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2-Phenyl-5,6-dihydro-2*H*-thieno[3,2-*c*]pyrazol-3-ol Derivatives as New Inhibitors of Bacterial Cell Wall Biosynthesis

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Abstract—Twenty-five 2-phenyl-5,6-dihydro-2*H*-thieno[3,2-*c*]pyrazol-3-ol derivatives were synthesized for evaluation as new inhibitors of bacterial cell wall biosynthesis. Many of them demonstrated good inhibitory activity against *Staphylococcus aureus* MurB, MurC and MurD enzymes in vitro and antimicrobial activity against gram-positive bacteria including MRSA, VRE and PRSP. However, when they were tested in the presence of 4% bovine serum albumin, the MIC values increased to greater than 128 μg/mL against PRSP. None of the compounds demonstrated activity against gram-negative bacteria at MIC <32 μg/mL.

Since peptidoglycan is an essential bacterial cell-wall polymer, peptidoglycan biosynthesis provides a unique and selective target for antibiotic action.[1] Peptidoglycan biosynthesis requires more than 10 synthetic transformations, each one of them requiring a specific enzyme.[1] These enzymes include MurA, MurB, MurC, MurD, MurE, MurF, MraY, MurG, and the transglycosylase and transpeptidase families of enzymes. Inhibition of any of these essential enzymes leads to loss of cell shape and integrity followed by bacterial death.[2,3] This applies in both gram-positive and gram-negative organisms. Of these enzymes, only MurA, the transglycosylases and the transpeptidases have been the targets of commerical antimicrobial agents. β-Lactam antibiotics inhibit transpeptidases; vancomycin inhibits transglycosylases; fosfomycin inhibits MurA.[4] Despite the unprecedented commercial success of β-lactam and glycopeptide antibiotics, their clinical use has recently been compromised by the emergence of resistant bacterial strains. Therefore, one of the attractive strategies to overcome resistance to β-lactam antibiotics and vancomycin is to find novel inhibitors of the cell wall biosynthetic enzymes other than the transpeptidases and the transglycosylases.[5] The objective of our bacterial cell-wall program was to identify novel inhibitors of the first eight enzymes of the peptidoglycan biosynthesis. In a previous publication,[6] we reported the urea, hydantoin and *N*-alkyl derivatives of muramycin C1 as novel inhibitors of MraY. Here, we report the synthesis and antimicrobial activity of 2-phenyl-5,6-dihydro-2*H*-thieno[3,2-*c*]pyrazol-3-ol derivatives as new inhibitors of *Staphylococcus aureus* MurB, MurC and MurD enzymes.

2-Phenyl-5,6-dihydro-2*H*-thieno[3,2-*c*]pyrazol-3-ol derivatives **5** were synthesized in four steps in ~60% overall yields. Reaction of methyl acrylates **1** with methyl thioglycolate in the presence of a catalytic amount of piperidine gave 3-methoxycarbonylmethylsulfanyl-propionic acid methyl esters **2** in almost quantitative yield. Cyclization of **2** with titanium tetrachloride and triethylamine in dichloromethane gave 3-oxotetrahydrothiophene-2-carboxylates **3** in ~60% yields.[7] Condensation of **3** with substituted phenyl hydrazines, followed by cyclization with sodium methoxide gave 2-phenyl-5,6-dihydro-2*H*-thieno[3,2-*c*]pyrazol-3-ol derivatives **5** in ~95% yield (Scheme 1).[8] Similarly, condensation of commercially available keto esters **6** with substituted phenylhydrazines, followed by cyclization with sodium methoxide gave 2-phenylbicyclopyrazol-3-ol derivatives **8** (Scheme 2). Reaction of 2-(3-cyanophenyl)-5,6-dihydro-2*H*-thieno[3,2-*c*]pyrazol-3-ol (**5l**) with potassium hydroxyde in *t*-butanol gave 3-(3-hydroxy-5,6-dihydro-thieno[3,2-*c*]pyrazol-2-yl)-benzamide (**5o**) in good yield (Scheme 3).[9] Oxidation of **5j** with H_2O_2/HCO_2H in dichloromethane gave sulfoxide **5u** in an excellent yield (Scheme 4).[10] However, further oxidation

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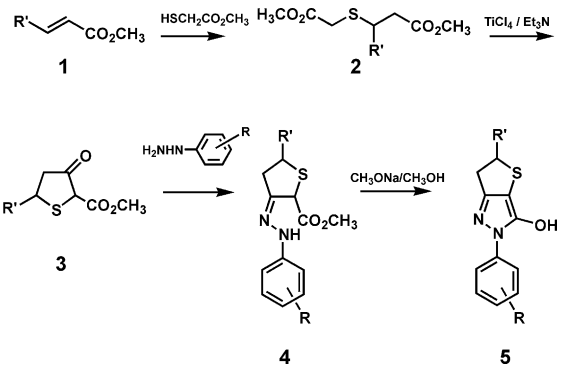
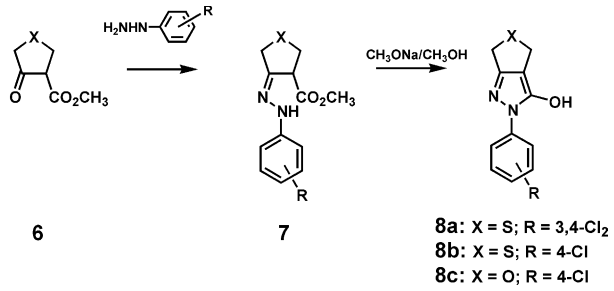
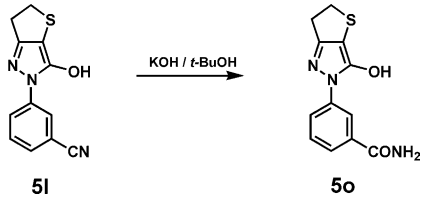


Scheme 1.



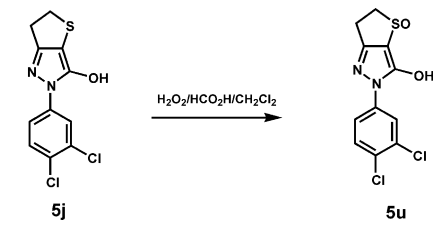
Scheme 2.



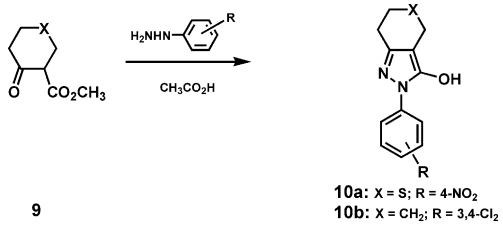
Scheme 3.

of **5u** with *m*-chloroperbenzoic acid failed to yield the corresponding sulfone derivative. Condensation of keto esters **9** with substituted phenylhydrazines in acetic acid gave 2-phenylbicyclopyrazol-3-ol derivatives **10** in good yield (Scheme 5).

The activity of **5c** against *S. aureus* MurB-MurD and gram-positive bacteria was discovered through biological screens. Using **5c** as the lead, 25 derivatives were synthesized and submitted for evaluation as new inhibitors of bacterial cell wall biosynthesis. Among the twenty five 2-phenyl-bicyclopyrazol-3-ol derivatives tested, five of them[11] (**5b**, **5c**, **5d**, **5e**, and **5k** with MIC values in the range of 0.25–32 μg/mL) demonstrated the best antibacterial activity against gam-positive bacteria including methicillin resistant *S. aureus* (MRSA), vancomycin resistant *Enterococus* (VRE) and penicillin resistant *Staphylococcus pneumoniae* (PRSP). However, when tested in the presence of 4% bovine serum albumin (BSA), the MIC values increased to >128 μg/mL against PRSP, suggesting that these compounds are highly protein bonded by BSA. These five derivatives also demonstrated good activity against at least one of *S. aureus* MurB-MurD enzymes (Table 1). In general,



Scheme 4.

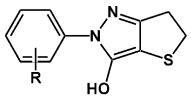


Scheme 5.

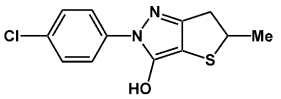
para-substituted derivatives were slightly more active or equally as active as *meta*-substituted derivatives. *ortho*-Substituted derivative **5p** was devoid of enzyme activity at IC_{50} ≤25 μg/mL, but demonstrated slight antibacteria activity. Di-substituted derivative **5q** was less active than mono-substituted derivatives **5k** and **5e**. As lipophilicity of compounds decreased (**5c**: clog P = 3.06 to **5f**: clog P = 2.53 to **5o**: clog P = 1.41 and **5j**: clog P = 3.62 to **5u**: clog P = 2.01), activity against the Mur enzymes and gram-positive bacteria decreased. The rates of passive diffusion of uncharged molecules across lipid membranes correlate reasonably well with their clog P. That is, the more polar a compound is, the less readily it enters and diffuses across the cytoplasmic membrane.[12] Suprisingly, *p*-isopropyl derivative **5h** and *t*-butyl derivative **5i** were devoid of enzyme inhibitory activity at ≤25 μg/mL and gave MIC >64 μg/mL. 5-Methyl derivative **5r** and 5-phenyl derivative **5s** were less active than the lead **5c**. The carbocyclic derivative **10b** is devoid of inhibitory activity against enzymes at ≤25 μg/mL and bacteria at <64 μg/mL. When the sulfur or oxygen was placed in the middle of the ring such as in **8a**, **8b**, **8c**, and **10a**, the compounds were also devoid of enzyme inhibitory activity at ≤25 μg/mL and gave MIC ≤32 μg/mL. (Table 2). None of twenty five 2-phenyl-bicyclopyrazol-3-ol derivatives tested demonstrated activity against gram-negative bacteria at <32 μg/mL.

In summary, several 2-phenyl-5,6-dihydro-2*H*-thieno-[3,2-*c*]pyrazol-3-ol derivatives were identified as a new class of bacterial cell wall biosynthesis inhibitors. They demonstrated good activity against *S. aureus* MurB, MurC and MurD and gram-positive bacteria including MRSA, VRE and PRSP. However, when they were tested in the presence of 4% bovine serum albumin, their MIC values increased to greater than 128 μg/mL against PRSP. Although compounds **5b**, **5c**, **5d**, **5e** and **5k** are less active than vancomycin against gram-positive bacteria, they are good leads for further investigation. None of them demonstrated activity against gram-negative bacteria at MIC <32 μg/mL.

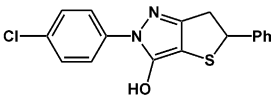
Table 1. Antimicrobial activities MIC (μg/mL) and IC_{50} values (μg/mL) of 2-phenyl-5,6-dihydro-2*H*-thieno[3,2-*c*]pyrazol-3-ol derivatives



5a-q



5r

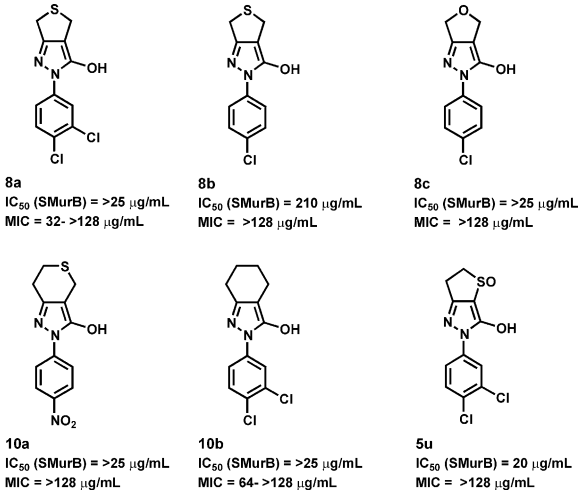


5s

R =	**5a** H	**5b** 4-F	**5c** 4-Cl	**5d** 4-Br	**5e** 4-CF_3	**5f** 4-CN	**5g** 4-NO_2	**5h** 4-*i*Pr	**5i** 4-*t*Bu	**5j** 3,4-Cl_2	**5k** 3-CF_3	**5l** 3-CN	**5m** 3-NO_2	**5n** 3-Cl	**5o** 3-$CONH_2$	**5p** 2-CF_3	**5q** 3,5-CF_3	**5r**	**5s**	**Vancomycin**
MIC (μg/mL):																				
S. aureus GC 1131 (MRSA)	16	2	16	16	8	32	64	128	>128	32	4	32	32	32	128	32	32	16	128	2
S. aureus GC 4543 (MSSA)	32	4	2	4	1	16	2	128	128	8	1	16	32	32	64	32	32	16	128	0.5
S. aureus GC 2216 (ATCC)	16	2	2	2	2	8	4	128	>128	8	2	8	16	8	128	32	32	32	64	0.5
E. faecalis GC 4555 (ATCC)	32	8	16	16	16	8	32	>128	>128	128	16	8	64	32	64	32	32	32	>128	2
E. faecalis GC 2242 (VRE)	4	8	16	32	8	8	64	>128	>128	64	8	8	32	8	32	16	16	2	64	>128
S. pneumo GC1894 (PRSP)	4	2	1	1	0.5	4	4	32	64	4	0.5	4	4	2	8	8	2	4	64	<0.12
S. pneumo GC1894 (PRSP)[a]	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	—
MSCNS GC 646	16	0.25	4	1	4	4	1	128	>128	16	2	2	32	32	>128	64	>128	16	128	
E. coli GC 4559	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
E. coli GC 4560	>128	32	128	128	128	64	>128	>128	>128	128	128	64	>128	>128	128	128	>128	>128	128	0.25
IC_{50} (μg/mL):																				
S. aureus MurB	>25	12	7.1	3.6	11	>25	5.6	>25	>25	11	21	14	6.2	>25	>25	>25	>25	>25	11	
S. aureus MurC	>25	>25	10.1	11.4	>25	>25	19.1	>25	>25	7	>25	13.3	23.1	15.8	>25	>25	>25	>25	>25	
S. aureus MurD	20.7	>25	11.5	9.0	12.3	12.0	9.5	>25	>25	14	19.2	11.4	9.1	8.3	12.5	>25	>25	17.1	24.5	

[a]Tested in the presence of 4% bovine serum albumin.

Table 2. Activity of other 2-phenyl-bicyclopyrazol-3-ol derivatives



Acknowledgements

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11. Spectra data of the five most active compounds are summarized as follows: **5b**, calcd for $C_{11}H_9FN_2OS$: 236.3, Electrospray-MS m/z 237.0 $(M+H)^+$, ^1H NMR (DMSO-d_6) δ 11.56 (1H, br), 7.66 (2H, m), 7.25 (2H, m), 3.58 (2H, t, $J=5.7$ Hz), 2.89 (2H, t, $J=5.7$ Hz). **5c**, calcd for $C_{11}H_9ClN_2OS$: 252.7, Electrospray-MS m/z 253.1 $(M+H)^+$, ^1H NMR (DMSO-d_6) δ 11.79 (1H, br), 7.69 (2H, d, $J=6$ Hz), 7.48 (2H, t, $J=6$ Hz), 3.58 (2H, t, $J=5.7$ Hz), 2.89 (2H, t, $J=5.7$ Hz). **5d**, calcd for $C_{11}H_9BrN_2OS$: 297.2, Electrospray-MS m/z 297.0 $(M+H)^+$, ^1H NMR (DMSO-d_6) δ 11.74 (1H, br), 7.64 (4H, m), 3.59 (2H, t, $J=5.7$ Hz), 2.89 (2H, t, $J=5.7$ Hz). **5e**, calcd for $C_{12}H_9F_3N_2OS$: 286.3, Electrospray-MS m/z 284.9 $(M-H)^-$, ^1H NMR (DMSO-d_6) δ 11.97 (1H, br), 7.94 (2H, d, $J=6$ Hz), 7.77 (2H, t, $J=6$ Hz), 3.60 (2H, t, $J=5.7$ Hz), 2.92 (2H, t, $J=5.7$ Hz). **5k**, calcd for $C_{12}H_9F_3N_2OS$: 286.3, Electrospray-MS m/z 284.9 $(M-H)^-$, ^1H NMR (DMSO-d_6) δ 11.94 (1H, br), 8.02 (2H, m), 7.64 (2H, m), 3.60 (2H, t, $J=5.7$ Hz), 2.91 (2H, t, $J=5.7$ Hz).
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