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Phenyl thiazolyl urea and carbamate derivatives as new inhibitors of bacterial cell-wall biosynthesis

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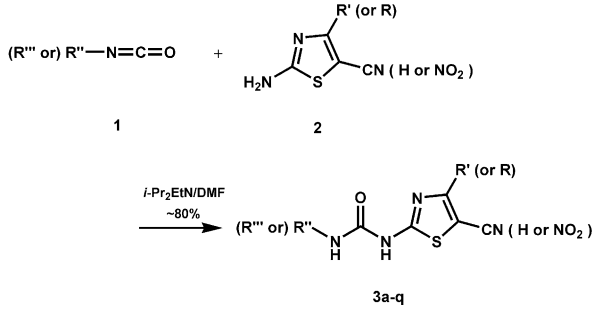
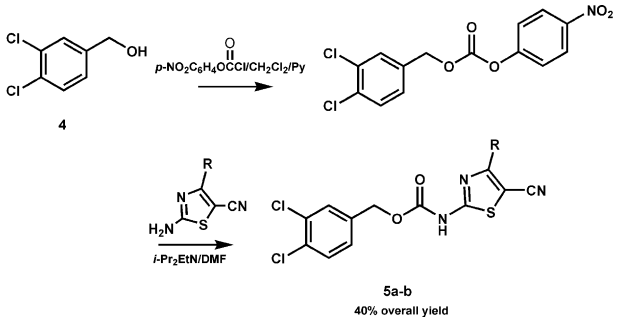
Abstract—Over 50 phenyl thiazolyl urea and carbamate derivatives were synthesized for evaluation as new inhibitors of bacterial cell-wall biosynthesis. Many of them demonstrated good activity against MurA and MurB and gram-positive bacteria including MRSA, VRE and PRSP. 3,4-Difluorophenyl 5-cyanothiazolylurea (**3p**) with clog P of 2.64 demonstrated antibacterial activity against both gram-positive and gram-negative bacteria.

Since peptidoglycan is an essential bacterial cell-wall polymer, peptidoglycan biosynthesis provides a unique and selective target for antibiotic action.[1] Peptidoglycan biosynthesis requires 10 synthetic transformations, each one of them requiring a specific enzyme.[1] These enzymes include MurA, MurB, MurC, MurD, MurE, MurF, MurG, MraY, and the transglycosylase and transpeptidase families of enzymes. Inhibition of any of these essential proteins leads to loss of cell shape and integrity followed by bacterial death.[2–4] This applies in both gram-positive and gram-negative organisms. Our bacterial cell-wall program is committed to identify the inhibitors of the first eight enzymes of the peptidoglycan biosynthesis. In a previous publication,[5] we reported 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 MurB, MurC, and MurD. Here we report the synthesis and antimicrobial activity of phenyl thiazolyl urea and carbamate derivatives as new inhibitors of MurA and MurB.

Thiazolyl urea derivatives **3** were prepared in one step in ~80% yield from reaction of substituted aminothiazole **2** with isocyanate **1** in the presence of Hunig's base in DMF (Scheme 1). Carbamate derivatives **5** were prepared in ~40% overall yields by reaction of 3,4-dichlorobenzyl alcohol (**4**) with *p*-nitrophenyl-chloroformate, followed by reaction with aminothiazoles in the presence of Hunig's base (Scheme 2). Since



Scheme 1.



Scheme 2.

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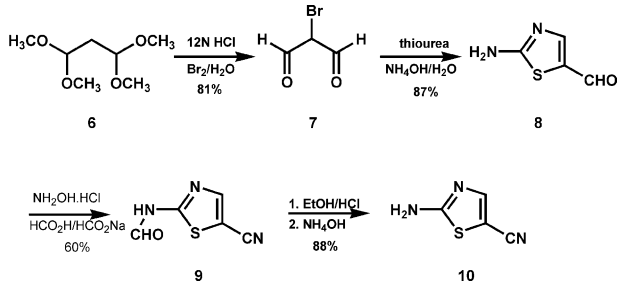
isocyanates and benzyl alcohols are commercially available, one of the major efforts has been the synthesis of substituted aminothiazoles.

2-Amino-5-cyanothiazole **10** was prepared in four steps in 37% yield (Scheme 3).[6]

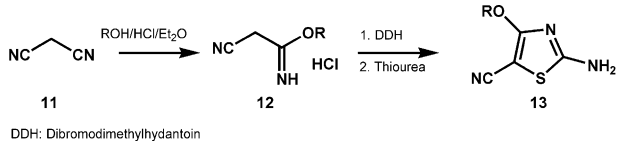
Three 2-amino-4-alkoxy-5-cyanothiazoles were prepared in two steps in about 40% overall yields (Scheme 4).[7]

A very effective new synthesis of 4-substituted 2-amino-5-cyanothiazoles was developed. 2-Amino-4-chloro-5-cyanothiazole **18** was prepared in four steps in 27% overall yield by the literature process.[8,9] Reaction of **18** with morpholine gave 2-amino-5-cyanothiazole **19** in about 85% yield (Scheme 5). This process was applied to the synthesis of other 4-substituted 2-amino-5-cyanothiazoles.[10]

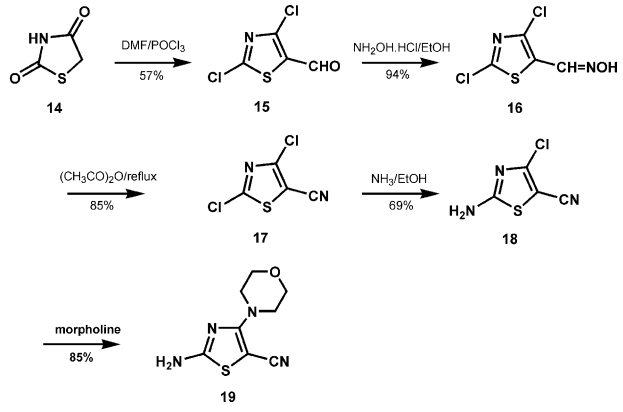
As shown in Table 1, 3,4-dichlorophenyl thiazolyl derivative **3a** demonstrated an IC_{50} value of 20 μg/mL against MurA and good antimicrobial activity against gram-positive bacteria including methicillin resistant *Staphlococcus aureus* (MRSA), vancomycin resistant



Scheme 3.



Scheme 4.



Scheme 5.

Enterococcus (VRE) and penicillin-resistant *Streptococcus pneumoniae* (PRSP) with MICs (minimum inhibitory concentrations) in the range of 0.5–4 μg/mL. Using **3a** as the lead, over 50 urea and carbamate derivatives were synthesized and submitted for evaluation as new inhibitors of bacterial cell-wall biosynthesis. The introduction of the 4-cyano group (**3b**) to **3a** slightly improved the activity against VRE and PRSP but slightly decreased the activity against MRSA. However, the introduction of the 5-cyano group (**3c**) to **3a** improved the activity against MRSA, VRE and PRSR by a factor of 2->4-fold and considerably improved IC_{50} values (4.5–12.6 μg/mL) against MurA and MurB. The introduction of a 4-*t*-butyl group to **3c** further improved both MIC and IC_{50} values. Of 19 derivatives tested, the derivative **3d** demonstrated the best antibacterial activity against gram-positive bacteria with MICs in the range of <0.12–0.25 μg/mL and IC_{50} values in the range of 2.8–6.2 μg/mL. Attempts to further improve the MIC and IC_{50} values of **3d** by the replacement of the 4-*t*-butyl group with 4-iso-butoxyl, 4-ethoxyl, 4-methoxyl, 4-chloro or 4-morpholino groups in the thiazolyl ring failed. The 5-nitrothiazoyl derivative **3o** was as active as the 5-cyanothiazoyl derivative **3c**. Antibacterial activity was diminished, when the 3,4-dichlorophenyl group of **3c** and **3d** was replaced by 3,4-dichlorobenzyl group (**3q** and **3n**).

The MIC values of **3c** and **3d** against PRSP were ≤0.12 μg/mL. However, when **3c** and **3d** were tested in the presence of 4% bovine serum albumin, their MIC values increased to 128 μg/mL. Since this increase in MIC might be due to serum protein binding, attempts were made to minimize the serum protein binding problem by increasing the polarity of compounds. The 3,4-dichlorophenyl group of **3d** was replaced by 3,4-dimethylphenyl, 3,4-difluorophenyl, 3-cyanophenyl, 3-pyridinyl, or 3,4-dichlorobenzyl group. These new derivatives (**3j–n**) failed to improve the MIC against PRSP when tested in the presence of 4% bovine serum albumin. In general, the antimicrobial activity decreased as the polarity increased. However, when the 3,4-dichlorophenyl group of **3c** with clog P of 3.44 was replaced by a 3,4-difluorophenyl group, the resulting compound

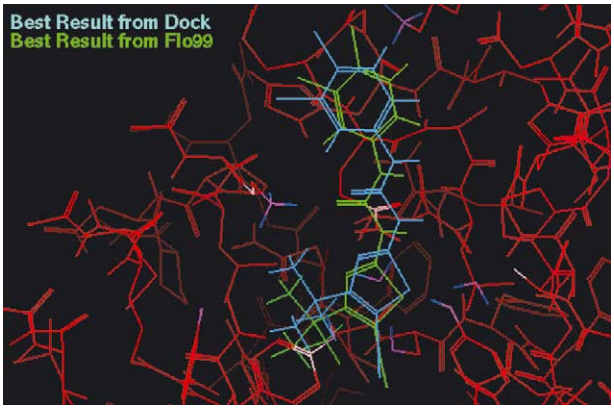
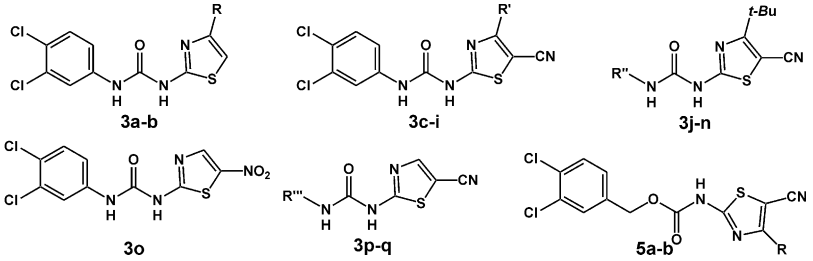


Figure 1. Preferred orientation of **3d** in the MurB crystal structure based on the Dock and Flo programs.

3p with clog P of 2.64 demonstrated antibacterial activity against both gram-positive and gram-negative bacteria with MIC values in the range of 0.5–8 μg/mL. It also demonstrated improved MIC (64 μg/mL) against PRSP when tested in the presence of 4% serum albumin.

Molecular modeling studies were carried to explore the potential binding preferences of this series within the MurB structure. An expanded set of conformations of *t*-butyl analogue **3d** were generated using an in-house script described previously.[11] The resulting conformer database was docked into the MurB crystal structure[12] with the substrate removed, using Dock 3.5.[13] The same structures were docked together using Flo.[14] The lowest energy complex obtained by both methods (Fig. 1) suggest that **3d** prefers to bind to the substrate-binding region of the structure. The carbamate occupies the bisphosphate binding region of the MurB substrate, and mimics a number of its interactions, in particular, strong hydrogen bonds between the urea carbonyl and Lys217. There are also strong interactions Gln288 Tyr125 and Lys275. There are several alternative binding orientations available to the diaryl urea structure with slightly lower interaction energies. In some of these orientations the urea remains in the same place, but the aromatic ring and the heterocycle ring switch positions. In these orientations, the carbamate derivatives form stronger interactions with the enzyme than the corresponding urea derivatives due to the introduction of a hydrogen bond between the carbamate oxygen and Gln288. Therefore, carbamate derivatives **5a** and **5b**

Table 1. Antimicrobial activities (μg/mL) and IC_{50} values (μg/mL) of thiazolyl urea and carbamate derivatives

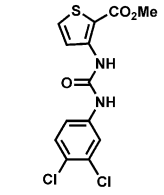


R, R′ or R″ =	**3a** H	**3b** CN	**3c** H	**3d** *t*-Bu	**3e** O*i*-Bu	**3f** OEt	**3g** Cl	**3h** OMe	**3i** morpholino	**3j** 3,4-diMePh
MIC (μg/mL)										
S. aureus GC 1131 (MRSA)	2	8	1	<0.12	0.5	1	0.5	>128	>128	0.5
S. aureus GC 4543 (MSSA)	2	4	0.5	<0.12	0.25	0.5	0.25	>128	>128	0.25
S. aureus GC 2216 (ATCC)	2	4	0.5	<0.12	0.5	0.5	0.25	>128	>128	0.25
E. faecalis GC 4555 (ATCC)	4	8	1	0.25	0.25	>128	0.5	32	>128	0.5
E. faecalis GC 2242 (VRE)	4	2	0.5	<0.12	0.25	0.5	0.25	4	128	0.5
S. pneumo GC1894 (PRSP)	0.5	<0.12	<0.12	<0.12	<0.12	<0.12	<0.12	>128	>128	<0.12
S. pneumo GC1894 (PRSP)[a]	>128	128	128	128	128	128	>128	>128	>128	128
MSCNS GC 646	2	4	1	<0.12	0.25	0.5	0.5	>128	>128	0.5
E. coli GC 4559	>128	>128	>128	>128	>128	>128	>128	>128	>128	>128
E. coli GC 4560	0.50	8	4	0.5	>128	0.5	0.5	>128	>128	2
MurA, *E. coli*: IC_{50} = μg/mL	20	>25	12.6	3.3	>25	>25	>25	21.6	>25	>25
MurB, *E. coli*: IC_{50} = μg/mL	>25	>25	4.5	6.2	5.8	23	>25	9.8	>25	6.2
MurB, *Staph*: IC_{50} = μg/mL	>25	14	5.6	2.8	—	—	>25	—	13	6.3
clog P	3.35	3.81	3.44	5.57	5.25	4.37	4.34	4.03	4.04	5.42

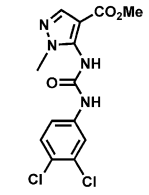
R″, R‴ or R =	**3k** 3,4-diFPh	**3l** 3-CNPh	**3m** 3-Py	**3n** 3,4-diClPh CH_2	**3o** —	**3p** 3,4-diFPh	**3q** 3,4-diClPh CH_2	**5a** H	**5b** *t*-Bu	Vancomycin
MIC (μg/mL)										
S. aureus GC 1131 (MRSA)	<0.12	>128	>128	1	1	4	>128	>128	2	2
S. aureus GC 4543 (MSSA)	<0.12	>128	>128	0.5	0.5	2	>128	>128	1	0.5
S. aureus GC 2216 (ATCC)	<0.12	>128	>128	0.25	0.25	4	>128	>128	8	0.5
E. faecalis GC 4555 (ATCC)	1	16	>128	1	1	4	>128	64	2	2
E. faecalis GC 2242 (VRE)	0.5	8	>128	0.5	1	4	64	64	1	>128
S. pneumo GC1894 (PRSP)	<0.12	8	>128	<0.12	0.25	0.5	64	128	0.25	<0.12
S. pneumo GC1894 (PRSP)[a]	128	>128	>128	128	128	64	128	>128	>128	–
MSCNS GC 646	0.5	>128	>128	1	0.5	4	>128	>128	>128	–
E. coli GC 4559	>128	>128	>128	>128	>128	8	>128	>128	>128	>128
E. coli GC 4560	1	>128	>128	2	2	4	>128	>128	>128	0.25
MurA, *E. coli*: IC_{50} = μg/mL	>25	>25	>25	20.8	4.6	>25	>25	>25	25	
MurB, *E. coli*: IC_{50} = μg/mL	>25	>25	>25	>25	3.8	19	>25	>25	18	
MurB, *Staph*: IC_{50} = μg/mL	—	>25	>25	11	3.0	—	24	24	—	
clog P	4.77	4.48	3.11	5.64	—	2.64	3.51	4.19	6.31	

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

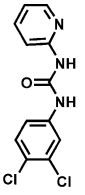
Table 2. Activity of other phenyl heteroaryl urea derivatives



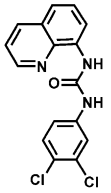
20
IC_{50} (MurB) = 18 μg/mL
MIC = 0.5- >128 μg/mL



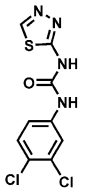
21
IC_{50} (MurB) = 23 μg/mL
MIC = 1-4 μg/mL



22
IC_{50} (MurB) = 17 μg/mL
MIC = 0.5-8 μg/mL



23
IC_{50} (MurB) = >25 μg/mL
MIC = 32- >128 μg/mL



24
IC_{50} (MurB) = >25 μg/mL
MIC =16- >128 μg/mL

were prepared. There was no significant difference in activity between the carbamate derviatives (**5a** and **5b**) and the urea derivatives (**3q** and **3n**).

As is evident from Table 2, other phenyl heteroaryl urea derivatives investigated (**20**–**24**) were not as active as many of the phenyl thiazoyl derivatives listed in Table 1.

In summary, many substituted phenyl thiazolyl urea and carbamate derivatives were identified as a new class of bacterial cell-wall biosynthesis inhibitors. They demonstrated good activity against MurA and MurB and gram-positive bacteria including MRSA, VRE and PRSP. However, when tested in the presence of 4% bovine serum albumin, their MIC values increased to greater than 128 μg/mL against PRSP. The derivative **3p** with clog P of 2.64 demonstrated antibacterial activity against both gram-positive and gram-negative bacteria with MIC values in the range of 0.5–8.0 μg/mL. It also demonstrated improved MIC value (64 μg/mL) against PRSP when tested in the presence of 4% serum albumin. Since many compounds **3c**–**e**, **3j**, **3k**, **3n**, and **3o** are more active than vancomycin against gram-positive bacteria, they are good leads for further investigation. The compounds **3c** and **3d** demonstrated IC_{50} of 32.1 and 4.2 μg/mL in the soluble peptidoglycan screen using a strain of *S. epidermides*.[15] Thus, the cell wall biosynthesis inhibition (MurA and MurB) of **3c** and **3d** is responsible for at least part of their antimicrobial activity. Recently, antimicrobial activity of some related diaryl ureas[16,17] have been shown to act by inhibition of bacterial respiration.[16] It is likely that the antimicrobial activity of the phenyl thiazolyl urea and carbamate derivatives are due to inhibition of both bacterial cell-wall biosynthesis and bacterial respiration.

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References and notes

1. Bugg, T. D. H.; Walsh, C. T. *Nat. Prod. Rep.* **1992**, *9*, 199.
2. Isono, K.; Uramoto, M.; Kusakabe, H.; Kimura, K.; Izaki, K.; Nelson, C. C.; McCloskey, J. A. *J. Antibiotics* **1985**, *38*, 1617.
3. Kimura, K.; Ikeda, Y.; Kagami, S.; Yoshihara, M. *J. Antibiotics* **1998**, *51*, 1099.
4. Green, D. W. *Expert Opin. Ther. Targets* **2002**, *6*, 1.
5. Li, Z.; Francisco, G. D.; Hu, W.; Labthavikul, P.; Petersen, P. J.; Severin, A.; Singh, G.; Yang, Y.; Rasmussen, B. A.; Lin, Y. I.; Skotnicki, J. S.; Mansour, T. S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 2591.
6. Frishberg, M. D. US Patent 4,324,899, 1982; *Chem. Abstr.* **1982**, *97*, 25169.
7. Vanmaele, L. European Patent Application 687,674A, 1995.
8. Beck, G. U. S. Patent 4,555,577, 1985; *Chem. Abstr.* **1984**, *101*, 211130.
9. Athmani, S.; Iddon, B. *Tetrahedron* **1992**, *48*, 7689.
10. Li, Z.; Lin, Y. I. Unpublished work.
11. Joseph-McCarthey, D.; Thomas, B. E., IV; Belmarsh, M.; Moustakas, D.; Alvarez, J. C. *Proteins* **2003**, *51*, 172.
12. Benson, T. E.; Walsh, C. T.; Hogle, J. M. *Biochemistry* **1997**, *36*, 806.
13. Makino, S.; Kuntz, I. D. *J. Comput. Chem.* **1997**, *18*, 1812.
14. McMartin, C.; Bohacek, R. S. *J. Comp. Aided Mol. Des.* **1997**, *11*, 333.
15. Yang, Y. Manuscript in preparation.
16. Proctor, R. A.; Dalal, S. C.; Kahl, B.; Peters, G.; Nichols, W. W. *Antimicrob. Agents Chemother.* **2002**, *46*, 2333.
17. Beaver, D. J.; Roman, D. P.; Stoffel, P. J. *J. Am. Chem. Soc.* **1957**, *79*, 1236.