Evaluation of the In Vivo Efficacy of Novel Monosubstituted Sulfonylureas against H37Rv and Extensively Drug-Resistant Tuberculosis

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SUMMARY: Sulfonylureas have been regarded as potential drug candidates against tuberculosis (TB) because they can inhibit the biosynthesis of branched-chain amino acids by targeting acetohydroxyacid synthase (AHAS). We demonstrated previously that novel monosubstituted sulfonylureas showed potent in vitro activities against TB. In the current study, we further explored the anti-TB activity of monosubstituted sulfonylureas in a mouse model. Compounds 30 and 31 exhibited the most efficacy: a single intragastric administration at a dose of 250 mg/kg led to a reduced lung bacterial count, and the dose of 500 mg/kg achieved a >99% reduction in bacterial load for both H37Rv and extensively drug-resistant isolates. These results indicate that these compounds are more potent than commercial sulfonylureas in vivo and may provide insight into the potential implications for the design of novel drugs to combat TB by targeting AHAS.

Recent appreciation of the widespread existence of multidrug-resistant tuberculosis (MDR-TB) and extensively drug-resistant tuberculosis (XDR-TB) has further heightened the awareness of the need for novel anti-TB agents (1,2). Small molecules with efficacy against TB with novel modes of action are considered to be candidates for neutopy anti-TB drug development. We have explored novel effective anti-MDR/XDR-TB compounds for years and have recently focused on targeting acetohydroxyacid synthase (AHAS) (3,4). AHAS has been regarded as a potential drug target against TB because it can catalyze the “first step” in the biosynthesis of branched-chain amino acids (5–8). Sulfonylureas are based on a scaffold of a central sulfonylurea bridge with an o-substituted aromatic ring attached to the sulfur atom and a heterocyclic ring attached to the nitrogen atom. Some sulfonylureas, such as sulfometuron methyl (SM), chlorimuron methyl, and metsulfuron methyl, have been proved to be capable of dergerming TB by targeting AHAS (5,6). Our previous studies found that some sulfonylurea compounds with a single substitution at the meta position of the heterocyclic ring exhibited significant and peculiar AHAS inhibitory activity, and we also found that monosulfuron and monosulfuron-ester (3), both of which contain a 4-monosubstituted pyrimidine, had observable activity against TB (Table 1). Based on these discoveries, we continued our work on the synthesis and biological evaluation of a large number of monosubstituted sulfonylureas, and we found that these structures generally exhibited activity against TB (4). The bactericidal effectiveness and low cytotoxicity of these compounds in THP-1 cells (9) suggest that they may be effective in mammals. In the current study, we evaluate the in vivo efficacy of our novel compounds using a mouse model.

The synthesis and in vitro and ex vivo activity of novel monosubstituted sulfonylurea analogs were described in our previous report (4). Among these analogs, the compounds with visible intracellular activities (>40% inhibition at a dose of 50 mg/L) were selected for use in the current study, and their structures are shown in Table 1. The standard Mycobacterium tuberculosis (MTB) strain H37Rv (ATCC 27294), which is susceptible to all anti-MTB drugs, was purchased from Beijing Institute for Tuberculosis Control. SM was obtained from Chem Service (Catalog no. PSI074). Minimum inhibitory concentration (MIC) of conventional drugs against H37Rv was as follows (mg/L): isoniazid (INH), 0.2; rifampicin (RFP), 0.4; ofloxacin (OLF), 1.0; amikacin (AMK), 1.0; kanamycin (KN), 4.0. One XDR isolate identified from the Chinese PLA 309 Hospital that was resistant to INH (MIC > 20 mg/L), RFP (MIC > 20 mg/L), OLF (MIC > 20 mg/L), and AMK (MIC > 20 mg/L) was also used (10). All the isolates were cultured in Lowenstein-Jensen medium before use.

Infections and mouse treatment models were performed using a previously reported method (5,11). Briefly, 4–6-week-old female BALB/c mice were infected with the tail vein injection of 0.2 mL of a suspension containing 5 × 10⁶ viable TB cells. All compounds were prepared in phosphate-buffered saline (PBS), and the intragastric administration of the compounds was started 1 week after infection (0.2 mL/day). The negative control group of infected mice was injected with PBS, whereas the positive control group received 50 mg/kg RFP twice every week (Monday and Thursday). Each treatment group was composed of 8 mice. The left lung lobes and spleen were removed aseptically on day 28.
Table 1. Structure and ex vivo activities of novel sulfonylurea compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>MIC for H37Rv (mg/L)</th>
<th>Intracellular inhibitions (%) at 50 mg/L</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfometuron methyl</td>
<td><img src="image" alt="Structure" /></td>
<td>10</td>
<td>70.1 ± 6.6</td>
<td>3, 4, 9</td>
</tr>
<tr>
<td>Monosulfuron-ester</td>
<td><img src="image" alt="Structure" /></td>
<td>16</td>
<td>N/D</td>
<td>3</td>
</tr>
<tr>
<td>Monosulfuron</td>
<td><img src="image" alt="Structure" /></td>
<td>32</td>
<td>N/D</td>
<td>3</td>
</tr>
<tr>
<td>Compound 3</td>
<td><img src="image" alt="Structure" /></td>
<td>10</td>
<td>43.9 ± 13.3</td>
<td>9</td>
</tr>
<tr>
<td>Compound 15</td>
<td><img src="image" alt="Structure" /></td>
<td>10</td>
<td>52.3 ± 10.3</td>
<td>9</td>
</tr>
<tr>
<td>Compound 20</td>
<td><img src="image" alt="Structure" /></td>
<td>20</td>
<td>40.4 ± 10.4</td>
<td>9</td>
</tr>
<tr>
<td>Compound 30</td>
<td><img src="image" alt="Structure" /></td>
<td>10</td>
<td>66.6 ± 9.5</td>
<td>9</td>
</tr>
<tr>
<td>Compound 31</td>
<td><img src="image" alt="Structure" /></td>
<td>10</td>
<td>64.4 ± 8.8</td>
<td>9</td>
</tr>
<tr>
<td>Compound 32</td>
<td><img src="image" alt="Structure" /></td>
<td>20</td>
<td>44.3 ± 14.2</td>
<td>9</td>
</tr>
</tbody>
</table>

post-challenge, homogenized, and plated on Middlebrook 7H10 agar medium (supplemented with oleic acid, albumin, dextrose, and catalase) with 10-fold serial dilutions. Media were incubated at 36.5°C, and colony-forming unit (CFU) counting was performed 3 weeks later. The results are expressed as means ± SDs of at least 3 independent experiments performed in triplicate. The differences among groups were determined using 2-tailed Student’s t-tests with SPSS software (Armonk, NY, USA). P values of <0.05 were considered to be significant. All experiments were performed according to principles of the Helsinki Declaration. The Ethics Committee of the PLA 309 Hospital approved the study.

In preliminary tolerance tests, no signs of overt toxicity were observed for any of the tested compounds at doses of 1 g/kg for 14 consecutive days. Next, the in vivo activity of the compounds was evaluated initially using doses of 500 mg/kg (Table 2). Compounds 30 and 31 and SM showed the most significant inhibition, and mice that received these treatments had lung bacterial counts that were >2 log (CFU) lower than that in the control group. Compounds 3 and 15 also showed visible efficacy, but their activities were weaker. Treatment with other compounds did not cause statistical differences in lung bacterial counts. The positive control administered RFP showed bactericidal efficacy against H37Rv but not XDR. Next, the dose-dependent effects of compounds 30 and 31 and SM against H37Rv were analyzed. As shown in Fig. 1, 250 mg/kg doses of compounds 30 and 31 caused a significant decrease in the number of viable bacteria, whereas treatment with SM led to only a slight change; therefore, compounds 30 and 31 exhibited more efficacy at this concentration.
The treatment of serious TB infections remains a major challenge, and novel drugs are needed urgently, particularly for MDR/XDR cases. Recent studies of the molecular mechanism of action of AHAS in TB have suggested that AHAS may be a potential target for antimicrobial drug development. Therefore, several research groups have attempted to discover novel AHAS inhibitors as anti-MTB agents. Choi et al. reported some inhibitors that they identified using high-throughput screening of a chemical library of 5,600 compounds and confirmed their anti-MTB activity in vitro (8). Sohn et al. tested a number of sulfonylurea derivatives and found that some showed efficacy against clinical TB strains (6). However, reports on the therapeutic effect of such compounds against TB in vivo are rare. Mouse models have been used extensively for the preclinical assessment of experimental compounds against TB, and the results obtained have always been predictive of what was achievable in humans. To the best of our knowledge, only SM has been confirmed to be capable of preventing the significant growth of TB in the lungs of mice, but a high concentration is required (500 mg/kg) (5). According to our previous study, monosubstituted sulfonylurea derivatives exhibit strong antimycobacterial potential in vitro and in macrophages. In the current study, the lung bacterial counts were reduced markedly by treatment with compounds 30 and 31 at doses of >250 mg/kg for 1 month. More than a 99% reduction in bacterial load was achieved with 500 mg/kg, which was more efficient than SM.

The recommended treatment for TB is the use of at least 4 drugs known to be effective; however, the elevated incidence of drug resistance has made treatment increasingly challenging, leading to a higher cost and a longer treatment time. XDR, the highly drug-resistant TB strain defined in 2006 (12), is susceptible to fewer than 3 remaining classes of second-line drugs, making it difficult to treat according to international standards. Patients with XDR-TB have a higher risk of death and treatment failure than those with MDR-TB. In the current study, the efficacy of sulfonylureas against H37Rv and XDR cases were similar. Conversely, RFP had much lower activity against XDR, suggesting that this compound has the potential to substitute RFP for combination therapy for XDR-TB. To confirm this hypothesis, the therapeutic effects of this drug combination, the potential shortened treatment duration, and the effectiveness against more clinical isolates are currently being assessed.

### Conflict of interest
None to declare.

### REFERENCES