(0.0504 ppm) was higher than in Oxoid MHA (0.0196 ppm) or BD MHB II (0.0055 ppm), but the tigecycline MIC determined using the agar dilution method (Hopebio) was the lowest among the three media. The results were contrary to those of two previous studies, both of which found that the MICs of tigecycline were increased in media with a high manganese content. However, high manganese concentrations were found in MHA from Oxoid in one study and low manganese concentrations were found in MHA from Oxoid in another study. Therefore, it remains unclear whether manganese really interferes with the in vitro activity of tigecycline or not.

To investigate the impact of the ion content of media on the MICs of tigecycline for Acinetobacter spp., MnCl₂, ZnCl₂ or MgCl₂ (analytical reagent) was used to supplement MHB II to achieve Mn, Zn and Mg concentrations of 1024 mg/L. Tigecycline MICs were determined and were found to be unchanged. Hence, the discrepancy in MICs was unlikely to be due to the Mn, Zn or Mg content. Additionally, the amount of beef extract powder is different in the three media; it is still unclear whether this discrepancy is related to the different tigecycline susceptibilities. Further studies are warranted.

In conclusion, we found that MIC values varied significantly when different methods or MH media were used. The results of tigecycline susceptibility for Acinetobacter spp. should be interpreted with caution. The content of Mn, Zn and Mg in different media may be irrelevant to the discrepancy in tigecycline MICs.

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References

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In vitro and intracellular activity of 4-substituted piperazinyl phenyl oxazolidinone analogues against Mycobacterium tuberculosis

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Keywords: oxazolidinones, M. tuberculosis, antimycobacterial activity, multidrug resistant, extensively drug resistant

Sir,
The recent appreciation that the widespread existence of multidrug-resistant (MDR) tuberculosis (TB) and extensively drug-resistant (XDR) TB represent an international public health threat means that new anti-TB drugs are urgently needed to treat MDR and XDR TB cases. Oxazolidinones are a novel class of

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antimicrobial inhibitors of bacterial ribosomal protein synthesis. The first oxazolidinone antibacterial agent that was approved for clinical use, linezolid, has excellent activity against TB, regardless of drug resistance patterns. However, reports also demonstrated that the duration of linezolid treatment may have to be limited because of potentially serious neurological, ophthalmological and haematological toxicities. So there is a need to develop second-generation oxazolidinones. PNU-100480 (sutezolid, Phase 2), an oxazolidinone analogue with a thiomorpholine moiety, exhibited MIC₉₀ values of ≤0.50 mg/L for a panel of organisms consisting of five drug-susceptible and five MDR strains of TB. DA-7867 is a new hetero-ring-substituted pyridine-containing oxazolidinone, which showed more potent activity than linezolid against methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, penicillin-resistant *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis*. In addition, other currently developed oxazolidinones, such as AZD5847 (posizolid, Phase 1), DA-7157 and RBx 8700, have also been reported to be more potent and/or have lower toxicity than linezolid.

The ability of the bacteria to invade and survive inside cells may be involved in the persistence of TB. Although hundreds of compounds have activity in vitro against TB strains, the presence of intracellular bacteria makes the prediction of optimal chemotherapy complicated and uncertain, because the intracellular activities of compounds depend on a series of pharmacokinetic and pharmacodynamic factors such as penetration, accumulation and bioavailability of the drugs inside cells, as well as the responsiveness of the bacteria to their action. Therefore, it is of far greater significance that these compounds are capable of killing intracellular TB in human macrophages.

In our previous studies, we synthesized various oxazolidinone analogues and set up a reserved compounds library. The present work was conducted for further identification of an anti-TB candidate through in vitro and intracellular screening of the library.

Standard strain H37Rv (ATCC 27294) was purchased from the Beijing Institute for Tuberculosis Control. Eighty-four TB clinical isolates, which were identified from the 309th Hospital of Chinese People’s Liberation Army, and characterized by our team for their susceptibility to the routine antibiotics and linezolid, were used here. Linezolid was obtained from Promega (part no. PS1074). An oxazolidinone analogue library was set up in the School of Pharmaceutical Engineering, Shenyang Pharmaceutical University. In vitro activities, cytotoxicities and intracellular activities of the compounds were determined as described previously. Using high-throughput screening for in vitro anti-TB activities against H37Rv, we identified a series of 4-substituted piperazinyl phenyl oxazolidinones with antimycobacterial activity (Table 1). Compounds sy142 and sy144 were the most active (MIC 0.5 mg/L). Compounds sy143, sy40, sy41 and sy146 possessed MIC values of 1–8 mg/L. As the quality control, the MIC of linezolid for H37Rv was 0.5 mg/L, which was within the expected range. Considering the relationship between structure and activity, we found that piperazinyl was associated with improved activity, e.g. sy40, sy41, sy142, sy143 and sy144. However, it is worth noting that introducing a bulky group or polar group such as benzyl (sy38), morpholinyl (sy147) or alkylamino (sy148) into the N-heteroaryl group may cause a dramatic decrease in activity, or even a complete loss of activity. This finding could help guide further modification of the compounds.

We previously reported that linezolid had excellent bacteriostatic activity against 84 clinical TB strains isolated in The 309th Hospital of Chinese People’s Liberation Army, including 45 MDR (non-XDR) and 16 XDR cases, but the MIC values varied among different samples. In order to compare the activities of identified compounds with that of linezolid against clinical pathogens, the same strains were used here. The MIC ranges, MIC₉₀₅ and MIC₉₀₆ for strains with different drug resistance spectra were nearly the same (Table 1), although individual variations existed. Analogues sy142 and sy144 had activities similar to that of linezolid, whereas sy143 was slightly less potent. Meanwhile, rifampicin showed no antimicrobial action against the rifampicin-resistant strain at 2 mg/L.

Toxicity was evident 3 days after adding sy143 and sy146 to THP-1 cells, with 50% cytotoxic concentration (CC₅₀) values of 11.6 and 54.2 mg/L (Table 1). These compounds were therefore excluded from the intracellular activity assay. In the THP-1 assay, sy142 and sy144 displayed the most significant activity, with mycobacterial kill rates of >99% at a concentration of 2 mg/L. Compounds sy40 and sy41 also exhibited visible activity, but the activities were lower (Table 1). Comparing the intracellular and in vitro activity of the compounds demonstrated that they all killed 99% of intracellular bacteria at 4× MIC in vitro. As expected, all the compounds showed similar intracellular activities against clinical TB isolates, regardless of their susceptibility to standard antimycobacterial drugs (data not shown).

We simultaneously assessed the in vitro and intracellular combination of sy142 and sy144 with four first-line anti-TB drugs (isoniazid, rifampicin, streptomycin and ethambutol) and ofloxacin, but there was no evidence to support any existence of positive interaction. These results are similar to what was known for linezolid and sutezolid. Even so, we also anticipate that novel compounds may be a viable choice for combination therapy for TB. Next we plan to determine whether they have sufficient sterilizing activity and low toxicity in a murine model of TB infection when added to the standard first-line regimen, and to explore effective treatment strategies against XDR strains.

**Acknowledgements**

We thank Dr Zhen Liu for assistance with the intracellular (THP-1) studies.

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Table 1. In vitro and intracellular antimycobacterial activities of new oxazolidinone analogues against TB

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<th>Compound</th>
<th>Structure</th>
<th>In vitro MIC for H37Rv (mg/L)</th>
<th>In vitro MICs for clinical isolates (mg/L)</th>
<th>CC50 for THP-1 (mg/L)</th>
<th>Intracellular MIC99 (mg/L)</th>
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Table 1. Continued

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ND, not determined.

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Transparency declarations
None to declare.

References