phenyl-Sepharose 6 Fast Flow column (General Electric, Piscataway, NJ) equilibrated with buffer B (1.6 M $(NH_4)_2SO_4$, 25 mM Tris-HCl, pH 8.0, and 5 mM DTT). MurB was eluted with a linear gradient from buffer B to buffer A. The eluted fractions containing MurB were pooled and concentrated. MurB was further purified using ion-exchange column chromatography on MonoQ column (General Electric, Piscataway, NJ). MurB was eluted from the column by a gradient of KCl from 0 to 1 M in buffer A. The purity of *E. coli* MurB was analyzed using SDS−PAGE.

MurB Assay. MurB activity was determined using a continuous assay[4] monitoring the oxidation of NADPH as measured by reduction in the absorbance at 340 nm. The reaction mixture contained 50 mM Tris-HCl, pH 8.0, 10 mM KCl, 100 μM NADPH, and 50 μM EP−UNAC, in a total volume of 200 μL. The reaction was initiated by the addition of enzyme. The OD_{340} was monitored over 5 min. One unit of enzyme activity was defined as the amount of enzyme that catalyzed the oxidation of 1 μmol of NADPH/min. A molar absorption coefficient of 6220 cm^{-1} M^{-1} for NADPH absorption at 340 nm was used.

MurB inhibition Studies. The inhibition of MurB enzymatic activity was determined following an initial 20-minute preincubation of 20 nM of the enzyme with the inhibitor. The substrate mixture was then added to the enzyme−inhibitor mixture to a final concentration of 50 μM enolpyruvyl UDP-*N*-acetylglycosamine and 100 μM NADPH. Six concentrations of inhibitor, 1.6, 3.2, 6.4, 12.8, 25, and 50 μM, were used for each compound tested. The IC_{50} values were derived using the data analysis function of Microsoft Excel (Sigmoid Curve Hill analysis 0−100). Phosphomycin, a MurA inhibitor, was included as a negative control.

Determination of in Vitro Antibacterial Activity. The microorganisms used for antimicrobial activity testing comprised a spectrum of Gram-positive bacteria including species of *Staphylococci*, *Streptococci*, and *Enterococci*. The organisms included recent clinical isolates that are resistant to methicillin, penicillin, and vancomycin. Gram-negative bacteria, a modified *E. coli* with altered outer membrane permeability (imp),[26] and a yeast strain, *C. albicans*, were also included in the test panel. The minimal inhibitory concentration (MIC) was determined by the broth dilution method using Muller−Hinton II media (Baltimore Biological Laboratories) following the recommendations of the National Committee for Clinical Laboratory Standards.[27] An inoculum of 5×10^5 cfu/mL and a range of compound concentrations (0.06−128 μg/mL) were used. The MICs were determined after incubation for 18 h at 35 °C in an ambient air incubator.

Effect of Pyrazolidine-3,5-diones and 5-Hydroxy-1*H*-pyrazol-3(2*H*)-ones on Peptidoglycan Biosynthesis. Peptidoglycan biosynthesis was measured by determining the amount of soluble peptidoglycan (SPG) produced by a *S. epidermidis* strain. The method was originally reported by Boothby[28] and was modified to fit a 96-well microplate. Briefly, cells were grown in BHI to an OD_{600nm} of 0.6, harvested, washed twice with cold H_2O, and resuspended in cell wall enriched media[28] at 10% of the original volume. The reaction mixture contained 200 μL of cell suspension, 50 μg/mL chloramphenicol, 100 μg/mL penicillin G, 2.9 μM/40nCi [^{14}C]-*N*-acethylglucosamine, and test compound, at concentrations of 0.0, 3.1, 6.2, 12.5, and 25 μg/mL, in a total volume of 250 μL. After incubation with agitation at 37 °C for 1 h, the reaction mixture was centrifuged at 1500*g* for 20 min. A quantity of 200 μL of the supernatant was transferred into a Millipore MultiScreen 1.2 μm glass fiber filter plate. BSA (bovine serum albumin) and TCA (trichloroacetic acid) were added to each well, giving final concentrations of 0.4% and 5%, respectively. After a 30-min incubation at 4 °C, the plates were filtered, washed with 5% TCA, and counted on a Packard TopCount using MicroScint scintillation fluid (Perkin-Elmer Life Sciences, Boston, MA).

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Supporting Information Available: Elemental analysis and HPLC results for compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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