Short Communication

An Update on the Structure of Oxazolidinone Analogs and a Comparison with Linezolid in Terms of In Vitro and Intracellular Efficacy against Clinically Relevant Bacterial Species

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SUMMARY: Oxazolidinones constitute a novel class of antimicrobials as inhibitors of bacterial ribosomal protein synthesis. In this study, we identified 15 novel oxazolidinone analogs with potent antituberculosis activities. Compounds sy124 and sy125 showed the best activity in vitro (better than that of the linezolid) against various clinically relevant bacterial species, including most Gram-positive bacteria, Mycobacterium spp., and Helicobacter pylori. A cell-based assay indicated that these compounds have a strong ability to kill intracellular pathogens. Our results reveal that the newly identified compounds may be further developed as novel antimicrobial agents.

Oxazolidinones constitute a novel class of antimicrobials as inhibitors of bacterial ribosomal protein synthesis (1). Linezolid was the first oxazolidinone antibacterial agent that was approved for clinical use, and it exerts in vitro inhibitory activities against numerous clinically relevant bacterial species, including staphylococci (methicillin-susceptible [MSSA] and -resistant [MRSA]), enterococci (vancomycin-susceptible [VSE] and -resistant [VRE]), streptococci, Corynebacterium spp. and Mycobacterium tuberculosis (2). Recent studies also revealed that linezolid has obvious efficacy against clinical Helicobacter pylori (3). The clinical and commercial success of linezolid has prompted many pharmaceutical companies to investigate and develop oxazolidinone-like compounds. Several compounds have been developed as candidate therapeutics, but only tedizolid and radezolid have advanced to clinical trials (1). We have identified a series of novel 4-substituted piperazinyl phenyl oxazolidinone analogs with effects comparable to or better than those of the control linezolid against M. tuberculosis, nontuberculous mycobacteria, Staphylococcus aureus, and H. pylori (4–7). On the basis of these findings, we continued our work on the synthesis and biological evaluation of a large number of novel oxazolidinones and found that these compounds generally exert activities with a broad antimicrobial spectrum. In this work, we report oxazolidinones most recently identified by our team and their effects against various clinically important pathogens.

Oxazolidinone analogs in this study were synthesized in the Pharmaceutical Engineering Department, Shenyang Pharmaceutical University. Clinical strains of Gram-positive bacteria included S. aureus (45 isolates of MRSA and 33 isolates of MSSA), Staphylococcus epidermidis (12 isolates), Enterococcus faecalis (22 isolates, including 6 VRE), Enterococcus faecium (24 isolates, including 6 VRE), Streptococcus pneumoniae (12 isolates), Group A streptococci (15 isolates), and Group B streptococci (17 isolates). Clinical strains of Mycobacterium spp. included M. tuberculosis (84 isolates), M. abscessus (21 isolates), M. avium (31 isolates), M. chelonae (11 isolates), M. fortuitum (24 isolates), M. kansasii (26 isolates), and M. intracellulare (17 isolates). In total, 88 isolates of clinical H. pylori were included in this study. All the isolates were collected in the PLA 309th Hospital and were characterized for their susceptibility to the routine antibiotics and linezolid.

In vitro minimum inhibitory concentrations (MICs) of compounds against certain strains of pathogenic bacteria were determined by two-fold dilution in a specific medium in accordance with our previous report (4–7).

An assay of intracellular activity of the compounds against Gram-positive bacteria and mycobacterium were performed using THP-1 macrophages as described previously (4,8). For H. pylori, the intracellular model was set up using AGS cells according to another report (9). The minimum concentrations of the compounds that could cause a 2log10 CFU decrease (99%) after 24 h of incubation were defined as intracellular MIC90 and were determined to assess the efficacy of these compounds against intracellular pathogens.

Using high-throughput screening for in vitro antimicrobial activities against standard S. aureus (29213), we identified 15 novel and potent oxazolidinones (MIC ≤ 10 μg/mL, Table 1). Compounds sy124 and sy125 showed the best activities, with MICs at 0.5 μg/mL (linezolid MIC = 1 μg/mL) and were next analyzed for the in vitro efficacy against a number of clinically relevant bacterial species. As shown in Table 2, sy124 and sy125 manifested better antimicrobial activity than linezolid did, with the MIC values no more than 1 μg/mL.
against all the tested Gram-positive isolates, even in the cases of linezolid MIC being higher (linezolid MIC = 8 μg/mL). Against mycobacteria strains, sy124 and sy125 showed effects comparable with those of linezolid, i.e., against the *M. kansasii*, *M. fortuitum*, *M. abscessus*, and *M. chelonae* strains and better efficacy than linezolid against *M. tuberculosis*, *M. avium*, and *M. intracellulare*. Sy124 and sy125 also exerted antimicrobial action comparable to that of linezolid against *H. pylori*.

According to the intracellular assay, we found that sy124 and sy125 showed significant inhibitory activities in the MIC<sub>90</sub> range of 1–8 μg/mL against all the Gram-positive bacteria. Meanwhile, these 2 compounds were also found to have potent intracellular efficacy against *M.*

### Table 1. Structure of new identified oxazolidinone analogs and the MIC against standard *S. aureus*<sup>29213</sup>

| Compound | Structure | Purity (%) | MIC for standard *S. aureus* (29213) |
|----------|-----------|------------|-------------------------------------|
| sy-6     | ![Structure](image) | 99.3       | 10                                  |
| sy-10    | ![Structure](image) | 98.2       | 1                                   |
| sy-14    | ![Structure](image) | 98.7       | 5                                   |
| sy-25    | ![Structure](image) | 98.8       | 1                                   |
| sy-48    | ![Structure](image) | 98.5       | 10                                  |
| sy-58    | ![Structure](image) | 98.4       | 5                                   |
| sy-59    | ![Structure](image) | 98.1       | 10                                  |
| sy-60    | ![Structure](image) | 98.7       | 10                                  |
| sy-61    | ![Structure](image) | 99.2       | 10                                  |
| sy-84    | ![Structure](image) | 99.1       | 5                                   |
| sy-123   | ![Structure](image) | 98.9       | 5                                   |
| sy-124   | ![Structure](image) | 99.4       | 0.5                                 |
| sy-125   | ![Structure](image) | 99.3       | 0.5                                 |
| sy-126   | ![Structure](image) | 98.5       | 5                                   |
| sy-129   | ![Structure](image) | 98.3       | 1                                   |

MIC, in vitro minimum inhibitory concentration.
**Table 2. In vitro and intracellular efficacy of new oxazolidinone analogues against Gram-positive bacteria, mycobacterium, and H. pylori**

| Strain                  | Compound | In vitro MIC for clinical isolates (μg/mL) | Intracellular MIC99 for clinical isolates (μg/mL) |
|-------------------------|----------|------------------------------------------|--------------------------------------------------|
|                         |          | MIC range | MIC50 | MIC90 | MIC99 range | MIC99-50 | MIC99-90 |
| **Gram-positive**       |          |            |       |       |             |          |          |
| MRSA                    | Sy-124   | 0.5–1      | 1     | 1     | 1–8         | 2        | 4        |
|                         | Sy-125   | 0.5–1      | 1     | 1     | 1–8         | 2        | 4        |
|                         | linezolid | 0.5–8     | 2     | 4     | 1–> 16      | 2        | 4        |
| **MSSA**                |          |            |       |       |             |          |          |
| Sy-124                  | 0.5–1    | 1         | 1     | 1–8   | 2        | 4        |
| Sy-125                  | 0.5–1    | 1         | 1     | 1–8   | 2        | 4        |
| linezolid               | 0.5–2    | 1       | 2     | 1–16  | 2        | 8        |
| **S. epidermidis**      |          |            |       |       |             |          |          |
| Sy-124                  | 0.5–1    | 0.5      | 0.5   | 1–8   | 2        | 4        |
| Sy-125                  | 0.5–1    | 0.5      | 1     | 1–8   | 2        | 4        |
| linezolid               | 0.5–4    | 0.5      | 1     | 1–8   | 4        | 4        |
| **E. faecalis**         |          |            |       |       |             |          |          |
| Sy-124                  | 0.5–1    | 0.5      | 0.5   | 1–8   | 2        | 4        |
| Sy-125                  | 0.5–1    | 0.5      | 1     | 1–8   | 2        | 4        |
| linezolid               | 0.5–4    | 0.5      | 4     | 1–16  | 4        | 4        |
| **E. faecium**          |          |            |       |       |             |          |          |
| Sy-124                  | 0.5–1    | 0.5      | 0.5   | 1–8   | 2        | 4        |
| Sy-125                  | 0.5–1    | 0.5      | 1     | 1–8   | 2        | 4        |
| linezolid               | 0.5–4    | 1       | 1     | 1–16  | 4        | 4        |
| **S. pneumoniae**       |          |            |       |       |             |          |          |
| Sy-124                  | 0.5–1    | 0.5      | 1     | 1–8   | 2        | 4        |
| Sy-125                  | 0.5–1    | 0.5      | 1     | 1–8   | 2        | 4        |
| linezolid               | 0.5–2    | 1       | 1     | 1–8   | 4        | 4        |
| **Group A streptococci**|          |            |       |       |             |          |          |
| Sy-124                  | 0.5–1    | 1       | 1     | 1–8   | 2        | 4        |
| Sy-125                  | 0.5–1    | 1       | 1     | 1–8   | 2        | 4        |
| linezolid               | 0.5–2    | 1       | 1     | 1–8   | 4        | 4        |
| **Group B streptococci**|          |            |       |       |             |          |          |
| Sy-124                  | 0.5–1    | 1       | 1     | 1–8   | 2        | 4        |
| Sy-125                  | 0.5–1    | 1       | 1     | 1–8   | 2        | 4        |
| linezolid               | 0.5–2    | 1       | 1     | 1–8   | 4        | 4        |
| **Mycobacterium**       |          |            |       |       |             |          |          |
| *M. tuberculosis*       |          |            |       |       |             |          |          |
| Sy-124                  | 0.5–1    | 1       | 1     | 1–4   | 2        | 4        |
| Sy-125                  | 0.5–1    | 1       | 1     | 1–4   | 2        | 4        |
| linezolid               | 1–2      | 2       | 2     | 1–8   | 4        | 4        |
| **M. abscessus**        |          | 16–64     | 32    | 64    | >16        | >16       | >16       |
| Sy-124                  | 16–64    | 32       | 64    | >16   | >16        | >16       | >16       |
| linezolid               | 16–64    | 64       | 64    | >16   | >16        | >16       | >16       |
| **M. avium**            |          | 4–64     | 8     | 16    | >16        | >16       | >16       |
| Sy-124                  | 4–64     | 16       | 32    | >16   | >16        | >16       | >16       |
| linezolid               | 8–64     | 32       | 32    | >16   | >16        | >16       | >16       |
| **M. chelonae**         |          | 2–16     | 4     | 8     | 8–> 16     | >16       | >16       |
| Sy-124                  | 2–16     | 4       | 8     | 8–> 16| >16       | >16       | >16       |
| linezolid               | 2–16     | 4       | 8     | 8–> 16| >16       | >16       | >16       |
| **M. fortuitum**        |          | 0.5–8    | 4     | 8     | 2–> 16     | >16       | >16       |
| Sy-124                  | 0.5–8    | 8       | 16    | 2–> 16| >16       | >16       | >16       |
| Sy-125                  | 0.5–8    | 8       | 16    | 2–> 16| >16       | >16       | >16       |
| linezolid               | 0.5–8    | 4       | 8     | 2–> 16| >16       | >16       | >16       |
| **M. kanssaii**         |          | 0.5–1    | 1     | 1     | 1–8        | 2        | 4        |
| Sy-124                  | 0.5–1    | 1       | 1     | 1–8   | 2        | 4        |
| Sy-125                  | 0.5–1    | 1       | 1     | 1–8   | 2        | 4        |
| linezolid               | 0.5–1    | 1       | 1     | 1–8   | 4        | 4        |
| **M. intracellularare** |          | 4–32     | 8     | 16    | >16        | >16       | >16       |
| Sy-124                  | 4–32     | 16      | 32    | >16   | >16        | >16       | >16       |
| linezolid               | 8–64     | 32      | 32    | >16   | >16        | >16       | >16       |
| **Hericobacter**        |          | 0.25–32  | 8     | 32    | 4–> 16     | >16       | >16       |
| *H. pylori*             |          | 0.25–32  | 8     | 32    | 4–> 16     | >16       | >16       |
| linezolid               | 0.25–32  | 8       | 32    | 4–> 16| >16       | >16       | >16       |

MIC, in vitro minimum inhibitory concentration; MIC50, MIC90, in vitro MICs for 50% and 90% of the isolates, respectively; MIC99, intracellular minimum concentrations of the compounds which could cause a 2log10 CFU decrease (99%); MIC99-50, MIC99-90, intracellular MIC99 for 50% and 90% of the isolates, respectively.

tuberculosis and *M. kanssaii*. Nonetheless, only modest efficacy against other Mycobacterium spp. and *H. pylori* was observed at the concentration of 16 μg/mL. These intracellular results were in agreement with the in vitro MIC assay.

Comparing their structures, we found that a substitution on the phenyl ring with 2-thiazolyl or 4-thiazolyl rings yielded a better antibacterial activity. By comparing the structure of linezolid with that of other oxazolidinone compounds (5), we noticed that substitution with 2-thiazolyl or 4-thiazolyl rings on the phenyl ring resulted in a good antibacterial action (Table 1). Among
the resulting compounds, 4-thiazolyl derivatives were more beneficial than the 2-thiazolyl ones. The replacement of the thiazolyl ring with an alkoxy group such as methoxy (sy-125) or ethoxy (sy-124) yielded an activity superior to that or linezolid, but methyl (sy-25) and 4-pyridinyl group (sy-129) led to inferior or comparable activity relative to linezolid. For 2-thiazolyl derivatives, the chloro group (sy-10) was more beneficial than the morpholino group (sy-14). These phenomena may be explained as follows: alkoxy groups have stronger binding affinity for a receptor than other groups do. The newly identified structure-function relation may facilitate further modification of these compounds to design the second generation of oxazolidinone antibiotics.

Acknowledgments  This work was financially supported by the Chinese National Natural Science Foundation Project (No.81401635) and the Foundation of the PLA 309th Hospital (2015MS-002).

Conflict of interest  None to declare.

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