

## Supporting Information

# Rationally Designed Novel Phenyloxazoline Synthase Inhibitors: Chemical Synthesis and Biological Evaluation to Accelerate the Discovery of New Antimycobacterial Antibiotics

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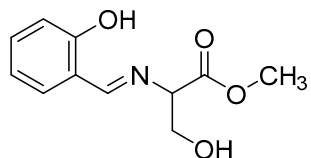
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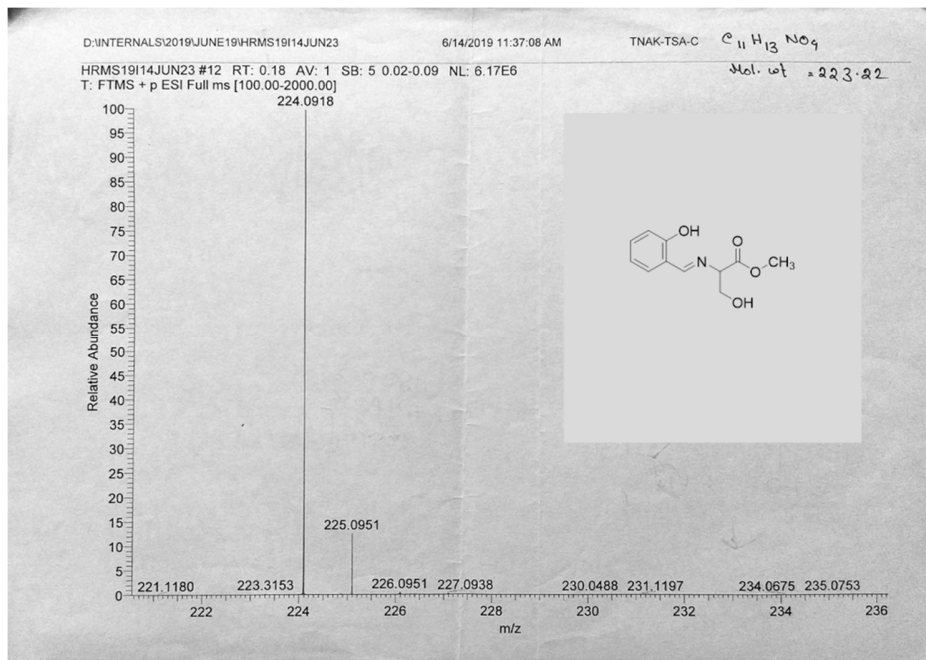
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### Supplementary Figure S1. <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, HRMS, Depts 135 and Depts 90 spectra of TSA (1-5):

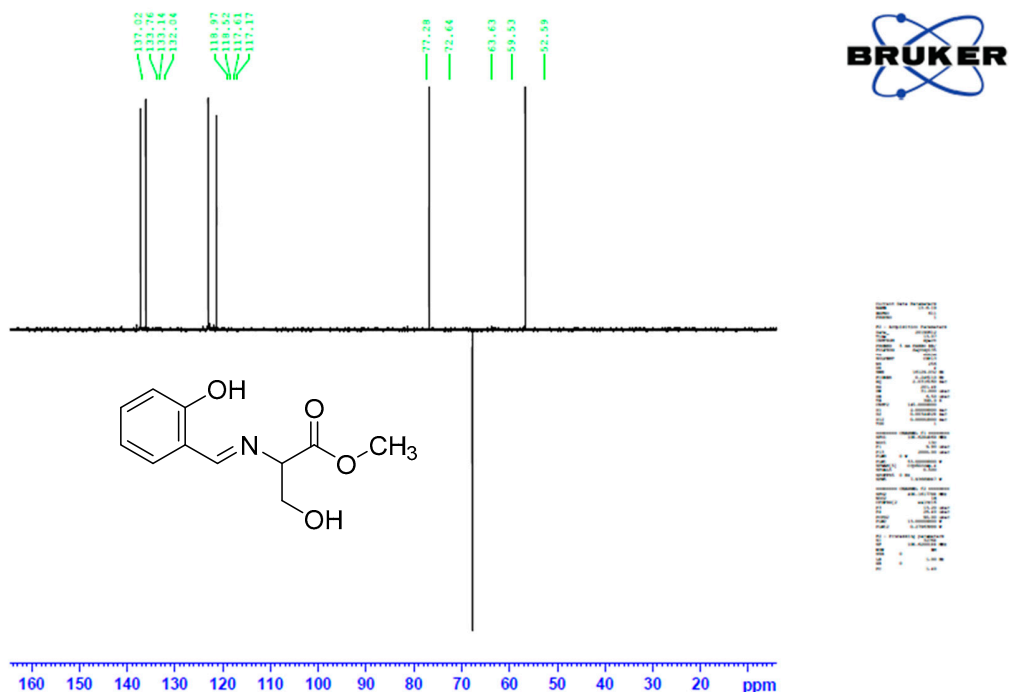
All the newly synthesized compounds were characterized by <sup>1</sup>H-NMR, <sup>13</sup>C-NMR, and HRMS spectroscopic analysis. Compound TSA-1 is additionally characterized by Depts 90 and Depts 135 analysis.

[illegible]

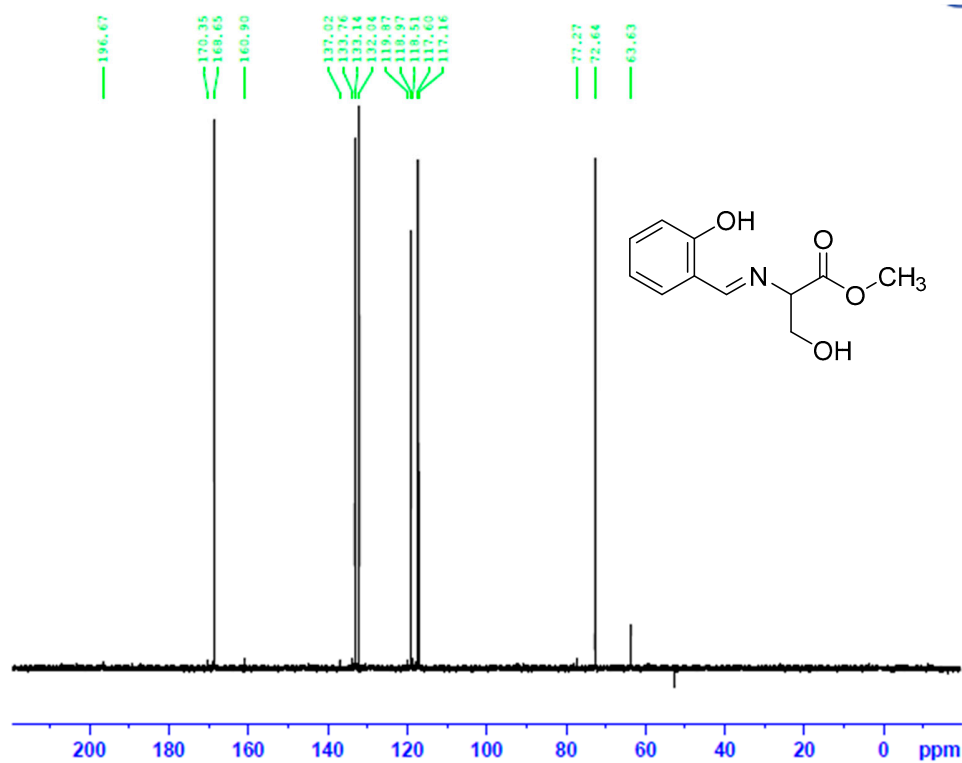
# 100 MHz <sup>13</sup>C-NMR spectrum of compound TSA-1 in CDCl<sub>3</sub>



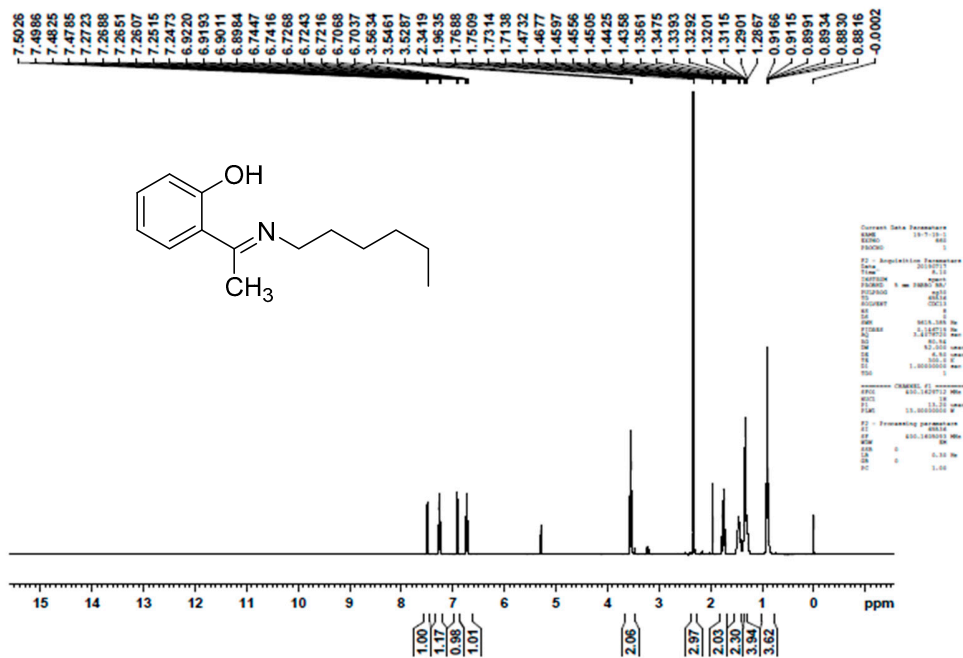
## HRMS spectrum of compound TSA-1



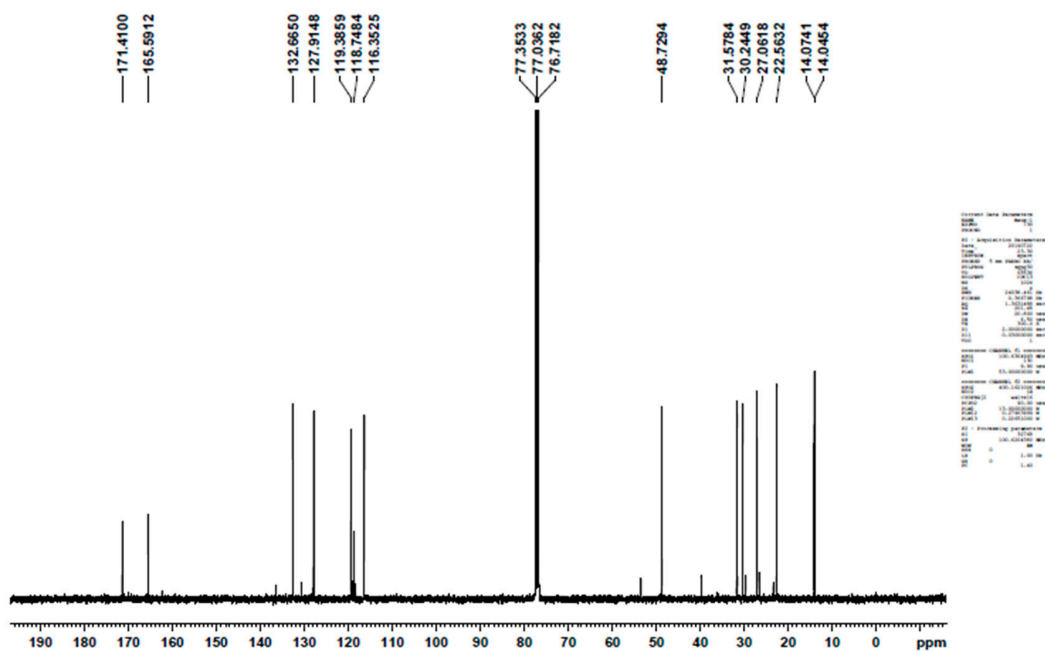
## Depts-135 spectrum of TSA-1



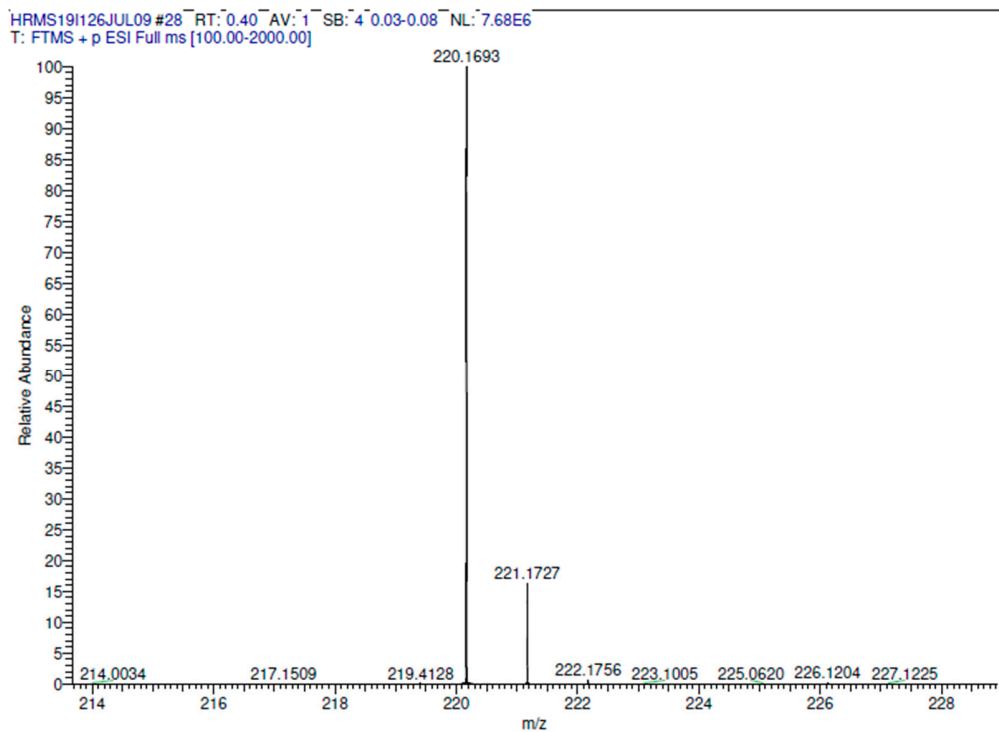
Depts-90 spectrum of TSA-1



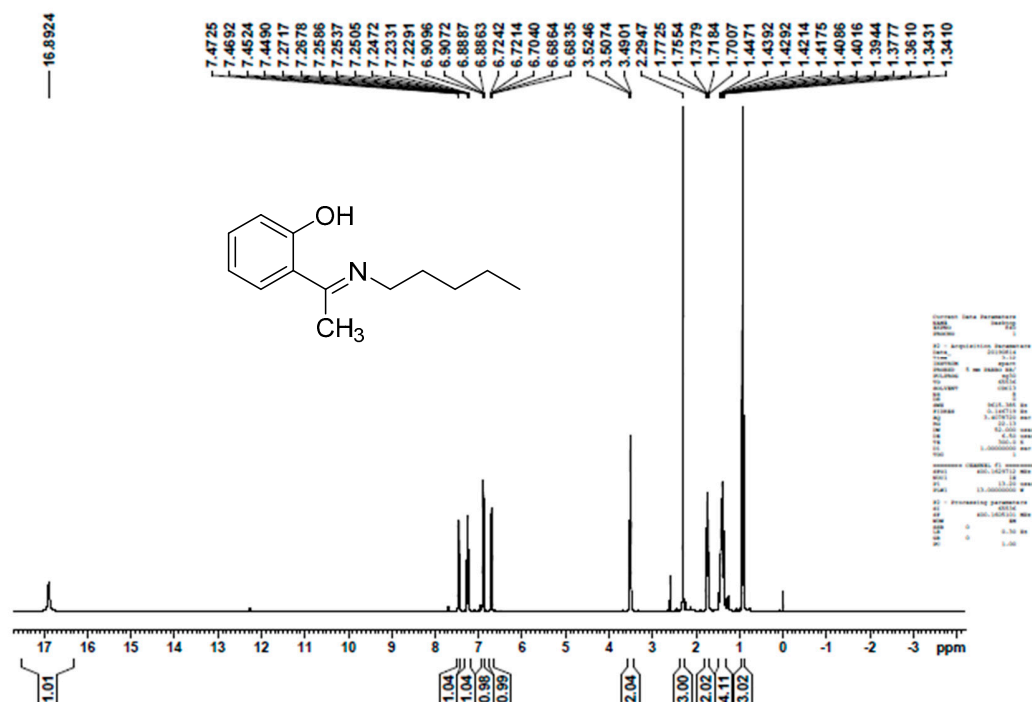
400 MHz <sup>1</sup>H-NMR spectrum of compound TSA-2 in CDCl<sub>3</sub>



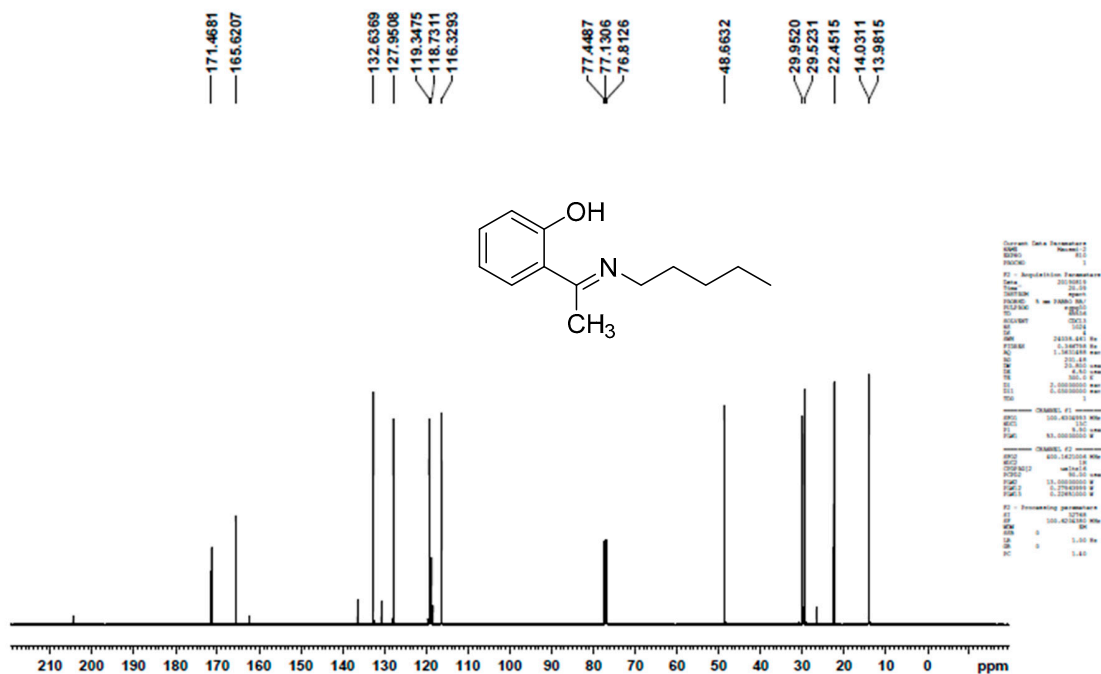
100 MHz  $^{13}\text{C}$ -NMR spectrum of compound TSA-2 in  $\text{CDCl}_3$



HRMS spectrum of compound TSA-2

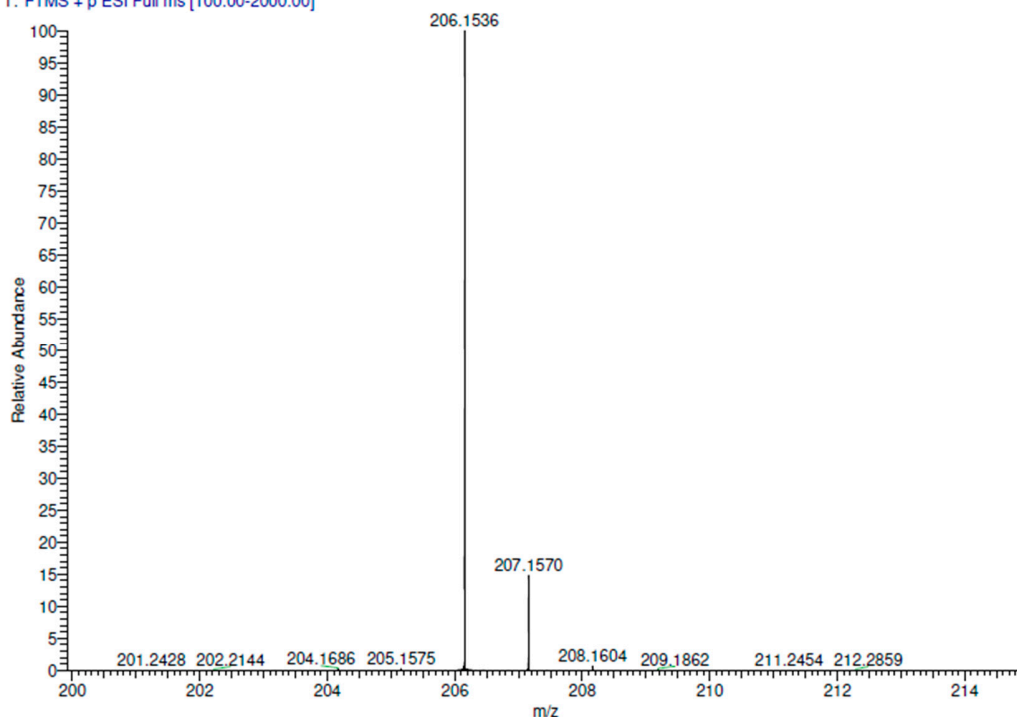


400 MHz  $^1\text{H}$ -NMR spectrum of compound TSA-3 in  $\text{CDCl}_3$

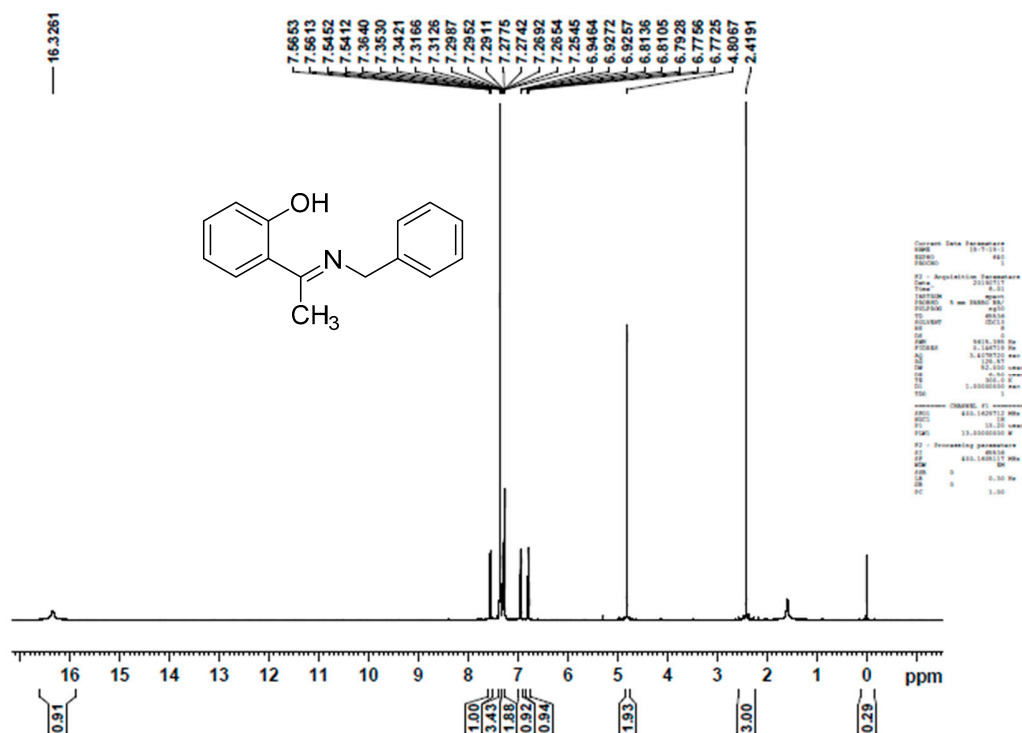


100 MHz  $^{13}\text{C}$ -NMR spectrum of compound TSA-3 in  $\text{CDCl}_3$

HRMS19119AUG01\_190821121931 #10 RT: 0.14 AV: 1 SB: 5 0.02-0.08 NL: 5.60E7  
T: FTMS + p ESI Full ms [100.00-2000.00]

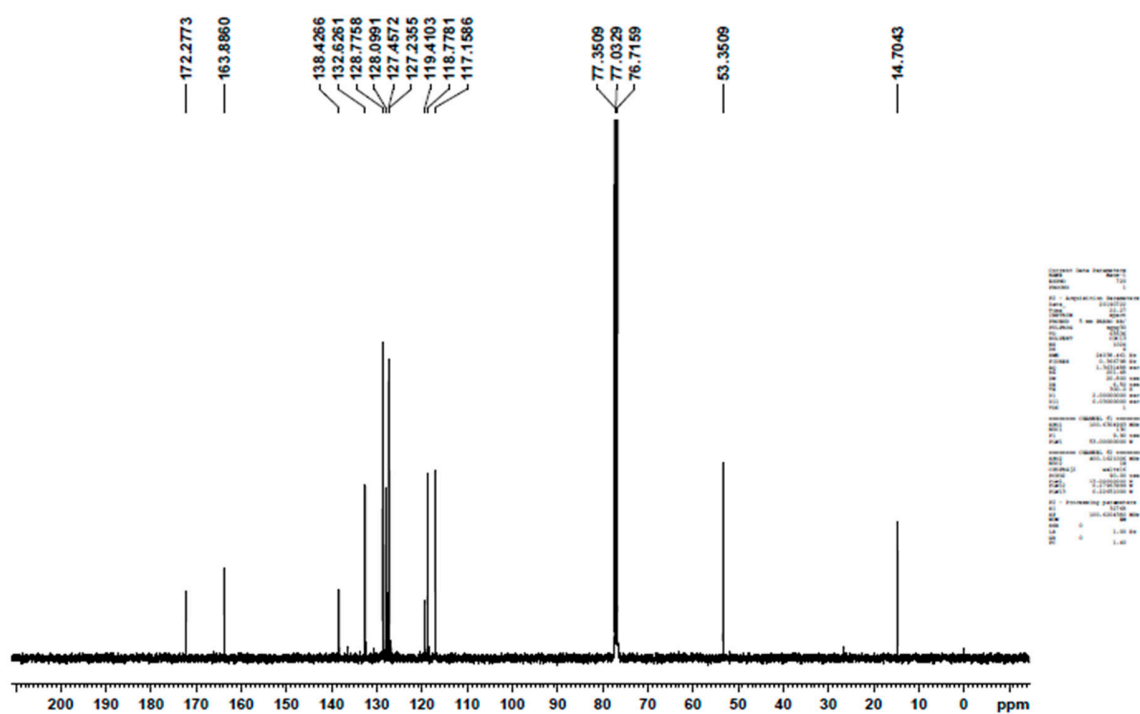


HRMS spectrum of compound TSA-3

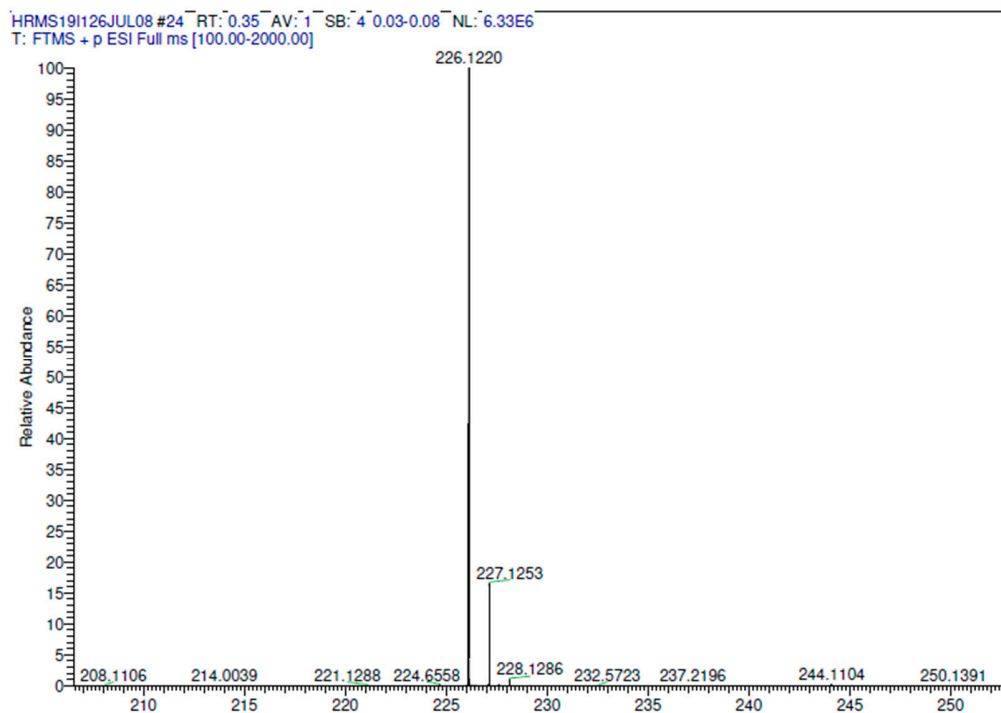




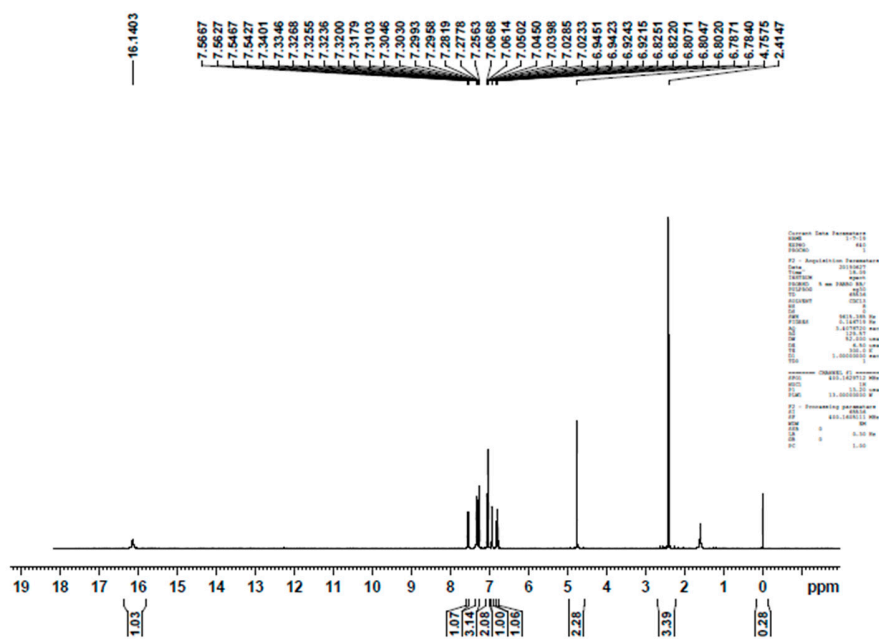
400 MHz  $^1\text{H}$ -NMR spectrum of compound TSA-4 in  $\text{CDCl}_3$



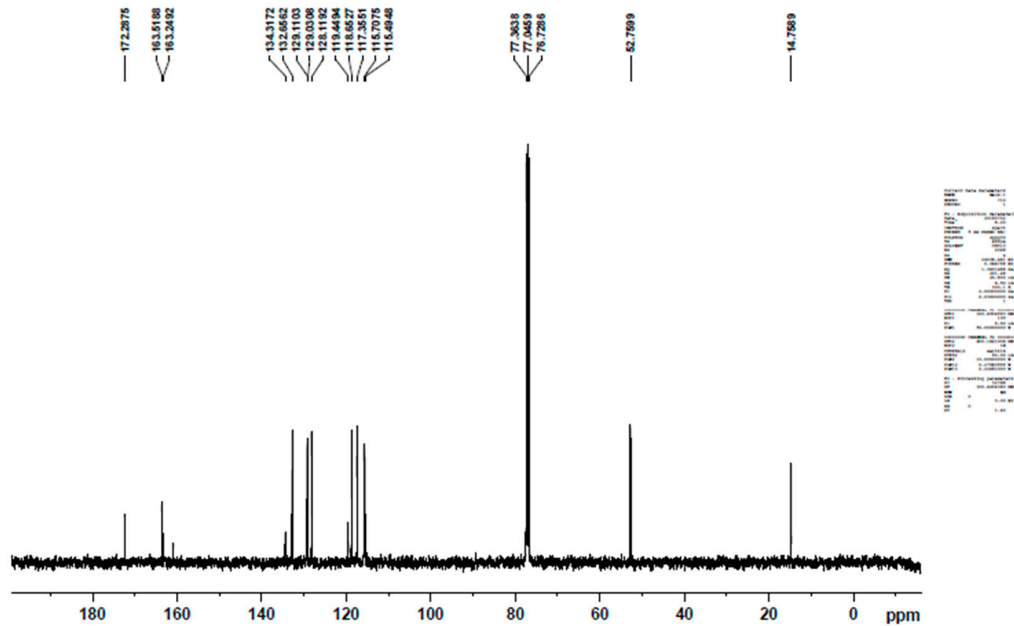
100 MHz  $^{13}\text{C}$ -NMR spectrum of compound TSA-4 in  $\text{CDCl}_3$



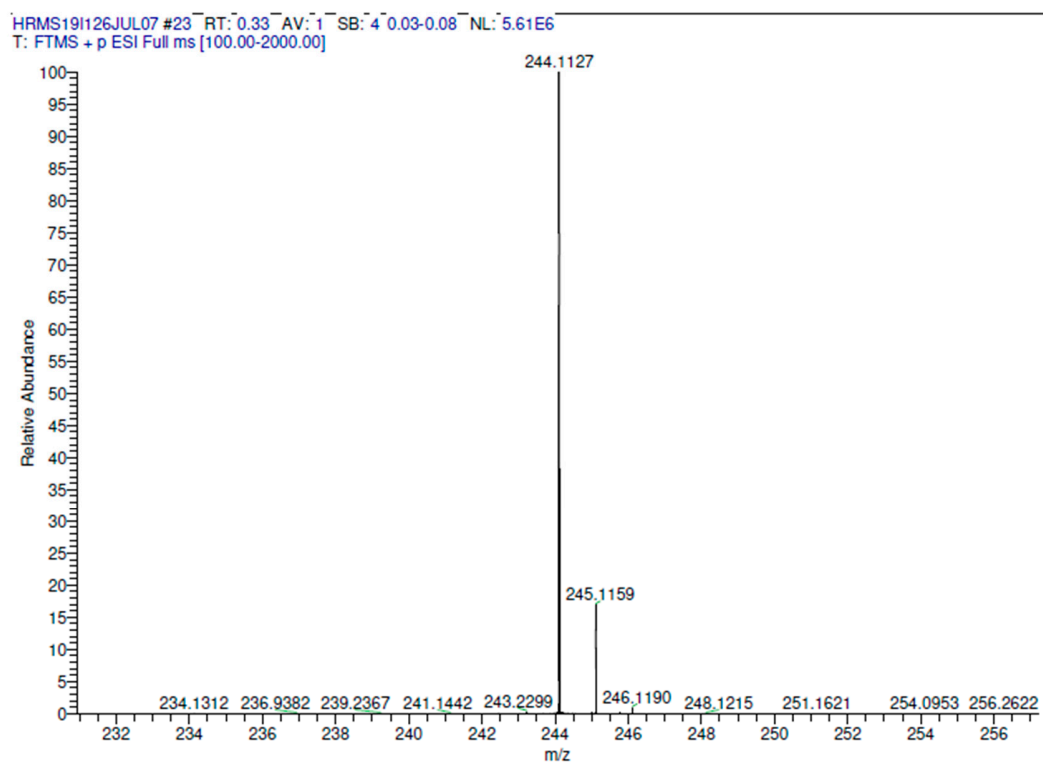
### HRMS spectrum of compound TSA-4



**400 MHz <sup>1</sup>H-NMR spectrum of compound TSA-5 in CDCl<sub>3</sub>**

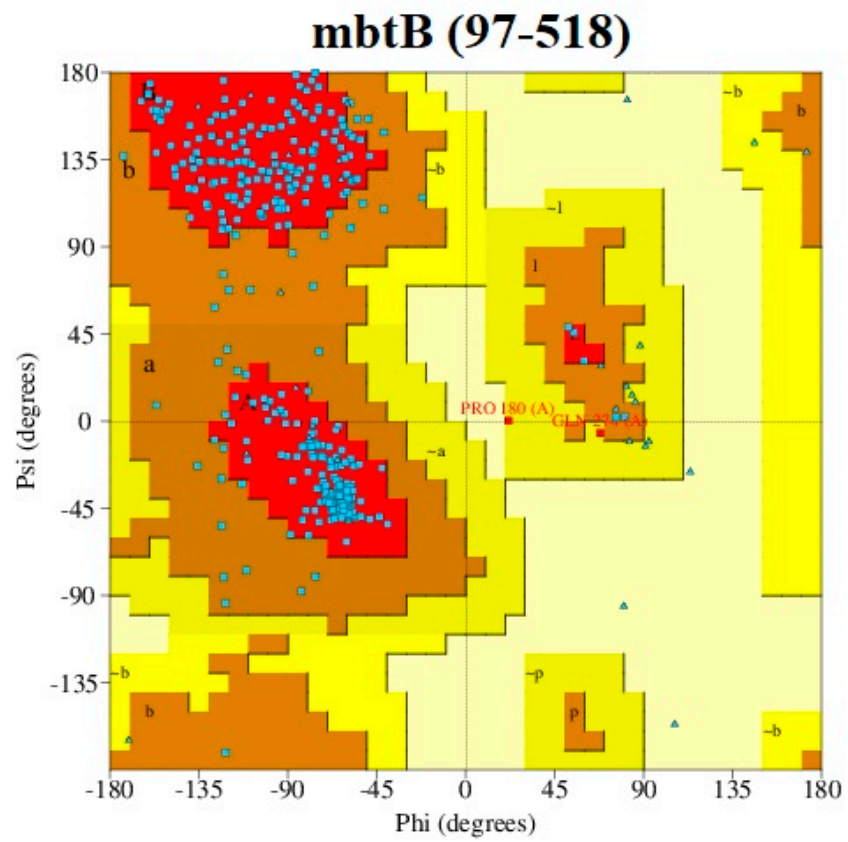


**100 MHz  $^{13}\text{C}$ -NMR spectrum of compound TSA-5 in  $\text{CDCl}_3$**

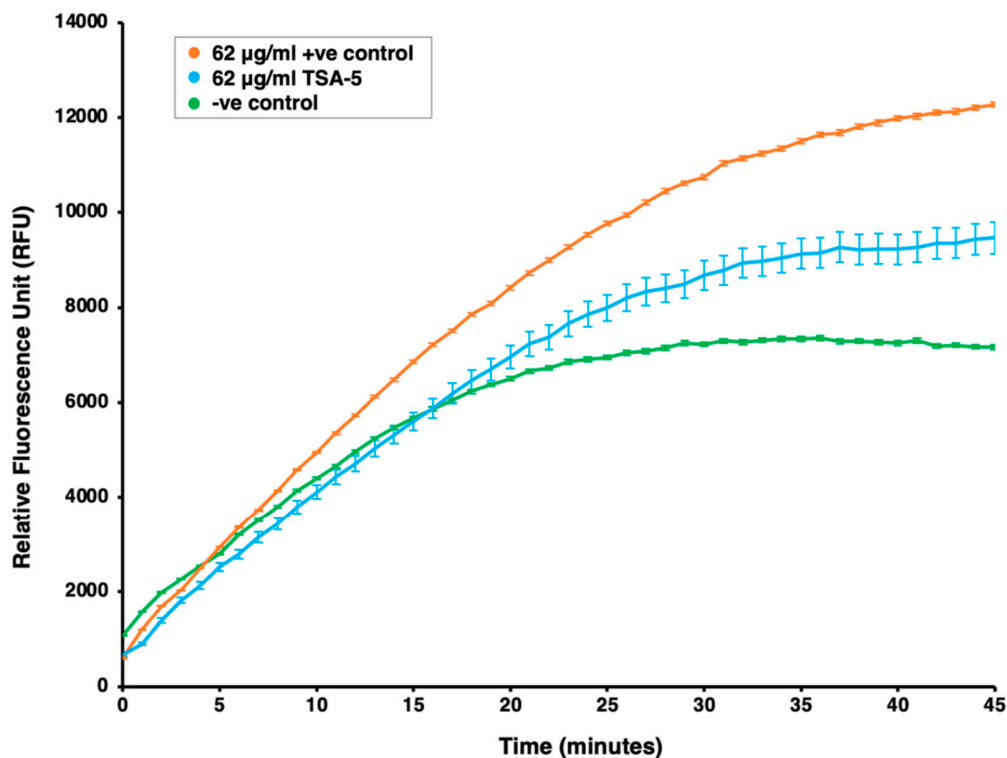


**HRMS spectrum of compound TSA-5**

1. **Supplementary Figure S1:** Ramachandran plot of the protein of interest, MbtB\_Cy



**Supplementary Figure S2:** Efflux-pump inhibition of TSA-5 against *M. abscessus*



**Supplementary Figure S3.** Accumulation of ethidium bromide (EtBr) in *M. abscessus* cells over a 45-minute time frame, in the presence of TSA-5, positive control (verapamil), and negative control (no inhibitor). Experiment was performed in three biological replicates (n=3), values represent the mean  $\pm$  SD.