Activity of the efflux pump inhibitor SILA 421 against drug-resistant tuberculosis

Sami O. Simons#1, Jette E. Kristiansen2, Gyorgy Hajos3, Tridia van der Laan4, József Molnár5, Martin J. Boeree1, Jakko van Ingen6, Jørn B. Christensen7, Miguel Viveiros8, Zsuzsanna Riedl3, Leonard Amaral8, and Dick van Soolingen6

1 Department of Pulmonary Medicine, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
2 Centre for Biomembrane Physics (Memphys), University of Southern Denmark, Odense, Denmark
3 Institute of Organic Chemistry, Research Centre for Natural Sciences, Hungarian Academy of Sciences, Budapest, Hungary
4 National Tuberculosis Reference Laboratory, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
5 Department of Medical Microbiology and Immunobiology, University of Szeged, Szeged, Hungary
6 Department of Clinical Microbiology, Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands
7 Department of Chemistry, University of Copenhagen, Copenhagen, Denmark
Unit of Mycobacteriology, Institute of Hygiene and Tropical Medicine, New University of Lisbon, Lisbon, Portugal

(⁎)Corresponding author:
Sami O. Simons, MD
Department of Pulmonary Medicine
Radboud University Nijmegen Medical Centre
PO Box 9101, 6500 HB Nijmegen
the Netherlands
Tel: +31 (0)243610325
Fax: +31 (0)243610324
E-mail: s.simons@long.umcn.nl
Dear editor,

Organosilicon compounds are efflux pump inhibitors with potency as an antituberculosis drug. Of the organosilicon compounds tested, SILA 421 has been shown to have a highest potency as an antituberculosis drug (1). It shares the common pathways for antimycobacterial killing with other efflux pump inhibitors: it revealed direct in vitro activity against \textit{M. tuberculosis} (1), it has been shown to modify resistance by inhibiting mdr-1 efflux pumps and has shown to enhance killing of \textit{M. tuberculosis} by macrophages (1). However, the activity of SILA 421 has only been tested against the \textit{M. tuberculosis} H37Rv type strain and one clinical XDR \textit{M. tuberculosis} isolate so far (1). We therefore assessed the antimycobacterial activity of SILA 421 against an extended set of \textit{M. tuberculosis} strains with varying drug susceptibility profiles. Secondly, we compared antimycobacterial activity of SILA 421 with that of thioridazine and other phenothiazines.

We selected 21 clinical \textit{M. tuberculosis} isolates with varying drug susceptibility profiles: five isolates were pansusceptible, 11 were monoresistant (6 isoniazid monoresistant, 4 rifampin monoresistant and 1 streptomycin monoresistant) and 5 were MDR \textit{M. tuberculosis} isolates. All isolates were extracted from a -70 °C freezer collection and sub-cultured on Ogawa medium. Inoculum preparation was performed as described previously aiming at 2 x 10^3 to 10 x 10^3 CFU (2). To determine the Minimal Inhibitory Concentration (MIC) we performed drug-susceptibility testing using a well-standardized Middlebrook 7H10 agar dilution method as described previously (2) using the following drug concentrations: 1, 2, 4, 8, and 16 mg/L. All drugs were received as chemically pure powder. SILA 421 was provided by Dr. G. Hajos (Institute for Biomolecular Chemistry, Budapest, Hungary). Chlorpromazine, desipramine, promazine and thioridazine were obtained from Sigma-Aldrich (Zwijndrecht, the Netherlands). Because the thioridazine S-enantiomer might induce less side-effects (4) we
studied both thioridazine enantiomers separately. The two thioridazine enantiomers were
prepared by Dr. J.B. Christensen (Dept. Chemistry, Copenhagen, Denmark). All drugs were
dissolved in distilled water to a stock solution of 10 g/L
The MIC of SILA 421 could be determined after a median of 14 days (range 14-18 days). The
MIC\textsubscript{50} was 4 mg/L and the MIC\textsubscript{90} was 8 mg/L. Table 1 shows the comparison of the MIC\textsubscript{50}
and MIC\textsubscript{90} of SILA 421 with the various phenothiazines. The MICs found for the
phenothiazines were comparable with those found in the literature (3). The MIC\textsubscript{50} of SILA
421 was similar to the MIC\textsubscript{50} of thioridazine and chlorpromazine. On the contrary, MIC\textsubscript{50} of
both desipramine and promazine were both much higher than the MIC\textsubscript{50} of SILA 421. When
comparing the MIC\textsubscript{90}, lowest MIC\textsubscript{90} was measured for SILA 421.
These data show that the SILA 421 is active in vitro against \textit{M. tuberculosis} isolates with a
wide variety of drug susceptibility patterns, thereby providing incremental evidence of its
antimycobacterial activity. Moreover, we showed that SILA 421 is equally active in vitro as
thioridazine, another well-known efflux pump inhibitor.
In a previous study, SILA 421 has been proposed to be more potent that thioridazine (1).
However, only two \textit{M. tuberculosis} isolates had been tested (1). The present study confirms
that SILA 421 may be a promising antituberculosis drug with activity against a wide variety
of \textit{M. tuberculosis} strains comparable to that of thioridazine. Further studies are warranted to
investigate the potency of SILA 421 as an antituberculosis drug in animals and in humans.
Besides its antimycobacterial activity, SILA 421 might also have another appealing feature.
Like all efflux pump inhibitors, SILA 421 will inhibit the activity of efflux pumps of MDR
mycobacteria, presumptively rendering mycobacteria more susceptible to the antituberculosis
drugs to which it was initially resistant as a consequence of their extrusion from the cell (5).
Thus, these drug resistance modifiers might be of interest not only for their immediate
antimycobacterial activity but also as an adjunctive agent to be added to new or existing antituberculosis regimens.

Of additional interest is our finding that the S-enantiomer of thioridazine is as effective as thioridazine itself against *M. tuberculosis*. It is thought this S-enantiomer might induce less neurological side effects owing to a lower affinity to the D2 dopamine receptor (4), putatively favoring the S-enantiomer over the racemate as an antituberculosis drug. However, it is unknown if the D2 dopamine receptor is the only target for neurological side-effects of thioridazine and it is unclear whether the pharmacokinetic and pharmacodynamic properties of the thioridazine enantiomers are equal. Notwithstanding these limitations, our findings raise hope that structural optimization of thioridazine-derivatives is possible without losing antimycobacterial activity (3.4).

In conclusion, the present study provides confirmatory and incremental evidence of the antimycobacterial activity of SILA 421 and the phenothiazines, such as thioridazine. Therapeutic opportunities lay in the structural optimization of these drugs as well as in their drug resistance modifying effect. Future studies should bring SILA 421 and the phenothiazines further up the drug development pipeline.
References


The MIC$_{50}$ and MIC$_{90}$ of SILA 421 and the various phenothiazines$^a$.

<table>
<thead>
<tr>
<th>Efflux pump inhibitor</th>
<th>MIC$_{50}$ (mg/L)</th>
<th>MIC$_{90}$ (mg/L)</th>
<th>Range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SILA 421</td>
<td>4</td>
<td>8</td>
<td>2 - 16</td>
</tr>
<tr>
<td>Thioridazine</td>
<td>4</td>
<td>16</td>
<td>2 - 16</td>
</tr>
<tr>
<td>Thioridazine, S-enantiomer</td>
<td>8</td>
<td>16</td>
<td>4 - 16</td>
</tr>
<tr>
<td>Thioridazine, R-enantiomer</td>
<td>8</td>
<td>16</td>
<td>4 - 16</td>
</tr>
<tr>
<td>Chlorpromazine</td>
<td>4</td>
<td>16</td>
<td>&lt;1 - 16</td>
</tr>
<tr>
<td>Desipramine$^b$</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>16 - &gt;16</td>
</tr>
<tr>
<td>Promazine$^b$</td>
<td>&gt;16</td>
<td>&gt;16</td>
<td>16 - &gt;16</td>
</tr>
</tbody>
</table>

$^a$ MIC$_{50}$, MIC at which $\geq$ 50% of isolates are inhibited. MIC$_{90}$, MIC at which $\geq$ 90% of isolates are inhibited.

$^b$ MIC distribution was based on 20 instead of 21 isolates.