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Synthesis and antibacterial activities of chiral 1,3-oxazinan-2-one derivatives

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Abstract—We report here the design, synthesis, and antibacterial activities of novel classes of compounds containing chiral 1,3-oxazinan-2-ones and oxazolidinones as the basic core structures. These compounds are tertiary amines containing the core structures and two aryl substituents. Several of these molecules exhibit potent antibacterial activities against the tested Gram-positive bacteria, including *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus subtilis*. These compounds represent new structure scaffolds and can be further optimized to give new antibacterial agents with structures significantly different from those of existing classes of antibiotics.

Antibiotic resistance is a growing problem that threatens human health globally. Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *enterococci* (VRE)[1,2] are very dangerous and can be life threatening especially for patients whose immune systems have been compromised due to HIV, surgery or any other illness. During the recent decades, the effort of discovering novel antibacterial agents has slowed down; in fact, oxazolidinones are the only new class of synthetic antibacterial agents over the past 30 years that possess totally new structures compared to existing antibacterial agents.[3–6] The first compound of this class, linezolid **1** (Fig. 1), was approved in 2000 for the treatment of multi-drug resistant bacterial infections including diseases caused by MRSA, VRE and *Streptococcus pneumoniae*. Oxazolidinones bind to the 50S subunit of the bacterial ribosome and inhibit protein synthesis at a very early stage by preventing the initiation of mRNA translation. Because they target the bacterial protein synthesis at an early stage, drug resistance was expected to be rare; however, resistance to zyvox (linezolid) has already been reported.[7,8]

Some structures of the existing small molecule antibacterial agents are shown in Figure 1, which include linezolid **1**, ciprofloxacin **2**, sulfonamide **3**, and chloramphenicol **4**. The very general features of these agents are that they all contain aromatic and/or heterocyclic structures and they have heteroatom substituents such as halo, amino, and hydroxyl groups. Linezolid **1** has the oxazolidinone as the core structure, which is important for its activity. Ciprofloxacin **2** is one example of the fluoroquinolone class of antibiotics.[9–13] They kill bacteria by inhibiting DNA gyrase enzyme which is essential for DNA replication. The structure of **2** contains a fused aromatic ring with a fluorine and a polar piperazine substituents. Sulfonamide **3** contains phenyl sulfonyl phenyl amines. The sulfonamides are inhibitors of the bacterial enzymes required for the synthesis of tetrahydrofolate,[14] an essential nutrient for bacterial growth. Chloramphenicol **4** contains substituted nitrophenol with dichloromethyl acetamido functional groups. It also inhibits bacterial protein synthesis and is a broad spectrum antibiotic for both Gram-positive and Gram-negative bacteria but with some serious side effects.[15] The general features of these compounds can be used to design new structures that can be potentially useful antibacterial agents.

Many new antibacterial agents are designed based on modification of the existing structural classes; since the antibiotic assay is easy to carry out, the modes of action of the agents often are discovered after finding them active. There are many methods of discovering new drugs. Identifying drug-like features in existing drug classes and incorporating them into a de novo design of a new compound could be an efficient method to discover new drugs with significantly different structure

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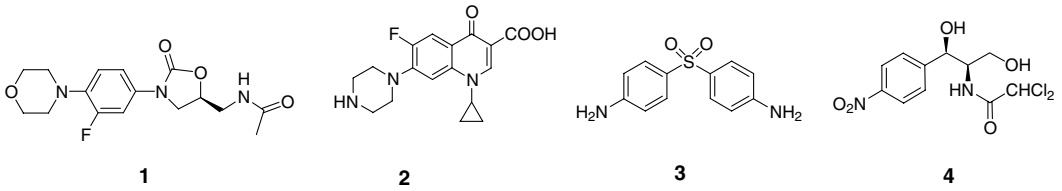


Figure 1. Structures of some common synthetic antibacterial agents.

motifs. With the advancement of synthetic organic chemistry, it is possible to create new structures that are potentially useful in medicine. This process is somewhat related to the concept of chemical structure evolution. As part of our effort to discover new classes of antibacterial agents, we designed and synthesized a small library of compounds containing very general structural features of existing synthetic antibacterial agents, using chiral cyclic carbamates as the core structures. The general structure (**5**) of the library compounds is shown in Figure 2.

The basic platform is a tertiary amine composed of a 1,3-oxazinan-2-one and two aromatic groups. The core structure is a homolog of the oxazolidinone in linezolid but with the opposite stereochemistry. Three sites $\mathbf{P_1}$–$\mathbf{P_3}$ can be optimized to obtain better potencies. Site $\mathbf{P_1}$ contains the chiral core structure 1,3-oxazinan-2-one or other chiral heterocycles, groups $\mathbf{P_2}$ and $\mathbf{P_3}$ can be substituted aromatic groups or heterocycles. There are several considerations in this structure platform. The first is that tertiary amines and heteroatom-substituted aryl groups are found often in different classes of drugs and the 1,3-oxazinan-2-one chiral core structure has been found in many natural product and drug classes as well. Therefore, it is foreseeable that this design could give rise to good biological activities. The second reason

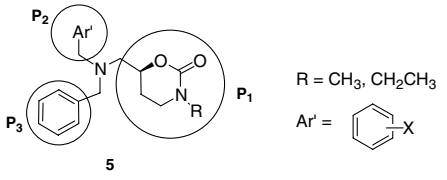


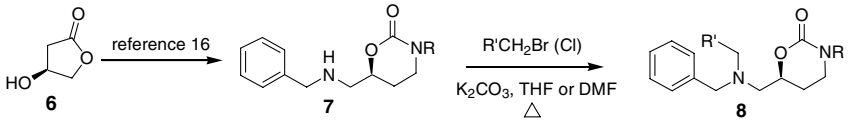
Figure 2. The general structures of the preliminary compound library.

is the easy access to the compounds synthetically. The third consideration is that we can easily modify the structures of the three sites by N-alkylation or N-arylation reactions. This will facilitate the process of finding potent agents.

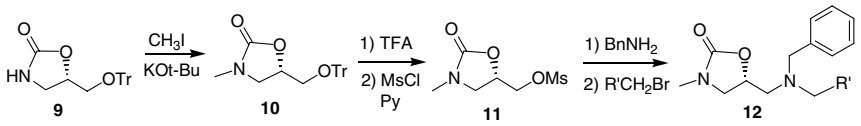
We carried out the synthesis of a small library with the general structure **5** by straightforward reactions and analyzed their antibacterial activities. The synthesis of 1,3-oxazin-2-one ring derivatives is shown in Scheme 1. The intermediate compound **7** was synthesized according to literature procedures using a carbohydrate derivative *S*-3-hydroxyl γ-butyrolactone **6** as the starting material.[16,17] Alkylation with alkyl halides using potassium carbonate in THF or DMF at 70–90 °C gave the desired product **8**. These compounds were synthesized by solution phase synthesis and purified by flash chromatography using silica gel.

For structure comparison purposes, we also synthesized a few compounds containing the oxazolidinone core structure. The synthesis of 5-membered ring oxazolidinone derivatives is shown in Scheme 2. Starting from the optically pure trityl-protected oxazolidinone **9**,[18] the nitrogen was alkylated by treating with a base and alkyl halides to give intermediate **10**. Deprotection of trityl group followed by mesylation afforded **11**. After displacement of the mesylate **11** with an amine and subsequent alkylation, we obtained compounds with the general structure **12**.

The library compounds synthesized containing general structures **A**–**C** are shown in Figure 3. The detailed structures of the R groups are listed in Table 1. The structures **A** and **B** are close analogs with the substituents on ring nitrogen differing from ethyl to methyl group. Structure **C** contains an oxazolidinone ring in-



Scheme 1. The synthetic route to the target compound library.



Scheme 2. Synthesis of oxazolidinone derivatives.

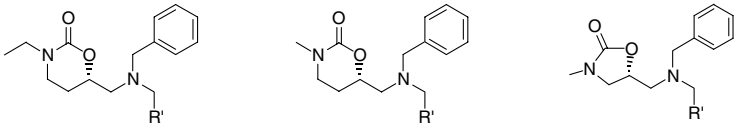


Figure 3. The structures of the small compound library synthesized.

Table 1. The minimum inhibition concentration (MIC, μg/mL) of the compound library against four bacterial strains

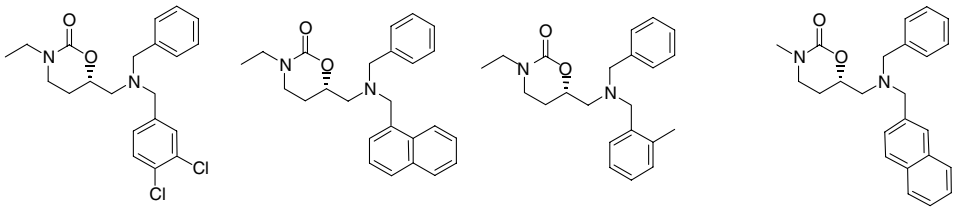
Compound	Structure of R′	*S. aureus* 29213	*S. aureus* 43300	*E. faecalis* 29212	*B. subtilis* PY79
A1	4-Nitrophenyl	N	N	45.0	N
A2	3-Nitrophenyl	90.0	22.0	47.0	108
A3	2-Nitrophenyl	72.0	100	43.0	100
A4	4-Cyanophenyl	180	59.5	12.0	10.0
A5	3-Cyanophenyl	238	N	45.0	238
A6	2-Cyanophenyl	130	180	33.0	160
A7	4-Fluorophenyl	64.0	47.0	35.0	42.5
A8	2,4-Difluorophenyl	45.0	50.5	59.5	38.0
A9	2,6-Difluorophenyl	47.0	70.0	59.5	77.0
A10	3,5-Difluorophenyl	40.0	45.0	37.0	43.0
A11	3,4-Dichlorophenyl	5.60	6.56	N	5.00
A12	2-Naphthalenyl	15.0	N	13.0	29.0
A13	1-Naphthalenyl	10.0	35.0	7.40	7.60
A14	3-Pyridinyl	N	N	238	N
A15	2-Pyridinyl	N	N	N	N
A16	3-Methoxyphenyl	90.0	90.0	80.5	95.0
A17	2-Methylphenyl	22.0	22.5	8.10	26.0
B1	4-Nitrophenyl	81.0	N	119	98.0
B2	3-Nitrophenyl	138	N	119	238
B3	2-Nitrophenyl	179	N	238	238
B4	4-Cyanophenyl	200	N	238	238
B5	3-Cyanophenyl	180	238	7.40	180
B6	2-Cyanophenyl	166	180	59.5	180
B7	4-Fluorophenyl	87.1	119	120	95.0
B8	2,4-Difluorophenyl	50.0	72.0	59.5	55.0
B9	2-Naphthalenyl	11.5	13.0	14.9	12.0
C1	4-Fluorophenyl	N	N	N	N
C2	2,4-Difluorophenyl	85.0	119	N	90.0
C3	3,4-Difluorophenyl	119	N	N	80.0
C4	4-Bromophenyl	20.0	59.5	N	22.0
C5	2-Bromophenyl	18.0	21.0	7.40	22.0

The MICs are reported as the concentrations at 50% inhibition of bacteria growth, N stands for no inhibition.

stead of a 1,3-oxazinan-2-one ring. The antibacterial activities of these compounds were evaluated by standard methods. These include the assay against several strains of Gram-positive bacteria from the American Type Culture Collection (ATCC), *S. aureus* 29213, *S. aureus* 43300, *E. faecalis* 29212, and *Bacillus subtilis* PY79. The inhibition of bacterial growth was monitored using a standard colorimeter at 600 nm using a serial dilution at concentrations of 238, 119, 59.5, 29.8, 14.9, 7.44, 3.72, 1.86, 0.93, and 0.46 μg/mL. Their minimum inhibition concentration (MIC) results at 50% growth inhibition are shown in Table 1. We also tested chloramphenicol as a control, it inhibits bacterial growth (MIC_{90}) at 7.44, 7.44, 4.0, and 2.0 μg/mL, respectively, to the four bacteria listed in Table 1.

The biological assay data have shown that several compounds have moderate to potent activity against all four strains of bacteria. Some active compounds also exhibited certain strain-specificity. It is common for some bacteria strains to be resistant and others susceptible to a particular antibiotic.[19,20] For instance, the tested strain *S. aureus* 29213 is methicillin susceptible, while *S. aureus* 43300 is methicillin resistant. The *E. faecalis* 29212 is vancomycin susceptible, another strain *E. faecalis* 51299 is vancomycin resistant. Compound **A12** showed promising activity against *S. aureus* 29213 but no activity against *S. aureus* 43300. Meanwhile, **A11** showed excellent activity against both strains of *S. aureus* and *B. subtilis* PY79 but no activity against *E. faecalis* 29212. These indicate that the potent compounds can be developed into narrow spectrum antibiotics. This may have some advantages in controlling the spread of resistance.

In Table 1, the most potent compounds are **A11**, **A13**, **A17**, and **B9**. Their concentrations of inhibition of *S. aureus* 29213 and *B. Subtilis* 79 at 90% are shown in Figure 4. Compounds **A17** and the oxazolidinone derivative **C5** also inhibit over 90% growth of *E. facecalis* 29212 at concentrations of 14.9 and 29.8 μg/mL, respectively. The most potent compound **A11** has MIC_{90} below

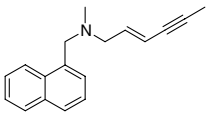


		A11	A13	A17	B9
MIC_{90} μg/mL	S. aureus 29213	9.85	14.9	29.8	14.9
	B. subtilis PY79	7.44	30.0	59.5	30.0

Figure 4. The compound structures and their concentrations of 90% inhibition of bacterial growth.

10 μg/mL, it is in the same range as chloramphenicol. These are reasonable good activities considering the simplicity of the structure and the differences with linezolid or other existing antibacterial agents.

The structure–activity relationship (SAR) of site $\mathbf{P_2}$ can be analyzed when $\mathbf{P_1}$ contains an 1,3-oxazinan-2-one ring. The nature of substituents and their positions on the benzyl group affected the activity significantly. A general trend is that non-polar groups are more favorable than polar groups on the benzyl ring. It seems that *ortho* position substituents on the $\mathbf{P_2}$ site favor the activity as well. *ortho* Methyl and halogen groups are generally good substituents. Fluoro substituents frequently appear in many antibacterial drug structures, however, for our tertiary amine systems, chloro and bromo substituted compounds seem to be more potent than fluoro-containing compounds. The polar nitro substituent is not very active. Naphthalenyl groups showed better activity than benzene ring only. When replacing one of the phenyl rings with a pyridinyl ring (**A14** and **A15**), the activity is completely lost. This also indicates that it is favorable to have a non-polar group at the site $\mathbf{P_2}$. For site $\mathbf{P_1}$, substituents on the 3-nitrogen of the 1,3-oxazinan-2-one ring also influence the activity. The similar structures with methyl instead of ethyl groups showed somewhat diminished activity. This trend can be proved by testing the propyl, butyl, and pentyl substituents in the future. For the oxazolidinone series, molecules with the same $\mathbf{P_2}$ and $\mathbf{P_3}$ groups indicated that the 6-membered ring 1,3-oxazinan-2-one is slightly more potent than the 5-membered ring oxazolidinones. But with bromo substituents on the benzene ring ($\mathbf{P_2}$), oxazolidinone compounds still showed good activity especially when the bromine is at *ortho* position.



Terbinafine

It is worth noting that compounds **A13** and **B9** have some structure similarities with an antifungal drug, the tertiary amine terbinafine. They all contain a naphthalenyl substituted tertiary amine as the general structure. Terbinafine inhibits the synthesis of ergosterol, an essential component for the fungi cell wall. It has a different mode of action compared to 'azole' antifungal agents. The structure resemblance to terbinafine may indicate that our tertiary amine systems also can have antifungal activities in addition to the observed antibacterial activities. These are currently under evaluation.

From the above discussions, we can draw several preliminary conclusions of SAR. For site $\mathbf{P_2}$ aromatic systems, naphthalene is more active than benzene; bromo, methyl are good candidates for the substituent on benzene ring. The 6-membered ring 1,3-oxazinan-2-one seems to have a slightly better activity than the oxazolidinone for the compounds tested so far. It is necessary to have one or two substituted aryl systems for antibacterial activities. The minimum inhibition concentrations of several compounds are around 10 μg/mL, which should be a good starting point for us to optimize the structure and obtain more potent antibacterial agents with this novel scaffold.

In conclusion, we have designed, synthesized, and evaluated the antibacterial activities of a small, focused compound library of tertiary amines containing novel chiral 1,3-oxazinan-2-one core structures and aryl substituents. Several compounds with the general scaffold have shown promising antibacterial activities. They exhibit promising activity against several types of Gram-positive bacteria including *S. aureus*, *E. faecalis*, and *B. subtilis*. The structure–activity relationship of one aryl substitution site $\mathbf{P_2}$ was evaluated thoroughly. We found that bromo, chloro, and methyl groups generally give rise to good antibacterial activity. The structures of these compounds and the structure–activity relationship observed from this library can be used to further optimize the structures to obtain potent novel antimicrobial drugs.

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Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2006.01.072.

References and notes

1. Pootoolal, J.; Neu, J.; Wright, G. D. *Annu. Rev. Pharmacol. Toxicol.* **2002**, *42*, 381.
2. Raad, I. I.; Hanna, H. A.; Hachem, R. Y.; Dvorak, T.; Arbuckle, R. B.; Chaiban, G.; Rice, R. B. *Antimicrob. Agents Chemother.* **2004**, *48*, 3583.
3. Brickner, S. J. *Curr. Pharm. Des.* **1996**, *2*, 175.
4. Diekema, D. J.; Jones, R. N. *Lancet* **2001**, *358*, 1975.
5. Hollingsworth, R. I.; Wang, G. *Kirk-Othmer Encyclopedia of Chemical Technology*; John Wiley & Sons, 2003, Available from: <http://www.mrw.interscience.wiley.com/kirk/>.
6. Bozdogan, M.; Appelbaum, P. C. *Int. J. Antimicrob. Agents* **2004**, *23*, 113.
7. Barbachyn, M. R.; Ford, C. W. *Angew. Chem., Int. Ed.* **2003**, *42*, 2010.
8. Hutchinson, D. K. *Curr. Top. Med. Chem.* **2003**, *3*, 1021.
9. Higgins, P. G.; Fluit, A. C.; Schmitz, F. J. *Curr. Drug Targets* **2003**, *4*, 181.
10. Zhanel, G. G.; Ennis, K.; Vercaigne, L.; Walkty, A.; Gin, A. S.; Embil, J.; Smith, H.; Hoban, D. J. *Drugs* **2002**, *62*, 13.
11. Drlica, K.; Malik, M. *Curr. Top. Med. Chem.* **2003**, *3*, 249.
12. Chen, Y.-L.; Fang, K.-C.; Sheu, J.-Y.; Hsu, S.-L.; Tzeng, C.-C. *J. Med. Chem.* **2001**, *44*, 2374.
13. Hu, X. E.; Kim, N. K.; Gray, J. L.; Almstead, J.-I. K.; Seibel, W. L.; Ledoussal, B. *J. Med. Chem.* **2003**, *46*, 3655.
14. Masters, P. A.; O'Bryan, T. A.; Zurlo, J.; Miller, D. Q.; Joshi, N. *Arch. Intern. Med.* **2003**, *163*, 402.
15. Mitscher, L. A.; Pillai, S. P.; Gentry, E. J.; Shankel, D. M. *Med. Res. Rev.* **1999**, *19*, 477.
16. Ella-Menye, J. R.; Sharma, V.; Wang, G. *J. Org. Chem.* **2005**, *70*, 463.
17. Wang, G.; Hollingsworth, R. I. *J. Org. Chem.* **1999**, *64*, 1036.
18. Wang, G.; Hollingsworth, R. I. *Tetrahedron: Asymmetry* **2000**, *11*, 4429.
19. Giacometti, A.; Cirioni, O.; Kamysz, W.; D'Amato, G.; Silvestri, C.; Del Prete, M. S.; Licci, A.; Riva, A.; Lukasiak, J.; Scalise, G. *Antimicrob. Agents Chemother.* **2005**, *49*, 1249.
20. Swenson, J. A.; Spargo, J.; Tenover, F. C.; Ferraro, M. J. *J. Clin. Microbiol.* **2001**, *39*, 3781.