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Discovery of potent anti-tuberculosis agents targeting aminoacyl-tRNA synthetases

G.P. Volynets[1], S.A. Starosyla[2], V.G. Bdzhola[1], S.M. Yarmoluk[1], M.A. Tukalo[1]

[1] Institute of Molecular Biology and Genetics, NAS of Ukraine
150, Akademika Zabolotnoho Str., Kyiv, Ukraine, 03143
[2] RECEPTOR.AI,
Boston, MA, USA
g.p.volynets@gmail.com

Background. The most serious challenge in the treatment of tuberculosis is the multidrug resistance of *Mycobacterium tuberculosis* to available antibiotics. This provides the stimulus for the search of novel molecular targets and approaches to overcome resistance emergence. Nowadays, *M. tuberculosis* leucyl-tRNA synthetase (LeuRS) is a clinically validated molecular target for antituberculosis drug discovery. The **aim** of our study was to develop small-molecular inhibitors of *M. tuberculosis* LeuRS with antibacterial activity. **Methods.** Molecular docking, pharmacophore screening and machine learning approaches were used for *in silico* design of *M. tuberculosis* LeuRS inhibitors. Molecular docking was performed with DOCK 4.0 software. Ligand-oriented pharmacophore models were built using web-server PharmaGist. Pharmacophore screening was conducted using PharmDeveloper program. The artificial neural networks were built using module net from R 3.6.1. The inhibitory activity of compounds toward recombinant aminoacyl-tRNA synthetase was investigated in aminoacylation assays. The antibacterial activity of compounds was studied toward pathogenic strain *M. tuberculosis* H37Rv in microdilution assay. **Results.** Using molecular docking approach we have found inhibitors of *M. tuberculosis* LeuRS among N-Benzylidene-N'-thiazol-2-yl-hydrazines with IC_{50} values in the range from 2.27 mM to 19.3 mM. The most promising compound among studied derivatives inhibits growth of pathogenic bacteria *M. tuberculosis* H37Rv with IC_{50} = 10 mM. Then, as a strategy to overcome resistance emergence in *M. tuberculosis* we used the idea of multitarget inhibition, since the probability of mutations simultaneously in both enzymes is negligible. As molecular targets we have selected *M. tuberculosis* LeuRS and methionyl-tRNA synthetase (MetRS). Using molecular docking approach we identified dual-targeted inhibitors among N-benzylidene-N'-thiazol-2-yl-hydrazines. The most promising compound among studied derivatives inhibits growth of *M. tuberculosis* H37Rv with IC_{50} = 1.56 mM. Applying a ligand-based pharmacophore approach we found five novel dual-targeted hit compounds from different chemical classes. The most active compound inhibits LeuRS and MetRS with IC_{50} values of 13 mM and 13.8 mM, correspondingly. Using the single-hidden-layer neural network models we identified dual-targeted inhibitors among 2-(quinolin-2-ylsulfanyl)-acetamides. The most active compound among studied derivatives inhibits MetRS and LeuRS with IC_{50} values of 33 mM and 23.9 mM, respectively. **Conclusions.** Using *in silico* approaches we identified several chemical classes of inhibitors targeting *M. tuberculosis* LeuRS and MetRS with IC_{50} values in micromolar concentration range. It was found that the most promising compounds which possess antibacterial activity belong to N-benzylidene-N'-thiazol-2-yl-hydrazine derivatives. Therefore, the compounds in this class can be valuable for further biological research and optimization. **Funding.** This work was supported by the National Research Foundation of Ukraine (grant № 2023.03/0175) and by Simons Support (grant № 1290589).

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