Deciphering the mechanism of action of novel anti-tuberculosis compounds is a key step in the drug development process. We have previously described a number of imidazo[1,2-b][1,2,4,5]tetrazines with a promising activity on Mycobacterium tuberculosis [1]. These compounds had predicted activity as serine–threonine protein kinase inhibitors, however spontaneous drug resistant Mycolicibacterium smegmatis mc² 155 (formerly Mycobacterium smegmatis) revealed only the mycobacterial mechanism of resistance to imidazo[1,2-b][1,2,4,5]tetrazines: mutations in MSMEG_1380 gene lead to overexpression of the mmpS5-mmpL5 operon in M. smegmatis, thus providing resistance to imidazo[1,2-b][1,2,4,5]tetrazines via enhanced efflux [2]. Here we report the RNA sequencing data of M. smegmatis mc² 155 culture treated with one of the imidazo[1,2-b][1,2,4,5]tetrazines for 1.5 h and the untreated culture as a control. The mapped reads showed that a total of 1386 genes are differentially expressed in this experiment. A further analysis of these data can shed light of the mechanism.
of action of imidazo[1,2-b][1,2,4,5]tetrazines. The data generated by RNA-seq (raw reads) have been deposited to NCBI sequence read archive (SRA) and have been assigned a BioProject accession number PRJNA615922.

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### Specifications Table

| Subject | Biochemistry, Genetics and Molecular Biology (General) |
|---------|------------------------------------------------------|
| Specific subject area | Transcriptomics |
| Type of data | Transcriptome sequences, tables, figure |
| How data were acquired | Illumina HiSeq 2500 sequencing platform |
| Data format | Raw Illumina HiSeq 2500 data in FASTQ format |
| Parameters for data collection | Comparison of *M. smegmatis* cultures treated with an imidazo[1,2-b][1,2,4,5]tetrazine with untreated control |
| Description of data collection | Total RNA extracted from six independent samples (three control replicates – untreated cultures, and three experimental replicates – treated with an imidazo[1,2-b][1,2,4,5]tetrazine) subjected to RNA sequencing. |
| Data source location | Vavilov Institute of General Genetics Russian Academy of Sciences, Moscow, Russia. |
| Data accessibility | Repository name: NCBI Sequence Read Archive – SRA |
| | Data identification number: PRJNA615922 |
| | Direct URL to data: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA615922 |

### Value of the Data

- These data show for the first time a transcriptomic response of *M. smegmatis* exposed to an imidazo[1,2-b][1,2,4,5]tetrazine - an anti-tuberculosis drug candidate.
- The data may be useful for researchers working on anti-tuberculosis drug development, as it may provide clues on imidazo[1,2-b][1,2,4,5]tetrazines’ mechanism of action.
- Analysis of differentially expressed genes upon exposure to imidazo[1,2-b][1,2,4,5]tetrazine may elucidate additional attractive biotargets in mycobacteria for drug development.

### 1. Data description

The dataset presented in this article represents raw RNA-seq reads from samples of *Mycobacterium smegmatis mc² 155* treated with compound *3a* (Fig. 1) – an anti-tuberculosis drug candidate of imidazo[1,2-b][1,2,4,5]tetrazines class [1] – at a final concentration of 256 μg/ml, and untreated control samples. The sample description together with NCBI accession numbers (BioProject, BioSample and SRA) are listed in Table 1. Sequencing and reads mapping statistics are summarized in Table 2. Reads mapping showed 1386 differentially expressed genes (DEGs)

| Sample ID | Group | *3a* concentration | Bioproject accession no. | Biosample accession no. | SRA accession no. |
|-----------|-------|--------------------|--------------------------|--------------------------|------------------|
| 1–1       | Control | 0 μg/ml           | PRJNA615922             | SAMN14476747             | SRX8018106       |
| 2–1       |        |                   |                         |                          | SRX8018107       |
| 3–1       |        |                   |                         |                          | SRX8018108       |
| 4–1       | Experiment | 256 μg/ml     | SAMN14476748             |                          | SRX8018109       |
| 5–1       |        |                   |                         |                          | SRX8018110       |
| 6–1       |        |                   |                         |                          | SRX8018111       |
Fig. 1. Chemical structures of the compound 3a [1].

| Sample ID | Total reads (raw) | Total reads (after trimming) | % Aligned Reads | Fragment Length (bp) |
|-----------|------------------|-----------------------------|-----------------|---------------------|
| 1–1       | 7560,080         | 7546,578                    | 98.0%           | 219.0               |
| 2–1       | 7389,426         | 7367,050                    | 98.2%           | 218.1               |
| 3–1       | 6420,732         | 6384,648                    | 98.5%           | 219.4               |
| 4–1       | 7416,774         | 7396,635                    | 98.8%           | 225.8               |
| 5–1       | 8184,700         | 8160,049                    | 98.9%           | 222.4               |
| 6–1       | 8448,563         | 8434,466                    | 99.1%           | 223.1               |

(671 downregulated and 715 upregulated) in the experimental group as compared to the control (Supplementary Table).

2. Experimental design, materials, and methods

2.1. Bacterial strains and growth conditions

_M. smegmatis mc²_ 155 strain was used in this work. _M. smegmatis_ cultures were grown in Middlebrook 7H9 medium (Difco Becton Dickinson, USA) supplemented with 0.5% (v/v) glycerol and 0.05% (v/v) Tween 80 at 37 °C and 250 rpm.

2.2. Experimental design

_M. smegmatis_ cultures were grown overnight in Middlebrook 7H9 broth to mid-log phase (OD600 = 1.0–1.2) and then compound 3a dissolved in DMSO was added to the medium to a final concentration of 256 μg/ml (4 × minimal inhibitory concentration [1]) for 1.5 h. The same amount of DMSO was added to the control samples. Afterwards cells were washed twice with fresh ice-cold Middlebrook 7H9 broth and total RNA was isolated. In total, 6 RNA samples were obtained — 3 biological replicates in the control and experimental conditions.

2.3. RNA extraction and sequencing

Cells from 10 mL culture were harvested by centrifugation for 10 min at 3000 × g and 4 °C, washed twice by 10 ml of fresh Middlebrook 7H9 broth and once by 1 ml of RNAprotect Bac-
teria Reagent (Qiagen, USA). Total RNA was extracted as described by Rustad et al. [3], with some modifications. In brief: *M. smegmatis* cells were homogenized in ExtractRNA reagent (Evrogen, Russia), followed by phenol (pH = 4.5)-chloroform/isoamyl alcohol (25:24:1) purification and precipitation with isopropanol (2:1, v/v). Remaining genomic DNA was removed by DNAse I, Amplification grade (Invitrogen, USA). Total RNA (1 μg) was used for library preparation. Ribosomal RNA was removed from the total RNA using the RiboMinus Transcriptome Isolation Kit, bacteria (Thermo Fisher Scientific) and libraries were prepared using the NEBNext® Ultra II Directional RNA Library Prep Kit (NEB), according to the manufacturer’s protocol. Libraries were subsequently quantified by Quant-IT DNA Assay Kit, High Sensitivity (Thermo Fisher Scientific). Finally, equimolar quantities of all libraries (12 pM) were sequenced by a high throughput run on the Illumina HiSeq using 2 × 100 bp paired-end reads and a 5% Phix spike-in control.

### 2.4. Transcriptome data analysis

Data processing and analysis was performed as described previously by Bespyatykh et al. [4]. Raw reads’ quality was assessed by FASTQC v0.11.7 [5], the remaining adapters were removed with Trimmomatic v0.33 [6]. Reads were mapped to the *M. smegmatis* mc² 155 reference assembly (GCF_000015005.1) and quantified with Kallisto v0.46.0 [7]. The Degust v4.1.1 web-tool [8] with integrated edgeR v3.26.8 package [9] was used for differential expression analysis. Only genes with count per million (CPM) ≥ 1 were analyzed further. Genes were filtered based on false discovery rate cutoff (FDR) ≤ 0.05 and minimum expression fold change (FC) ≥ 2.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships which have, or could be perceived to have, influenced the work reported in this article.

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### Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dib.2020.105805.

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