Supplementary Material

The Quality Control of Midecamycin and the Predictive Demarcation between Its Impurities and Components

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The method of Molecular docking

Molecular docking was carried out to investigate the binding mode between midecamycin A_1 and ribosome by using Autodock vina 1.1.2 [10]. The three-dimensional (3D) structure of the ribosome (PDB: 4V7U) was downloaded from RCSB Protein Data Bank [11, 12, 13]. The two-dimensional (2D) structure of the compounds were drawn by ChemBioDraw Ultra 14.0, and the 2D structures were converted to 3D structures, and then minimized by MM2 method using ChemBio3D Ultra 14.0 software. The AutoDockTools 1.5.6 package [14, 15] was used to produce the docking input files. The ligand was used for docking by merging non-polar hydrogen atoms and defining rotatable bonds. The search grid of the ribosome site was identified as center_x: -66.611, center_y: -56.398, and center_z: 4.489 with dimensions size_x: 15, size_y: 15, and size_z: 15. In order to improve the docking accuracy, the value of exhaustiveness was set to 16. For docking, the default parameters were employed if it was not mentioned. The best-scoring pose by the Vina docking score was confirmed and visually analyzed by using PyMoL 1.7.6 software (www.pymol.org). All the pictures were obtained with the rendering resolution 1500*1200.
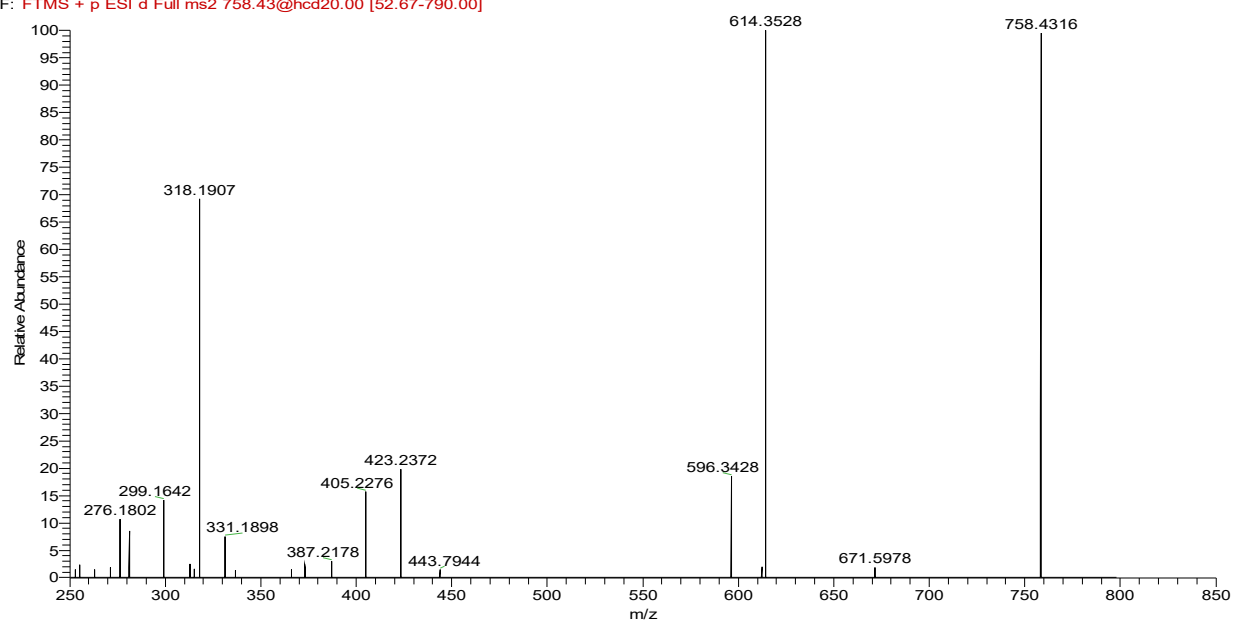
MS² spectrum of meleumycin D
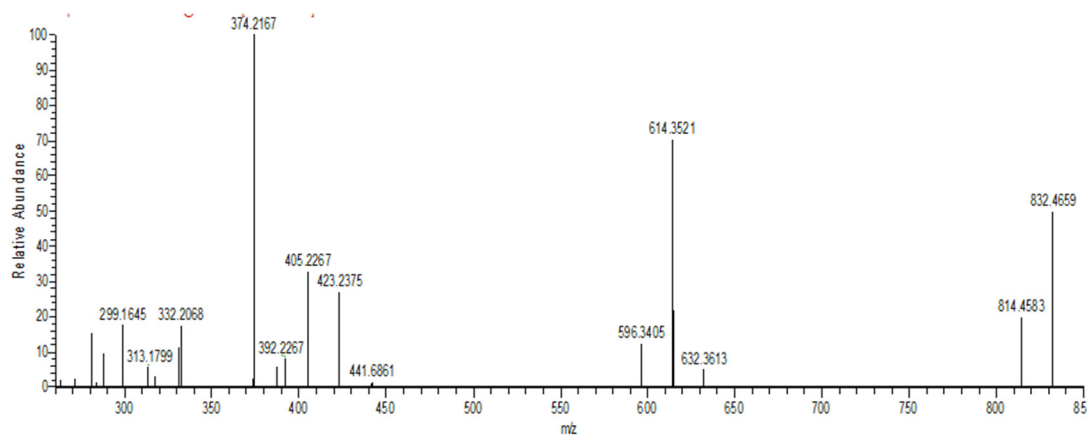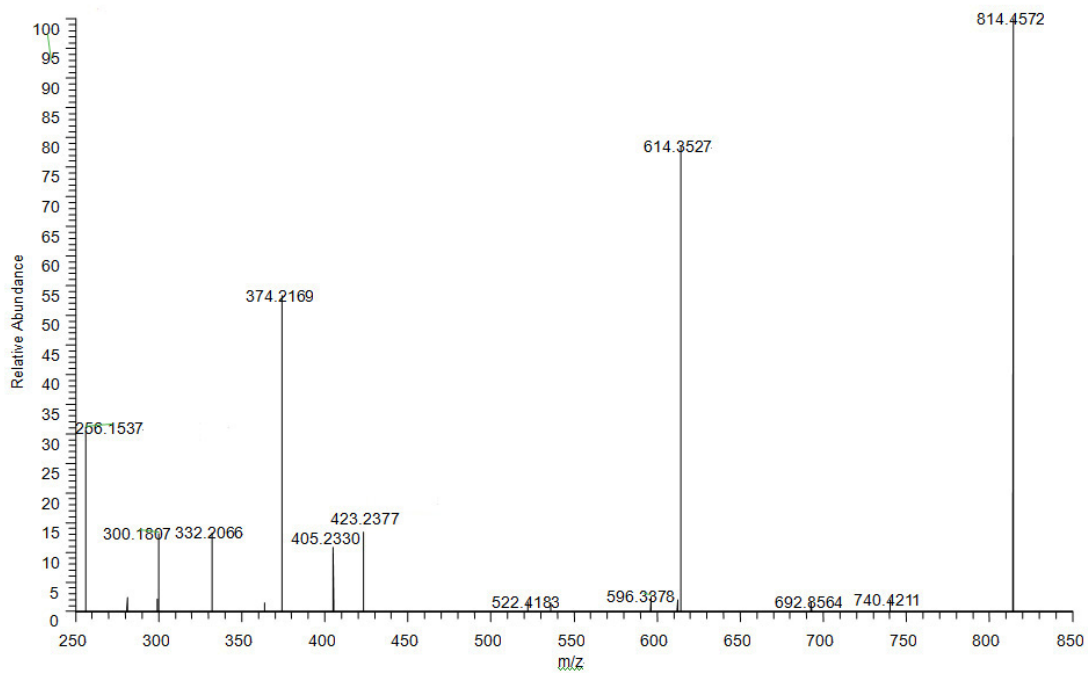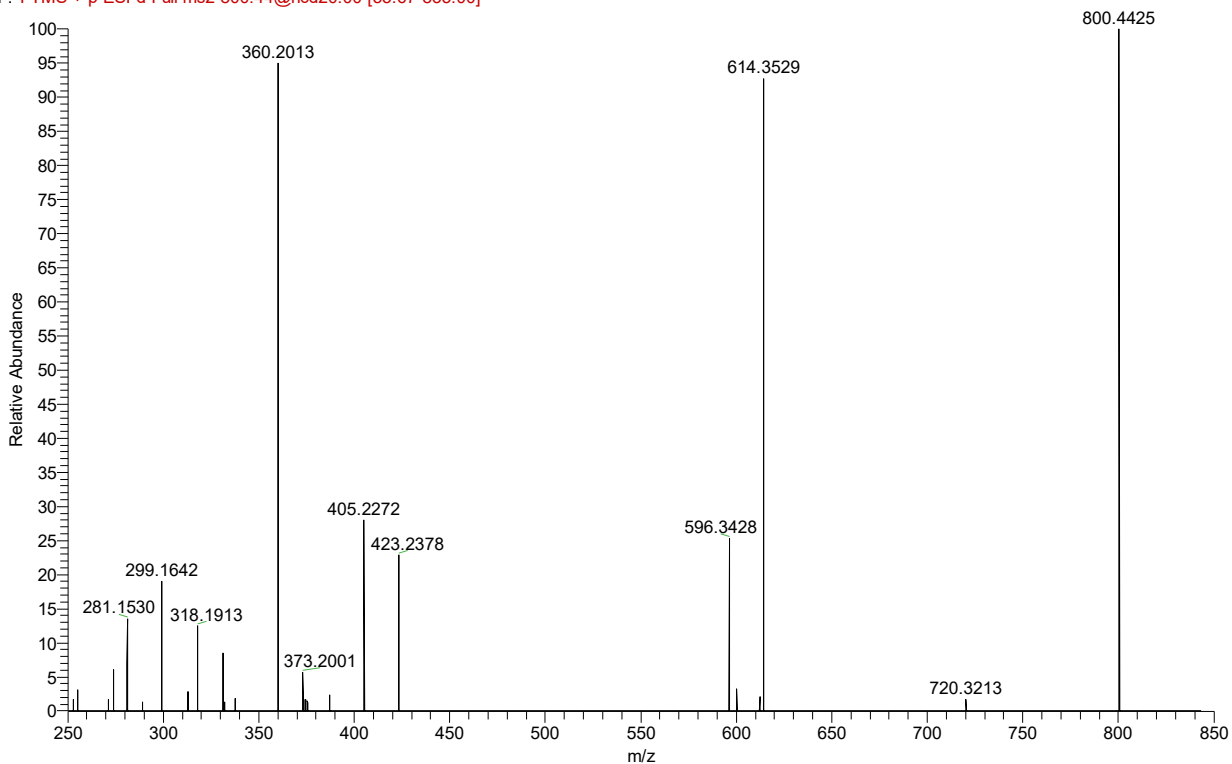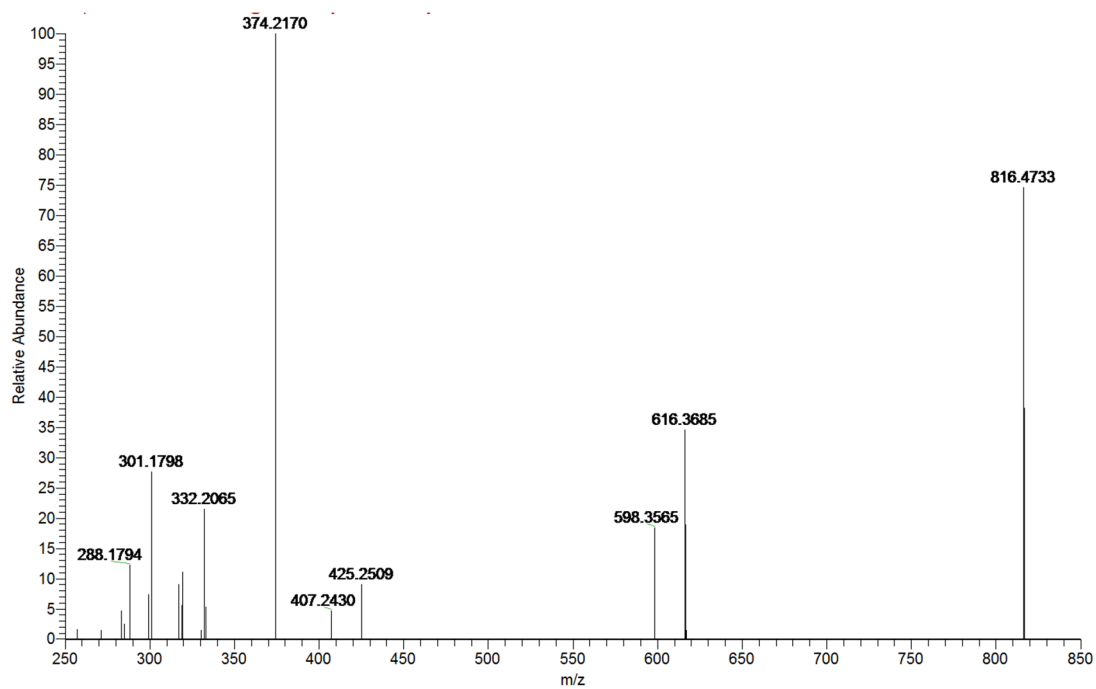
MS² spectrum of open loop midecamycin A

MS² spectrum of isomer of midecamycin A1

1mgmL5uLmaidis2 #5260   RT: 23.41   AV: 1   NL: 4.40E6
F: FTMS + p ESI d Full ms2 800.44@hcd20.00 [55.67-835.00]
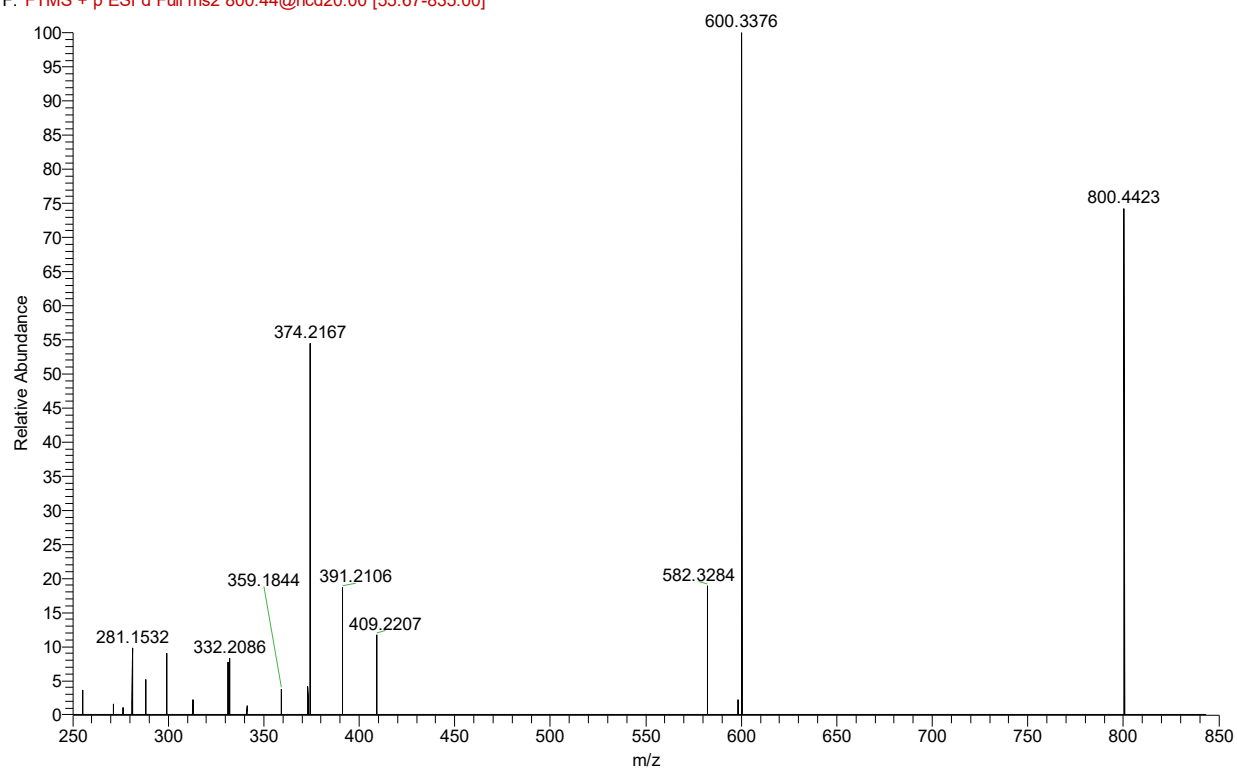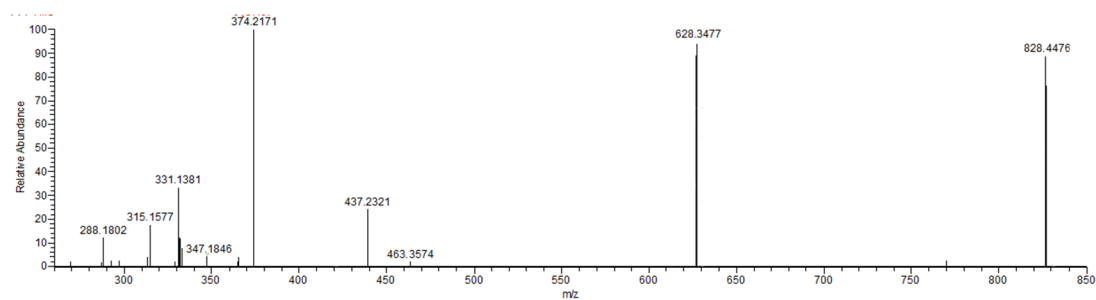
MS² spectrum of Meleumycin B₂

MS² spectrum of 6-hydroxyethyl-midecamycin A
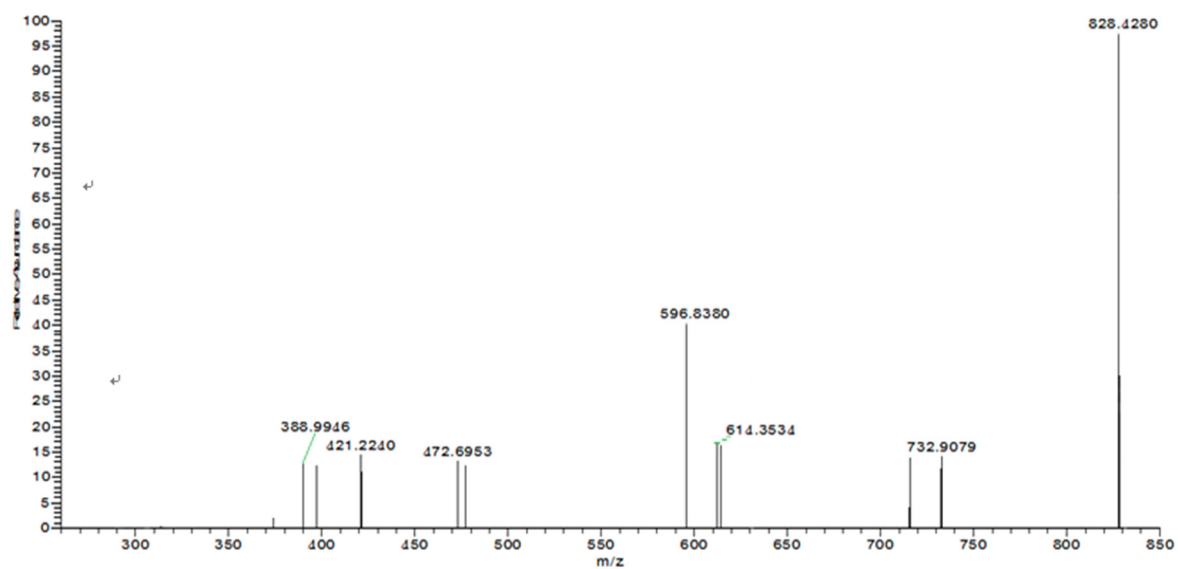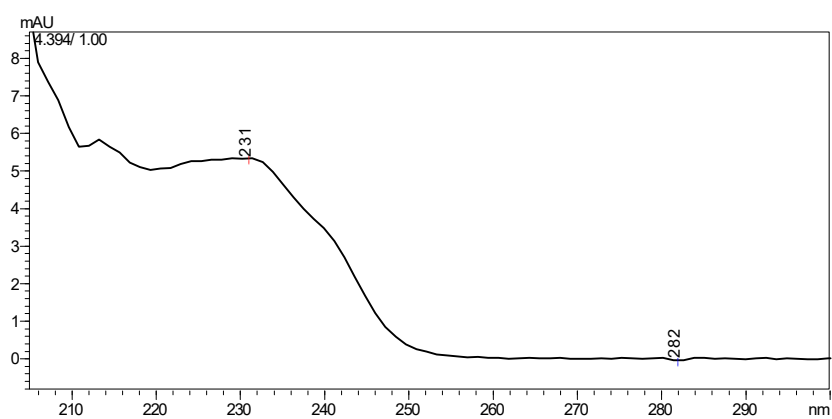
MS² spectrum of Leucomycin A₆

MS² spectrum of X

MS² spectrum of Midecamycin A₂

Figure S1. MS² spectrum of the impurities

Meleumycin D
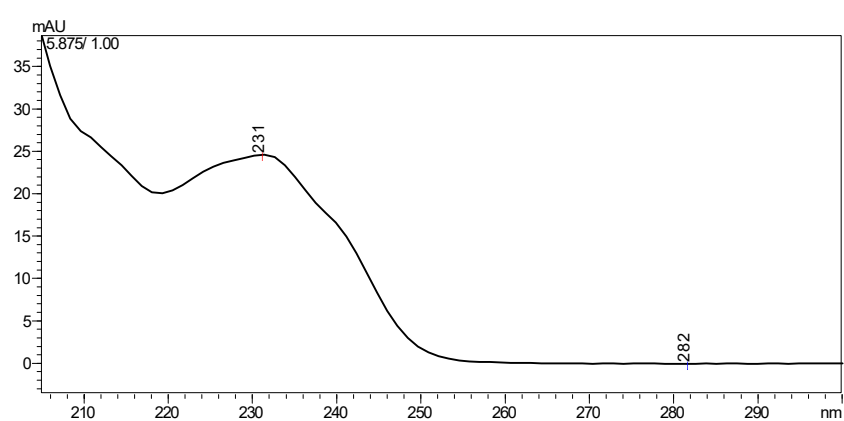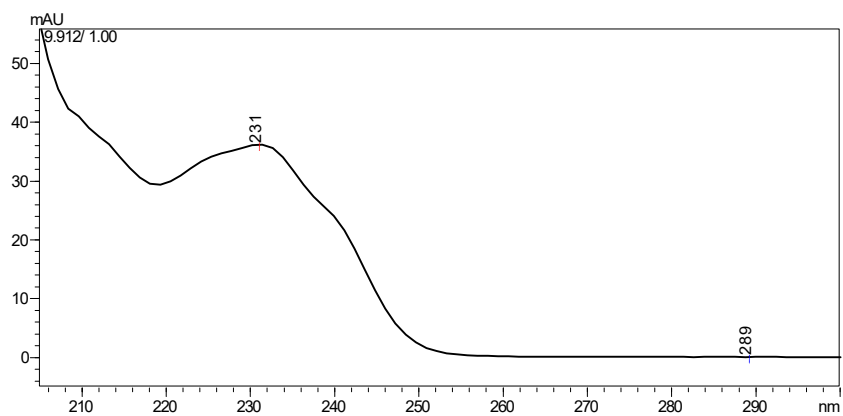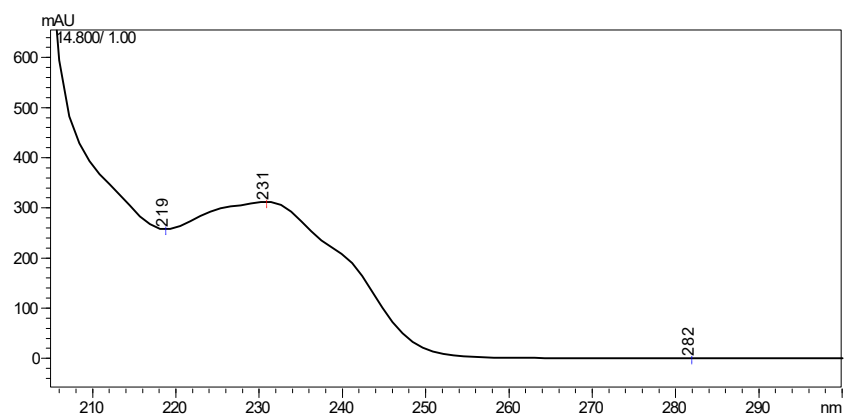

Open loop midecamycin A
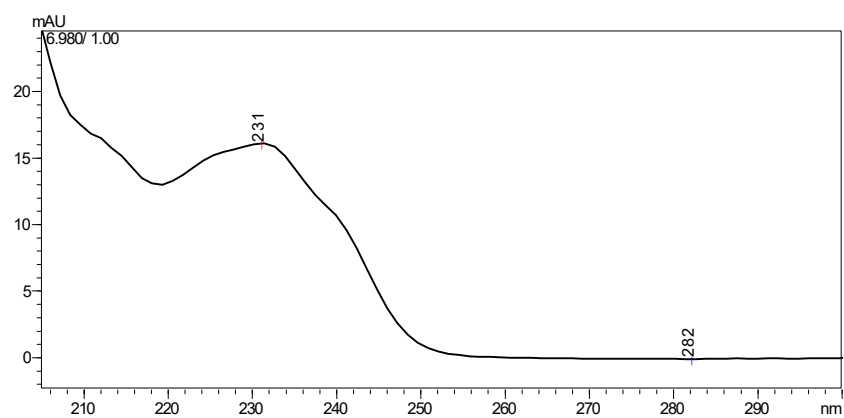
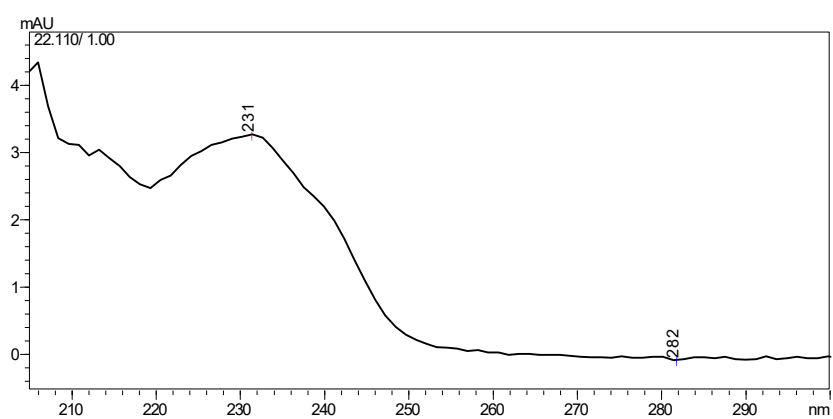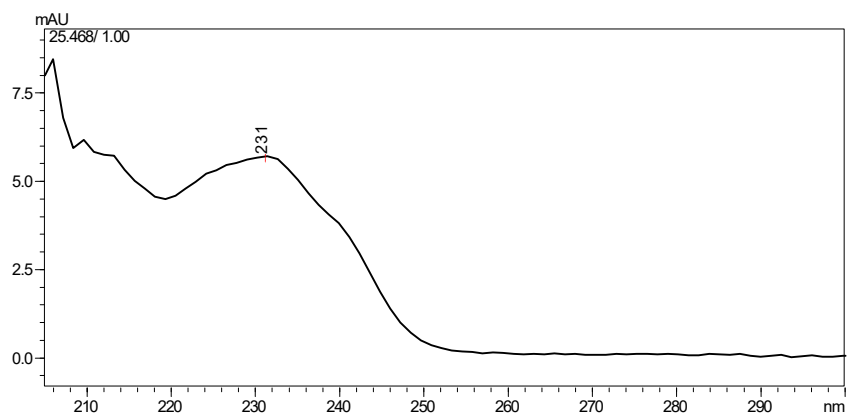Isomer of midecamycin A$_1$
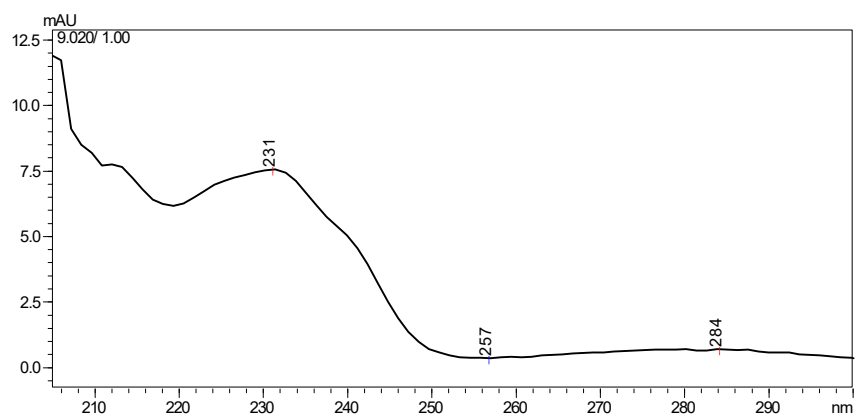
Meleumycin B$_2$
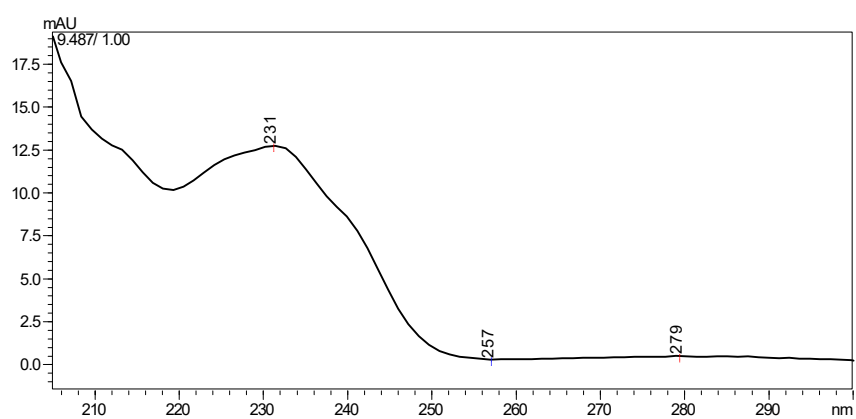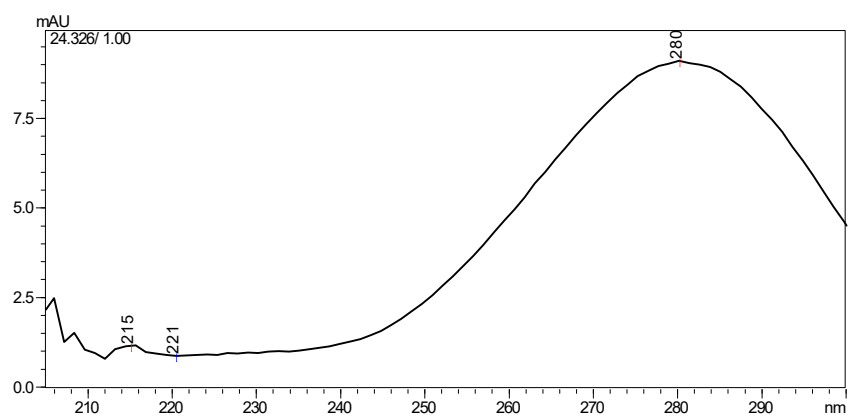

6-hydroxyethyl-midecamycin A
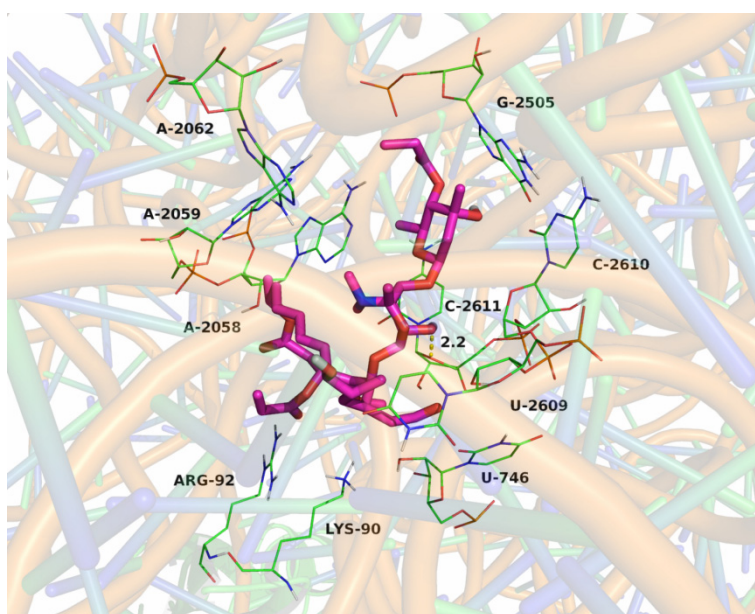

Leucomycin A$_6$

Midecamycin A₁
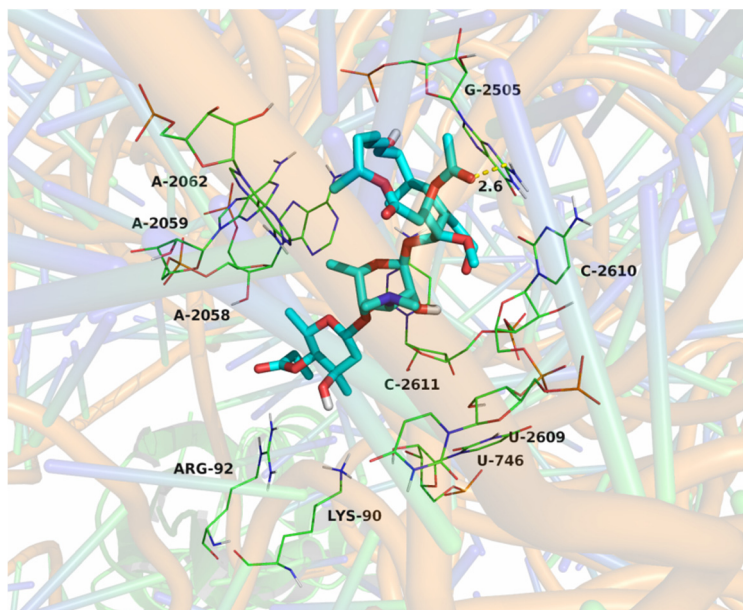

X

Midecamycin A₂

Midecamycin A$_3$

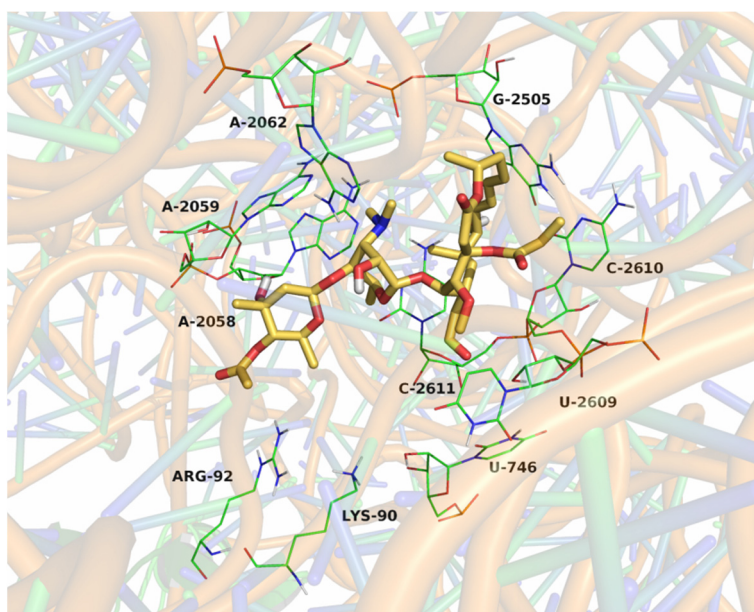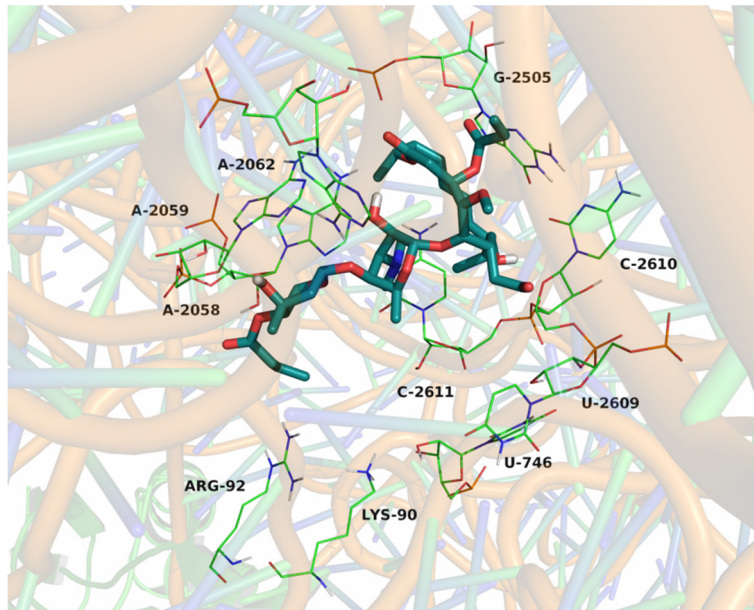# Figure S2. The UV spectrums of the impurities

Detailed analysis of the binding mode between the midecamycin A$_1$ and the ribosome.
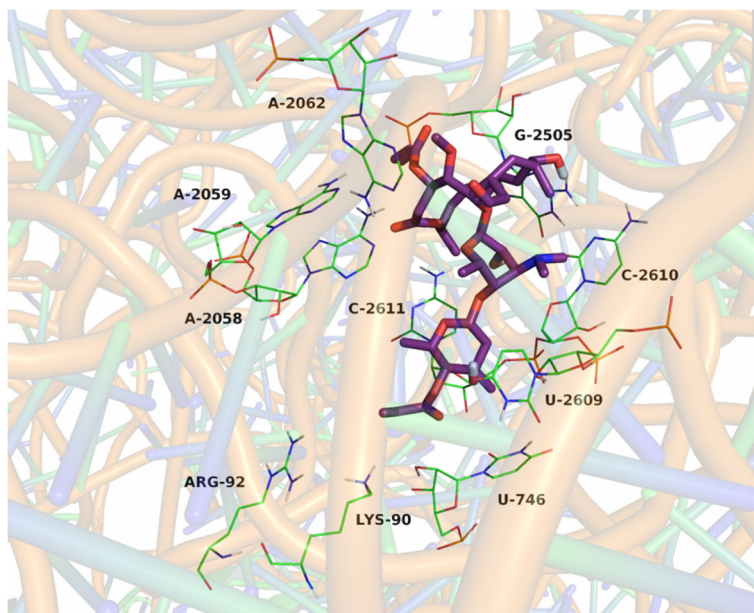
Detailed analysis of the binding mode between the Leucomycin A6 and the ribosome.

Detailed analysis of the binding mode between the Midecamycin B2 and the ribosome.
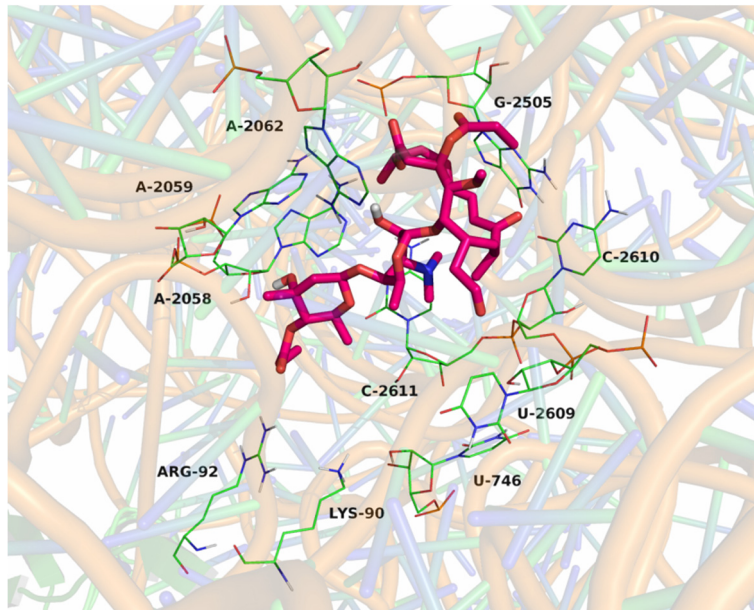
Detailed analysis of the binding mode between the Midecamycin A2 and the ribosome.
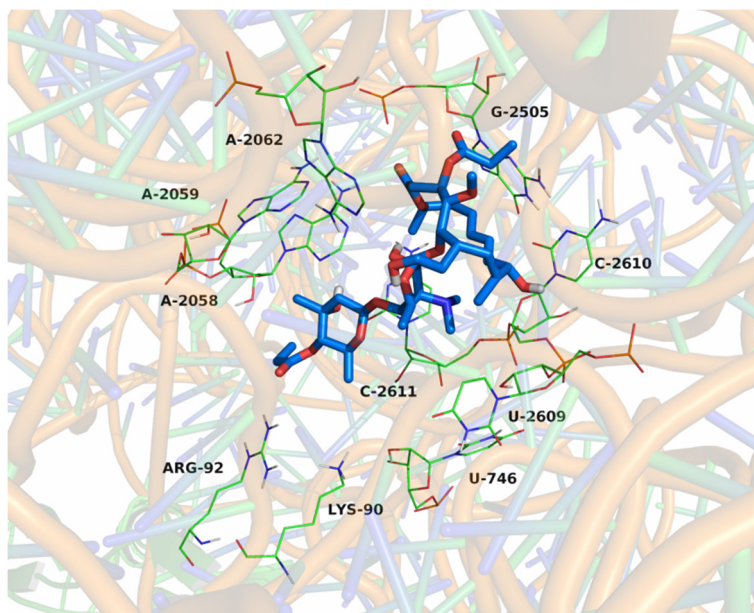

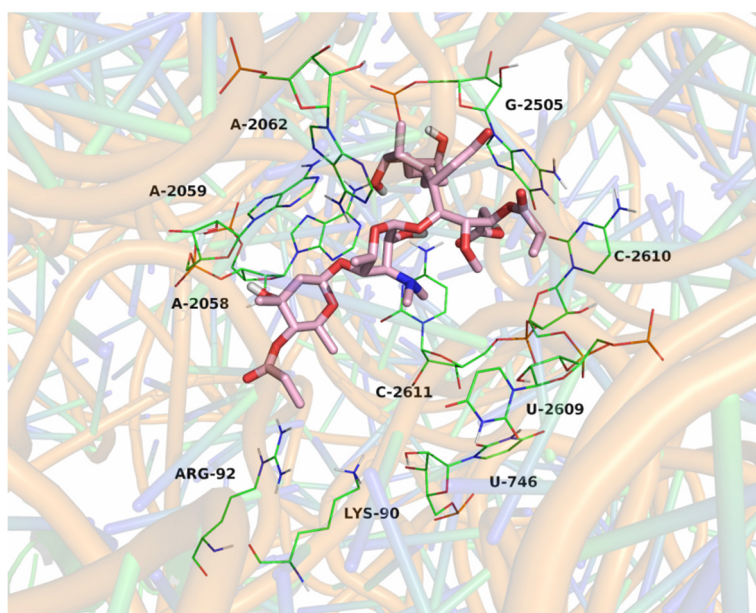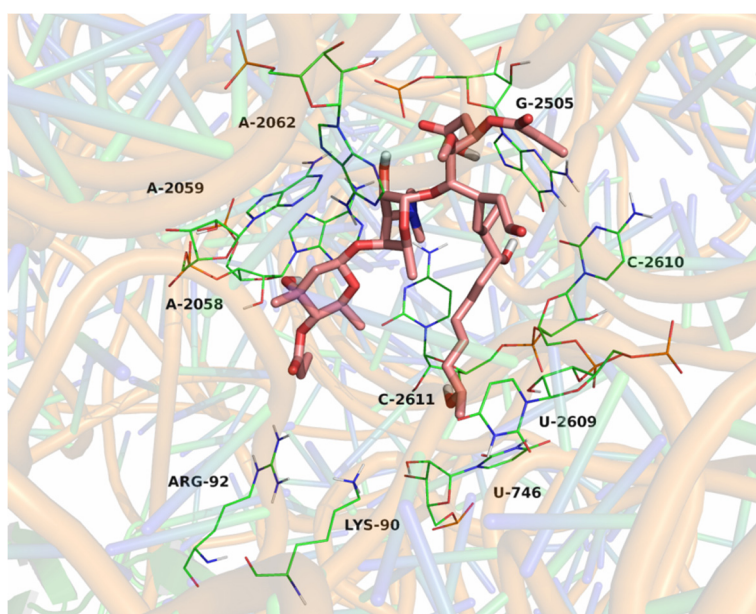Detailed analysis of the binding mode between the X and the ribosome.

Detailed analysis of the binding mode between the midecamycin A₃ and the ribosome.

Detailed analysis of the binding mode between the 6-Hydroxyeyhyl-Midecamycin A1

and the ribosome.

Detailed analysis of the binding mode between the Open loop-Midecamycin and the ribosome.

Detailed analysis of the binding mode between the Midecamycin D and the ribosome.

Figure S3. The 3D docking images of the components (or impurities)