Background
Para-aminosalicylic acid (PAS) was one of the first antibiotics to be used against tuberculosis (TB) and it is still one of the last remaining drugs available to treat extensively drug-resistant (XDR) disease (1, 2). Despite being on the market for decades, the mechanism of action of PAS is not completely understood yet (3). Sixteen new compounds against Mycobacterium tuberculosis were created in the laboratory as salicylate analogues (based on their chemical structures) and their antimycobacterial activity had never been tested before. The main aim of this project was to test the activity of these new analogues and understand their mechanism of action (including PAS).

Methods
The compounds (whose chemical structure is shown below) were tested using three different methods (spot culture, resazurin and BACTEC/MGIT system) (4, 5, 6). Additionally, resistant mutants were created against PAS and the most promising analogue; whole genome sequencing (WGS) was performed to understand their mechanism of action.

Results: susceptibility testing
1. Five PAS analogues (DE471, 1H2NA, 2H1NA, 3H1NA and AD25a) inhibited the growth of M. tuberculosis using the spot culture method (Table 1).
2. The five active compounds on the spot culture were further tested with the resazurin method for the determination of their critical concentrations (Table 2). One compound in particular, AD25a, showed the lowest critical concentration (0.04 µg/ml) among the analogues.
3. The compound AD25a was selected for further testing using the BACTEC/MGIT method (Table 3).

Table 1: Spot culture screening results. Five compounds inhibited the growth of M. tuberculosis H37Rv. The active compounds (DE471, 1H2NA, 2H1NA, 3H1NA and AD25a) are highlighted in grey.

Table 3: BACTEC/MGIT testing results. The table above shows the growth units of H37Rv over time (in hours) at different concentrations of AD25a (from 1.54 µg/ml to 0.05 µg/ml). When the growth control (GC) reaches 400 growth units, any concentrations with a growth unit less than 100 is considered as active against M. tuberculosis (susceptible). If the growth units are more than 100, the concentration tested is considered as non-active resistant. The lowest active concentration was 0.39 µg/ml (circled in yellow).

Conclusions
The complete performance in the mutation profiles suggests that AD25a has a mechanism of action different to that of PAS, despite AD25a being synthesized as a salicylate analogue. WGS analysis of PAS resistant mutants has also provided some interesting results. In particular, all our PAS mutants showed mutations in the rrn and rrl genes (16S and 23S RNA genes, respectively). These mutations should affect the ribosomes and the overall synthesis of proteins. This highlights a new potential mechanism of resistance for PAS that has never been observed before.

References