

Drug Screening & Validation

Virtual high-throughput screening (vHTS)

Step 1. Selection of two molecular libraries of 1000 compounds each (both for OXCT1 and ACAT1) from a small molecular screening collection of 30,000 compounds

Step 2. Further analysis of predicted binding affinity and visual inspection
Compounds performing well in all analysis steps were selected for assay



Phenotypic drug screening

ATP-depletion assays
on human breast cancer cells (MCF7)
84 Compounds (OXCT1); 143 compounds (ACAT1)
Observed 8 hits at 20 μ M



Functional validation

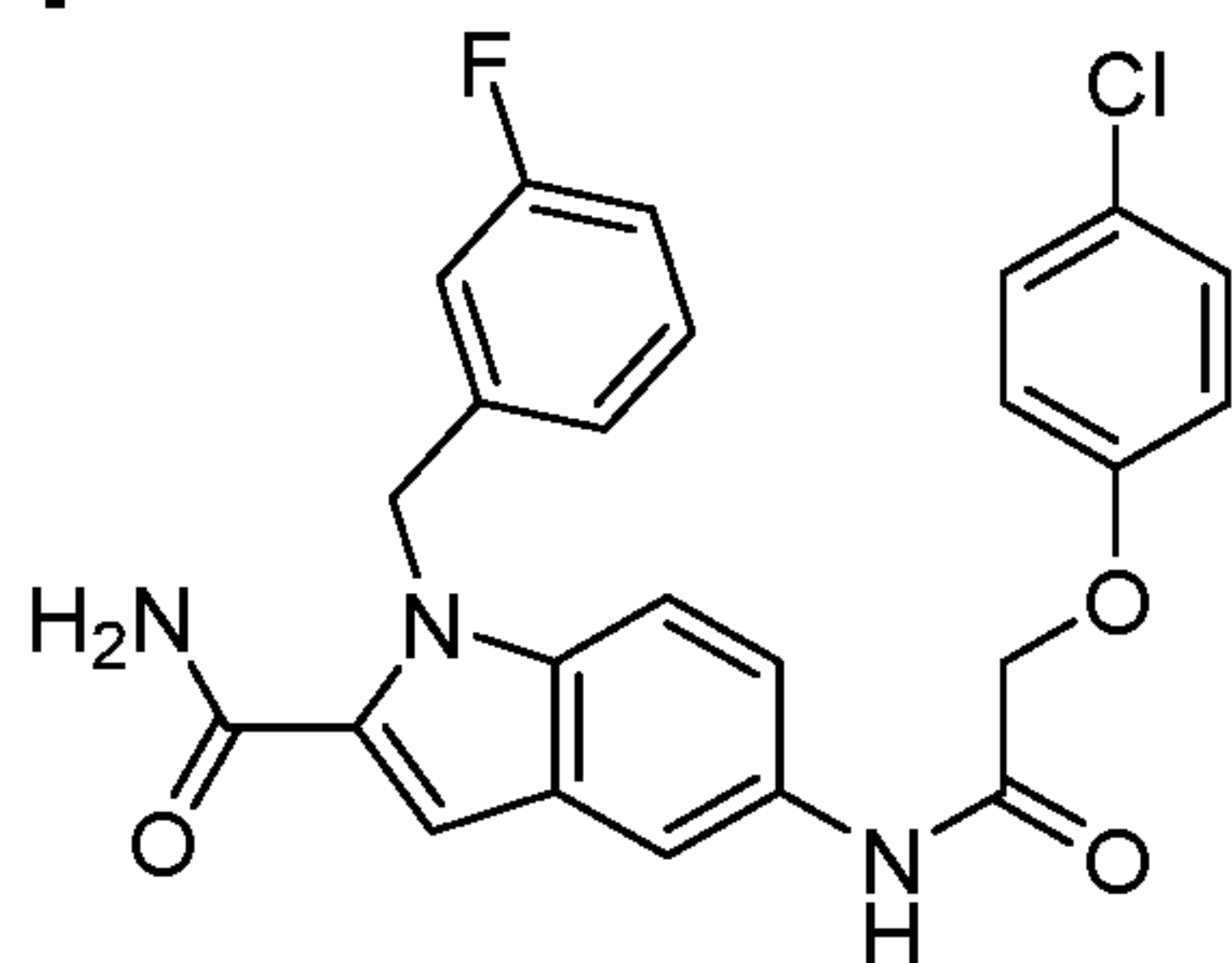
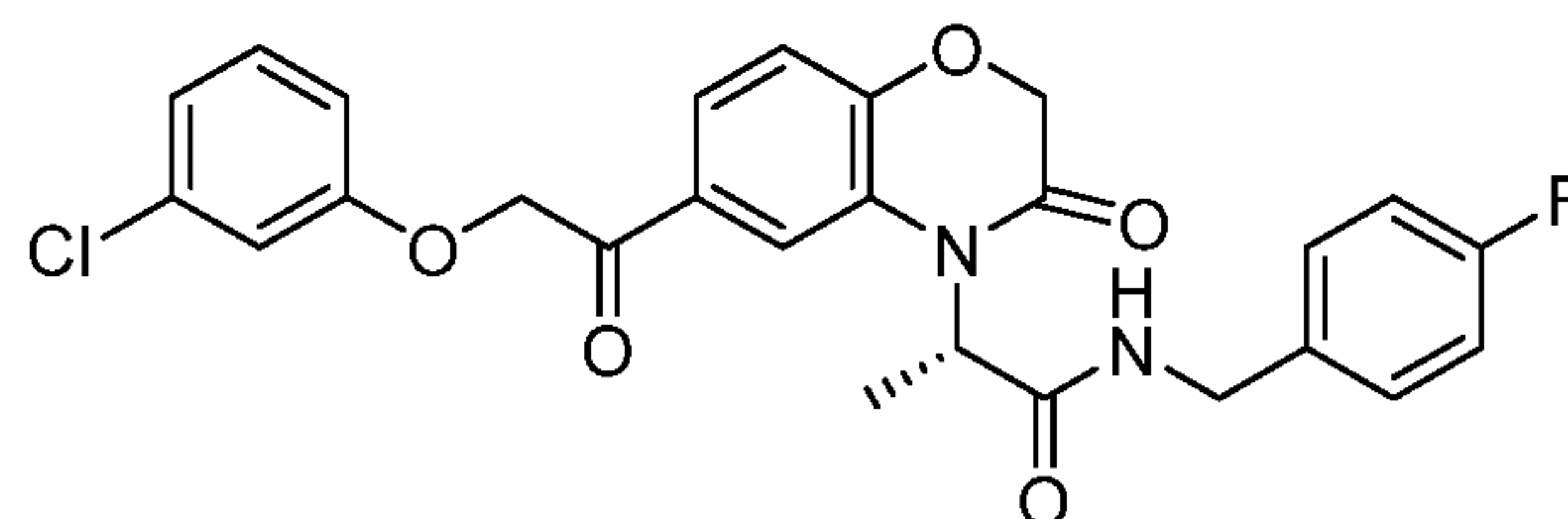
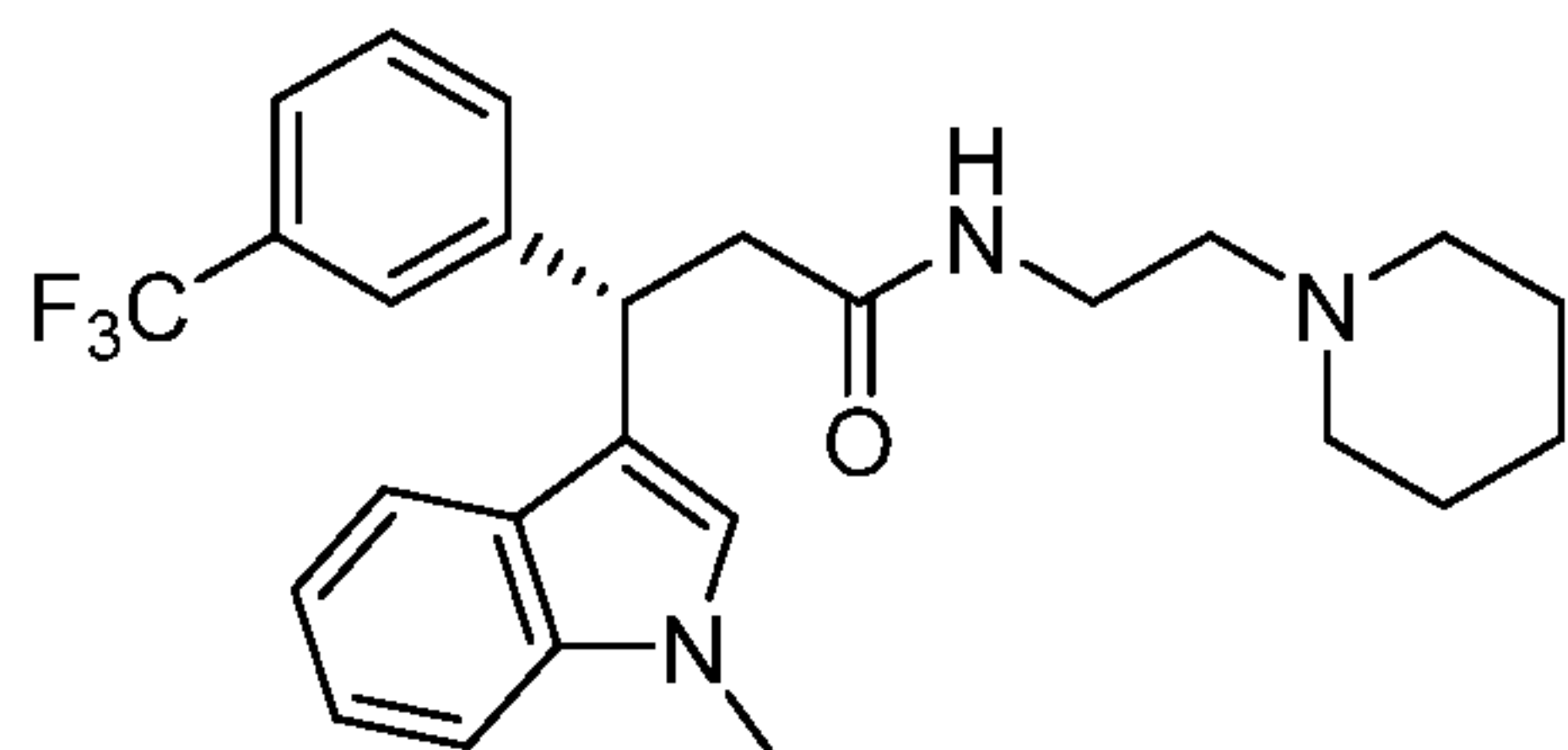
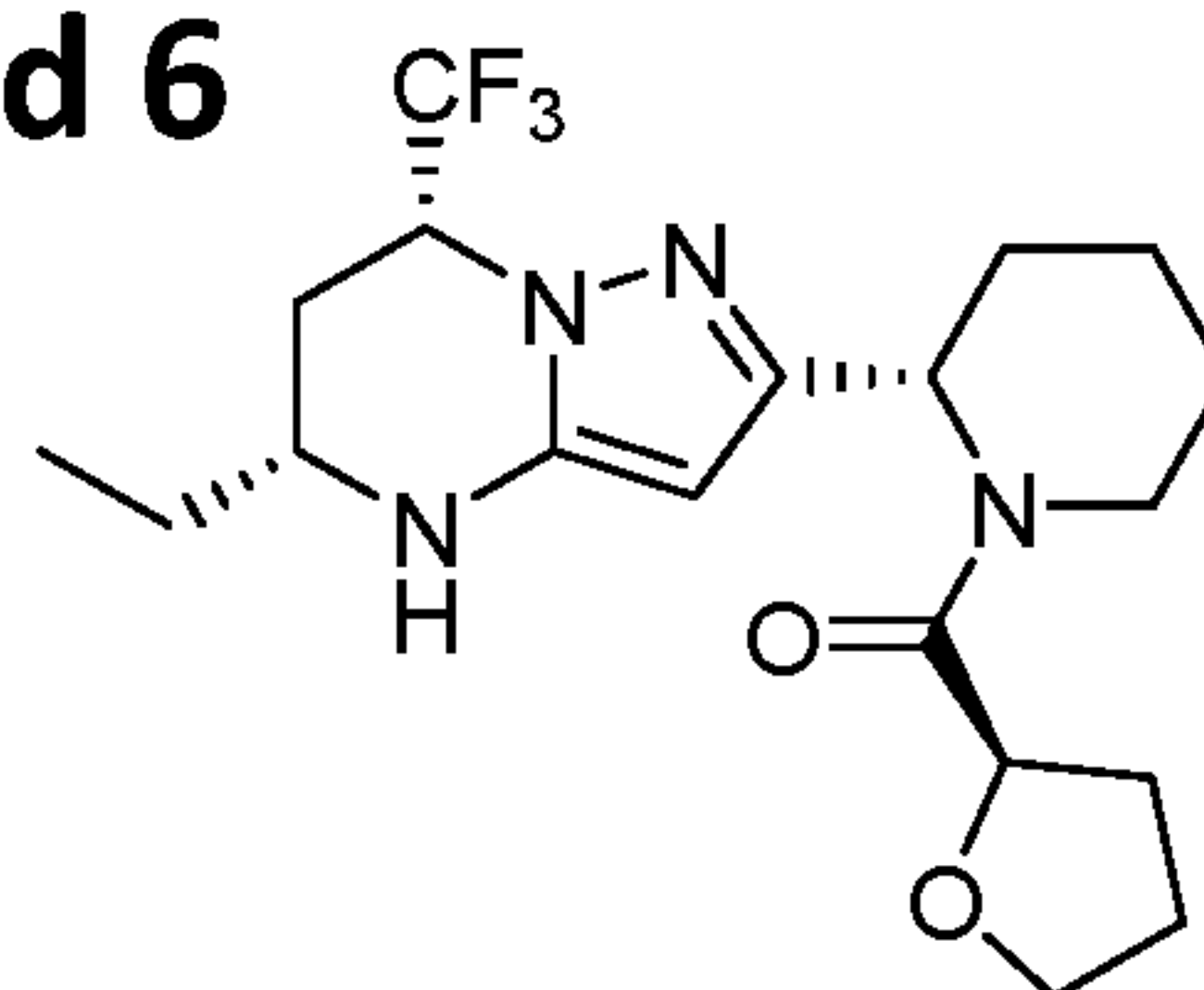
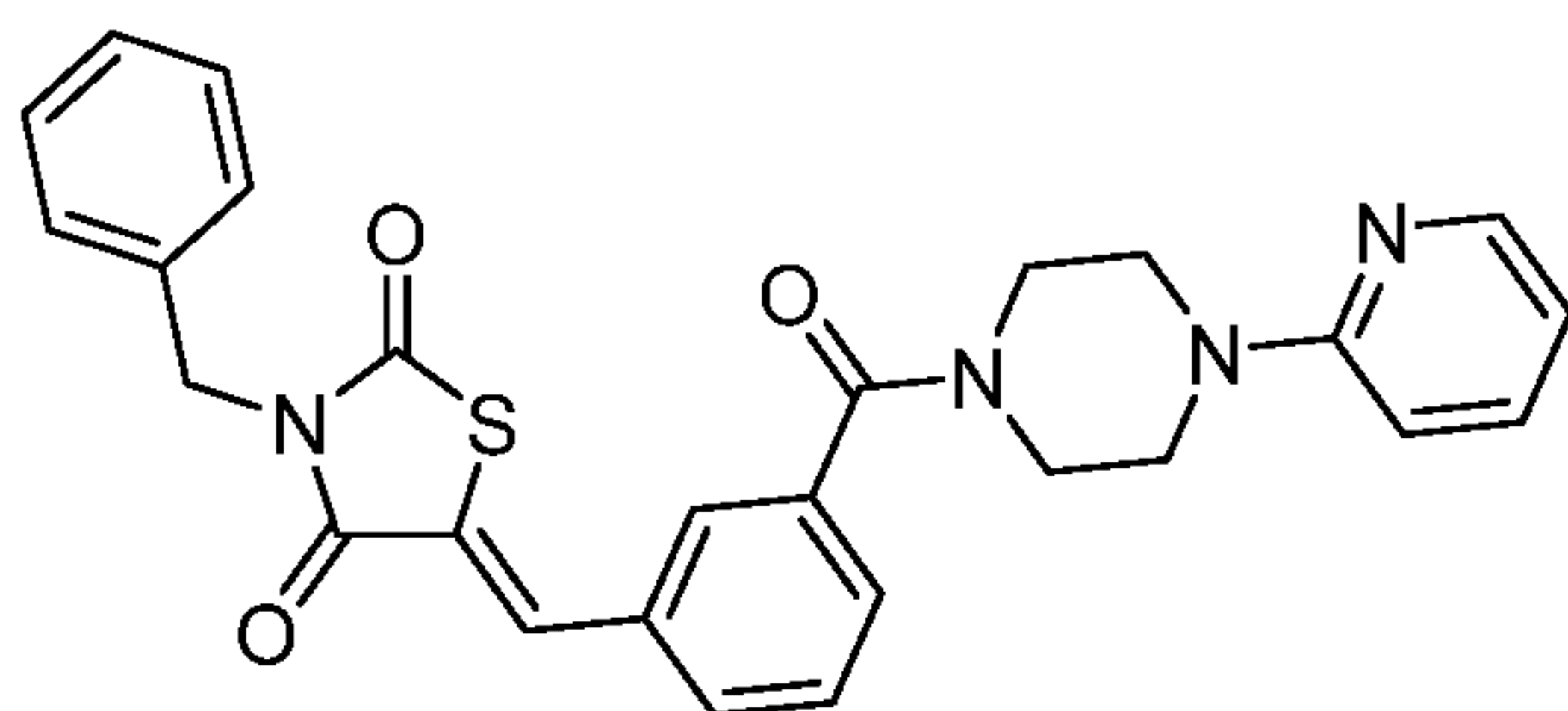
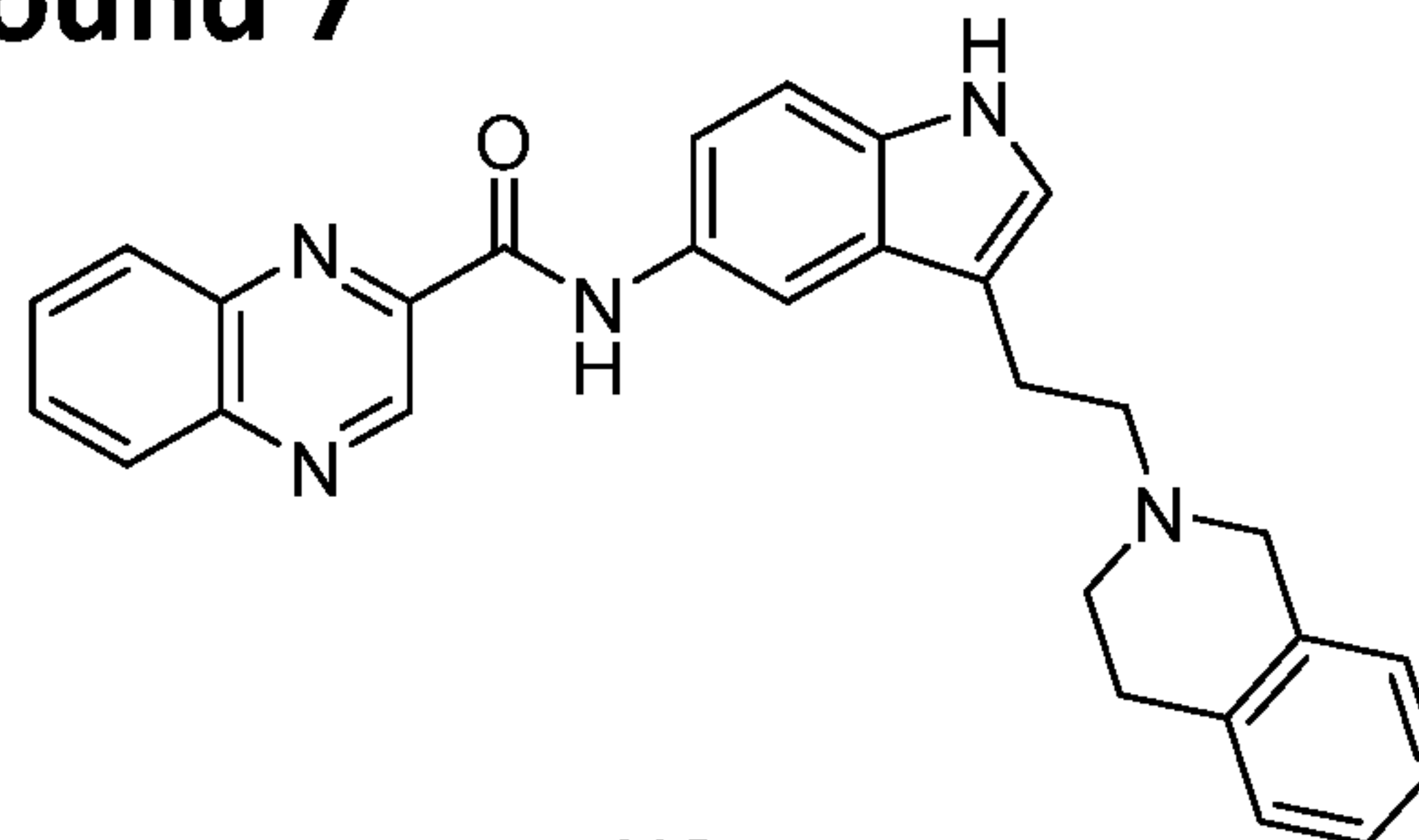
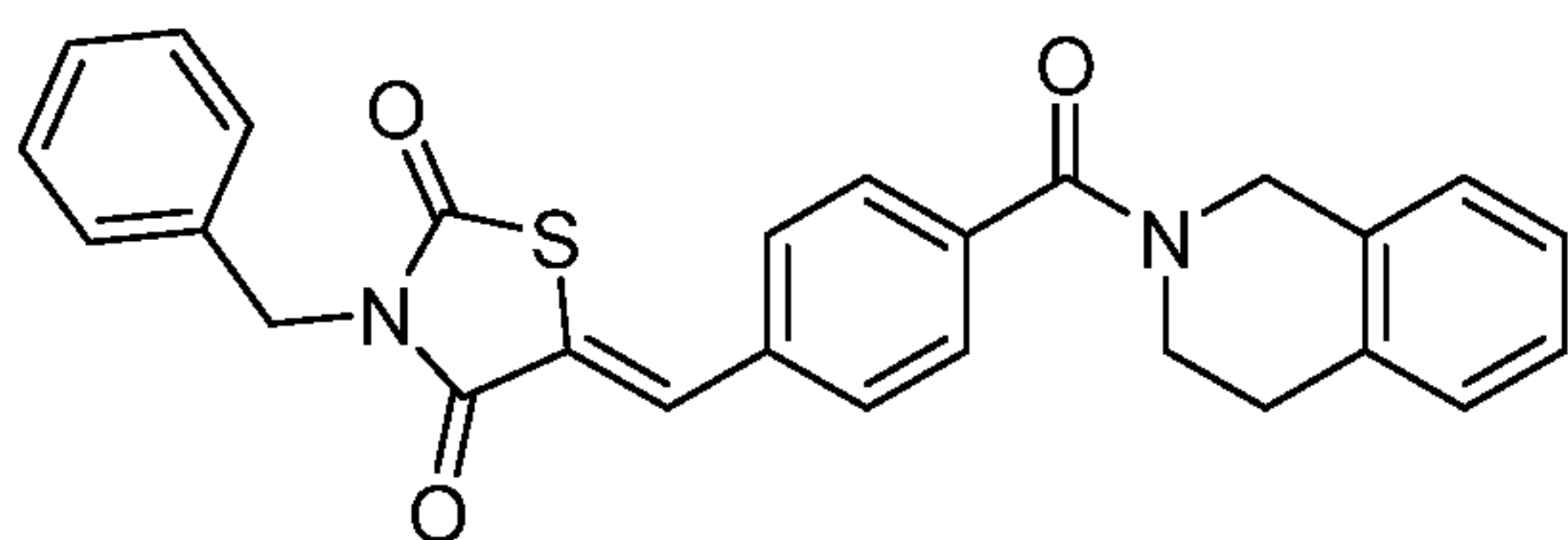
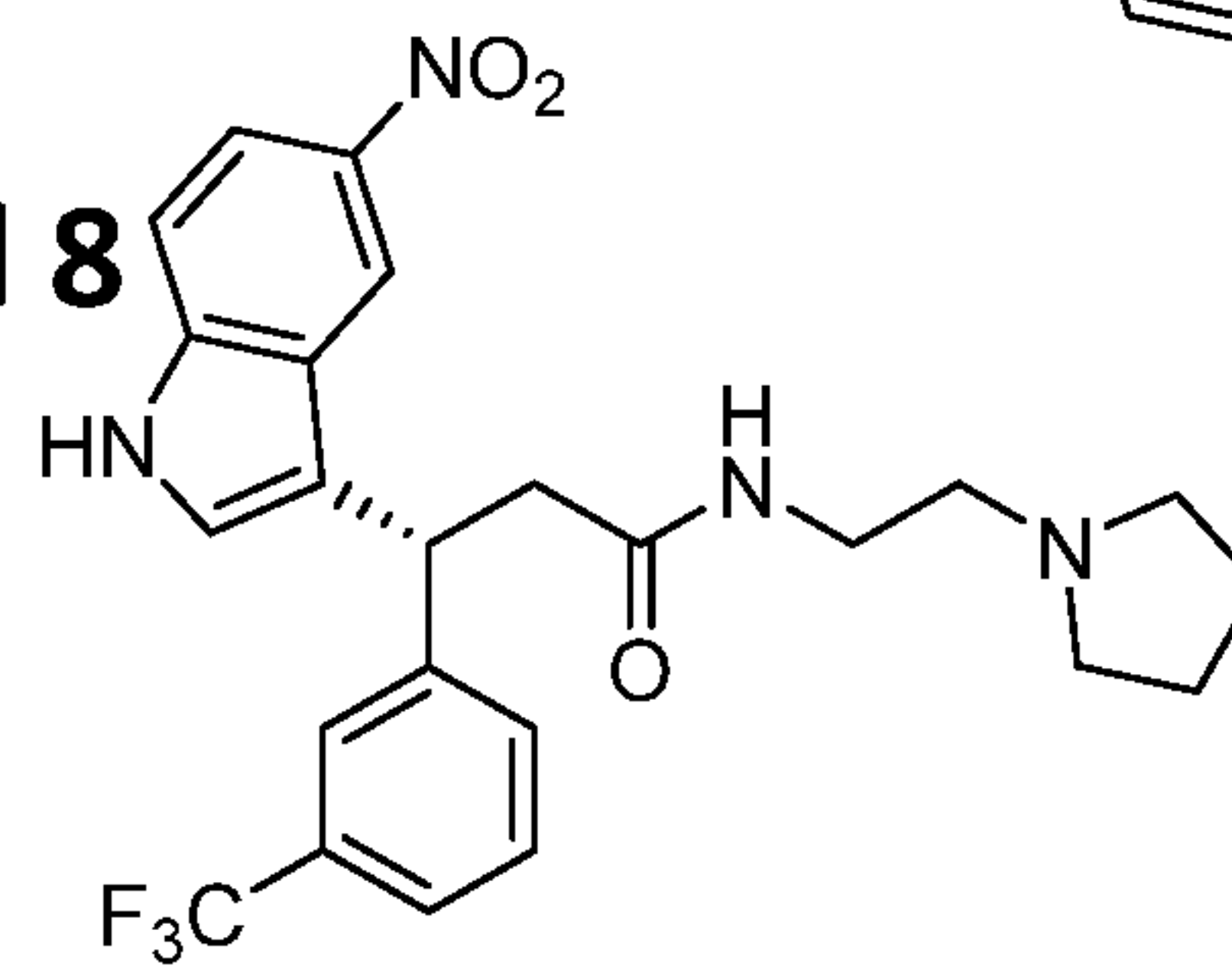
Mammosphere assays
Metabolic Flux analysis
OCR/ECAR



Mitoketoscins

FIG. 1

Mitoketoscins

Compound 1**Compound 5****Compound 2****Compound 6****Compound 3****Compound 7****Compound 4****Compound 8****FIG. 2**

3D-Spheroid CSC Assay (Mammospheres)

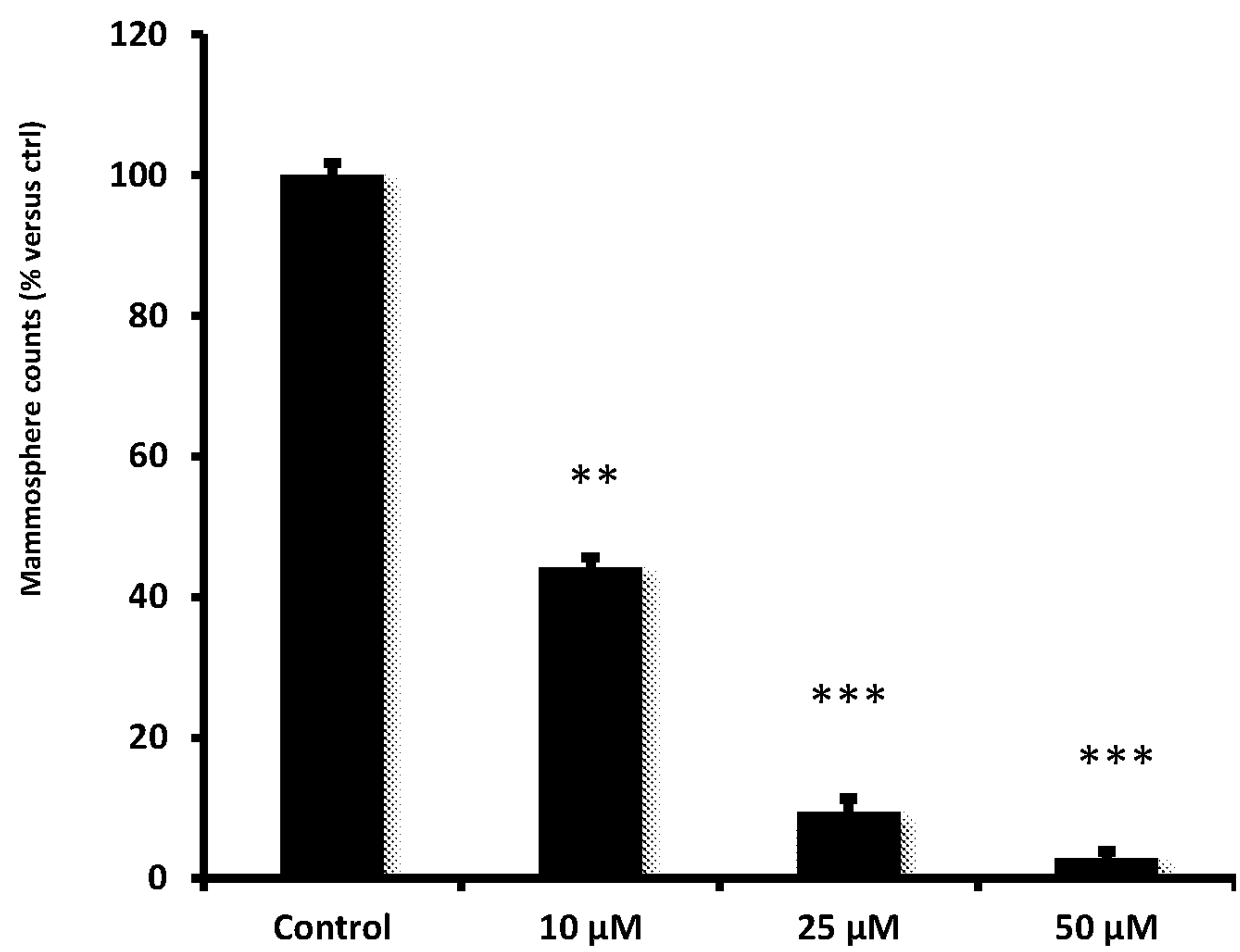


FIG. 3A

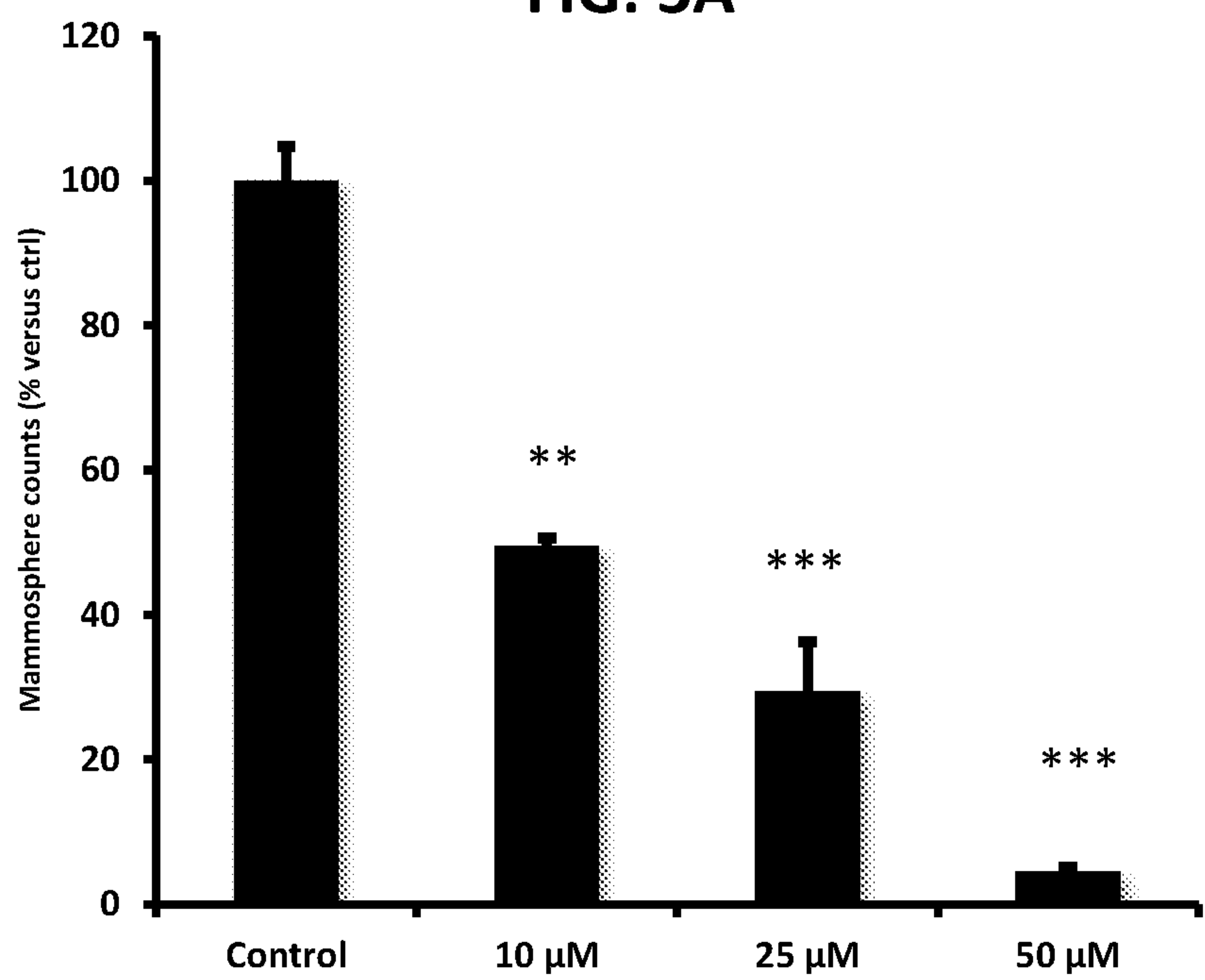


FIG. 3B

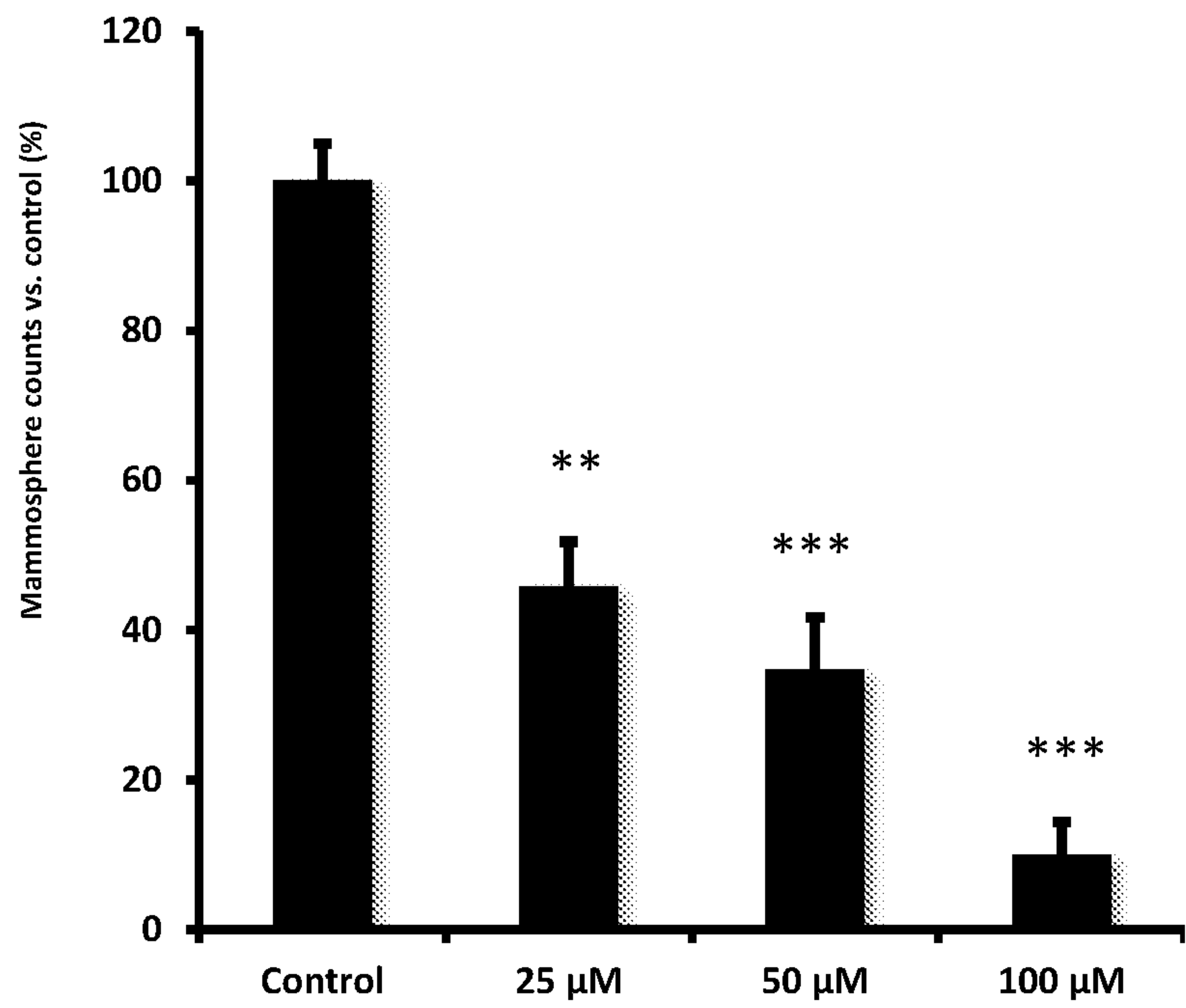


FIG. 3C

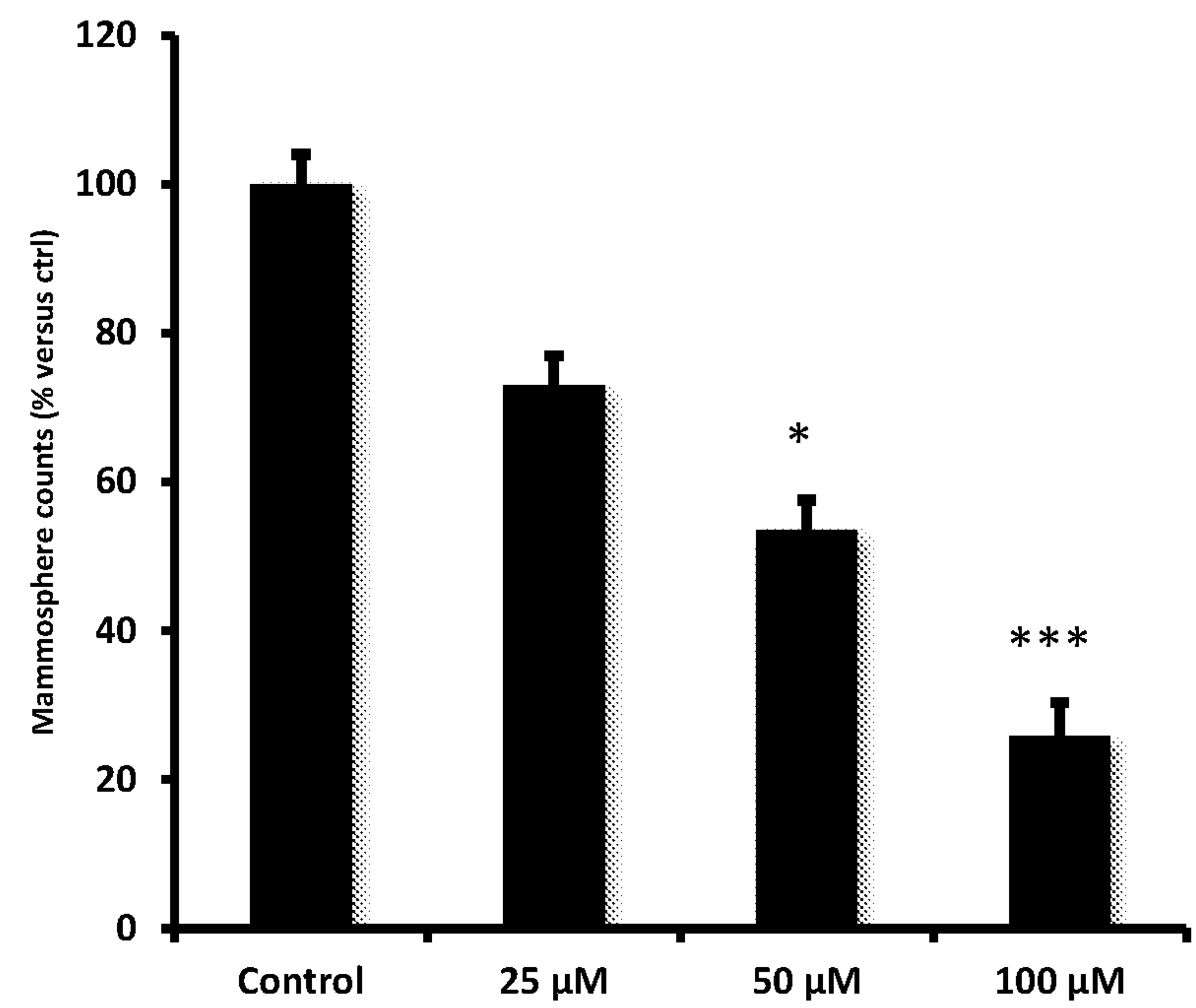


FIG. 3D

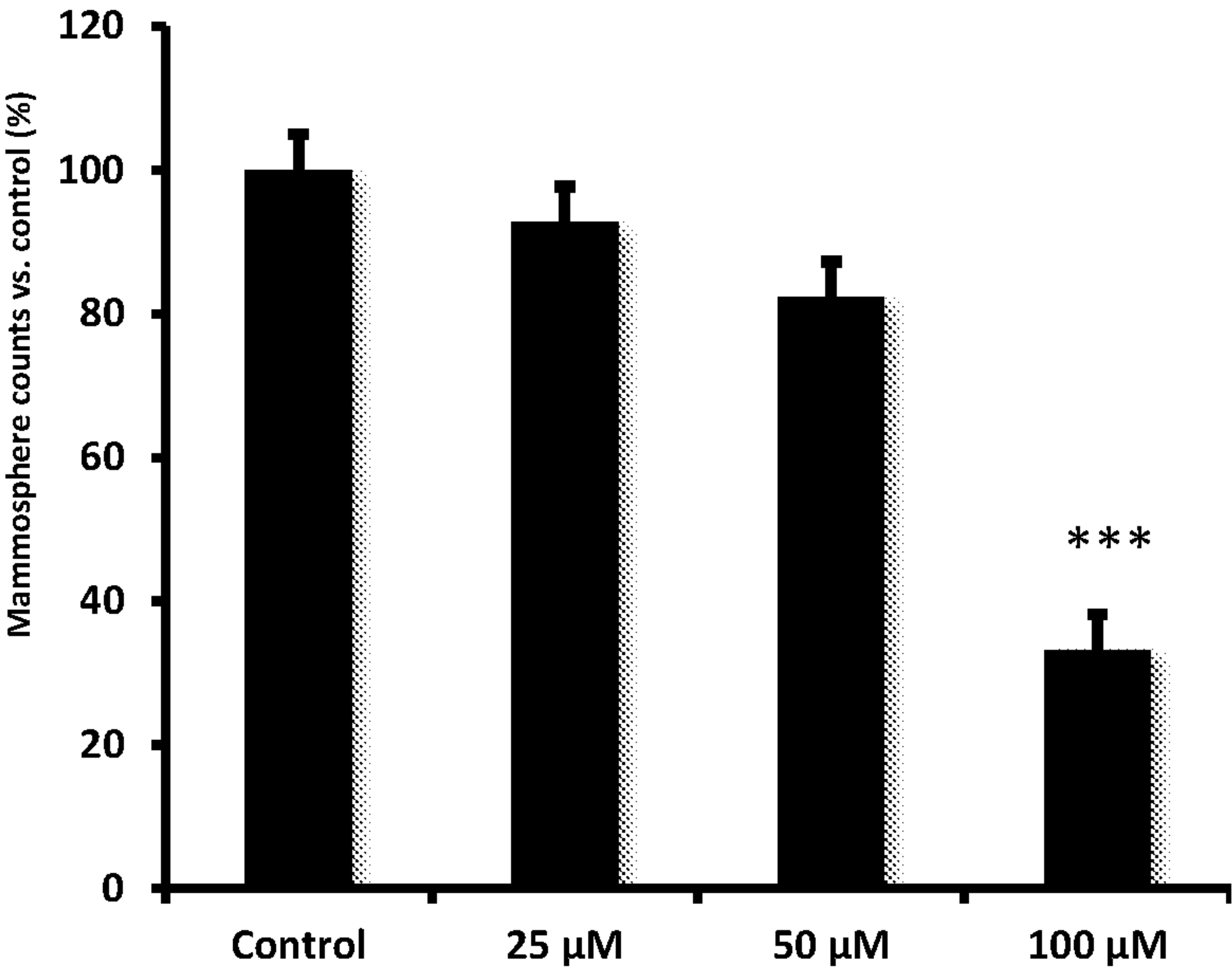


FIG. 3E

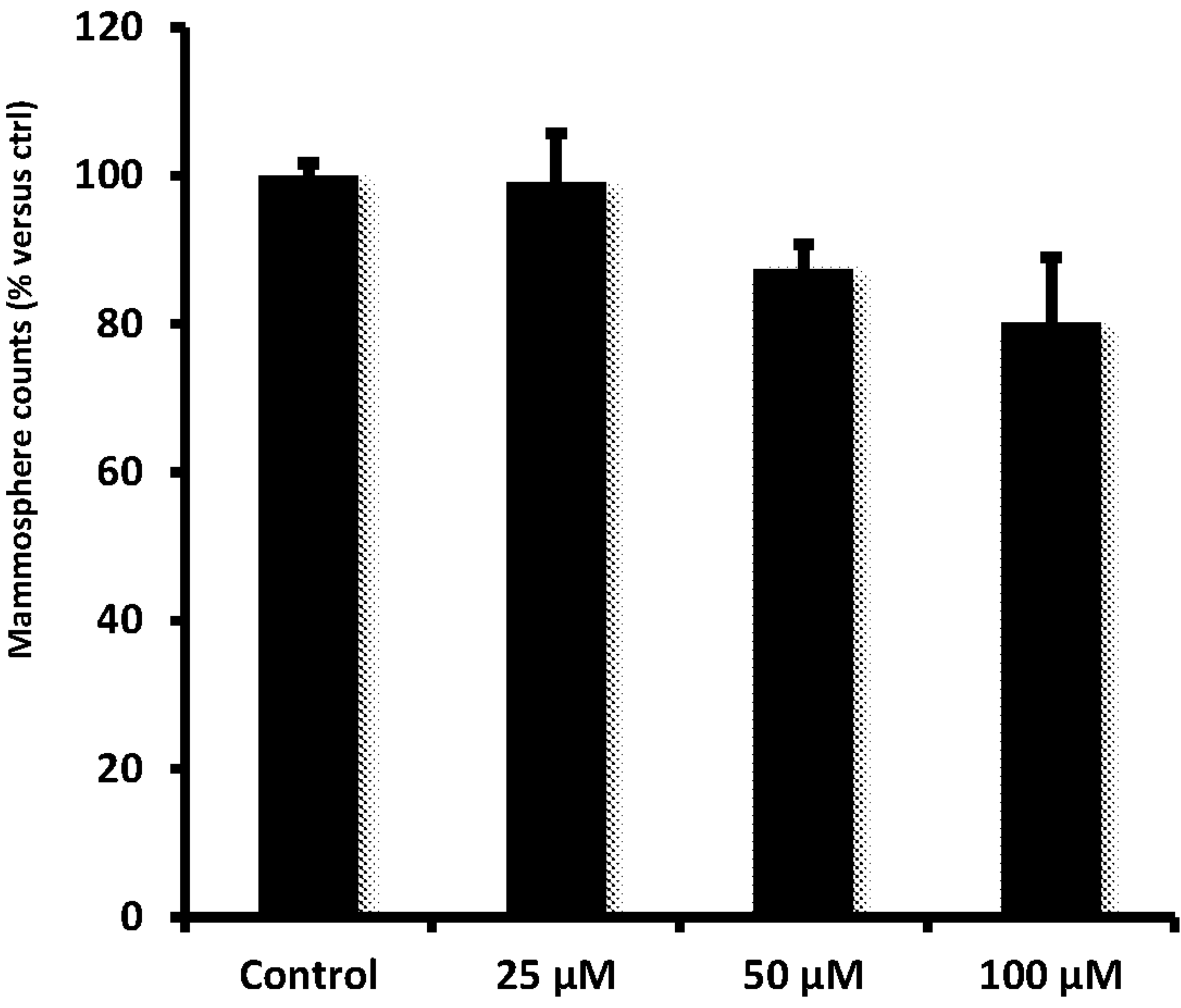


FIG. 3F

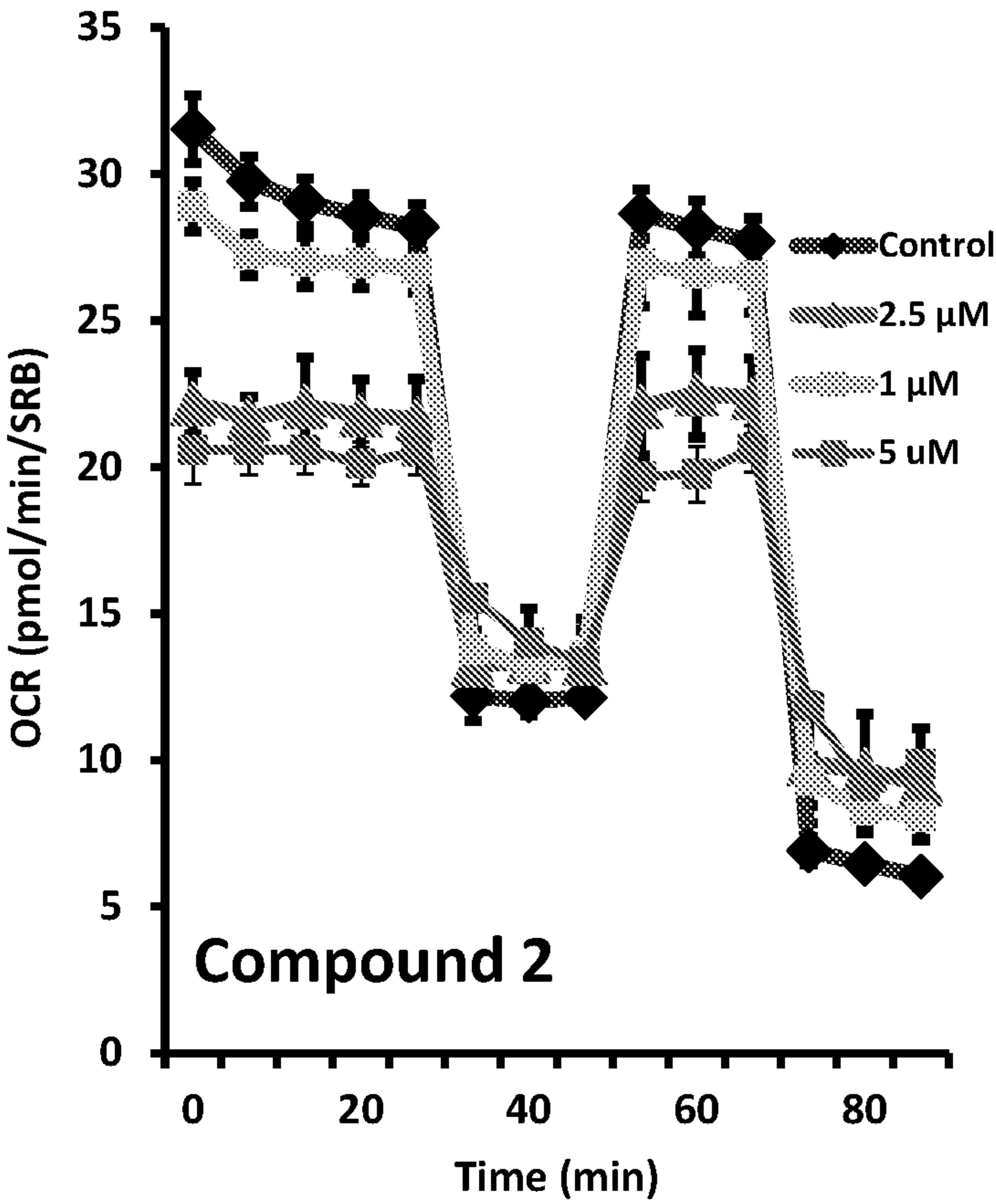


FIG. 4A

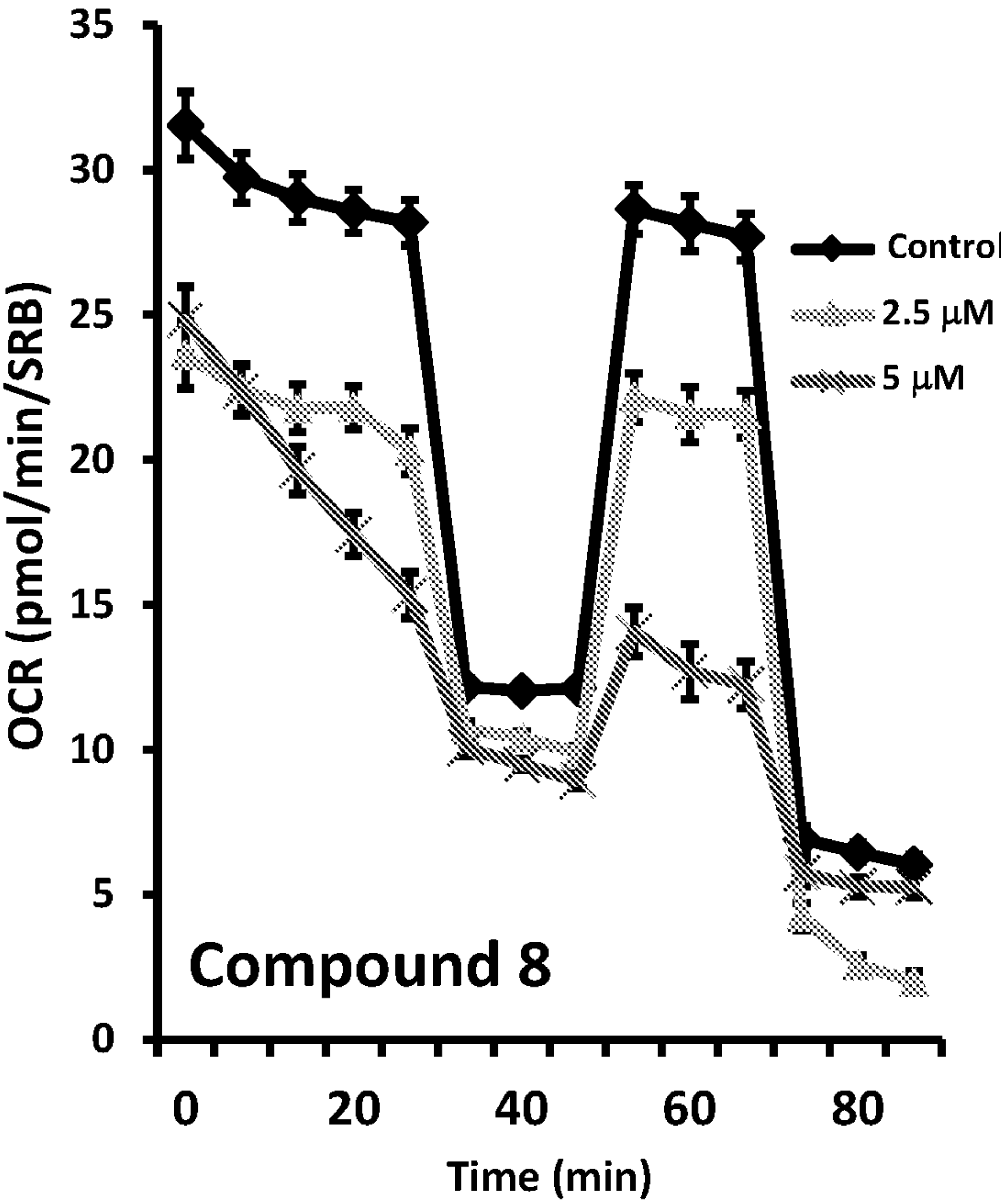


FIG. 4B

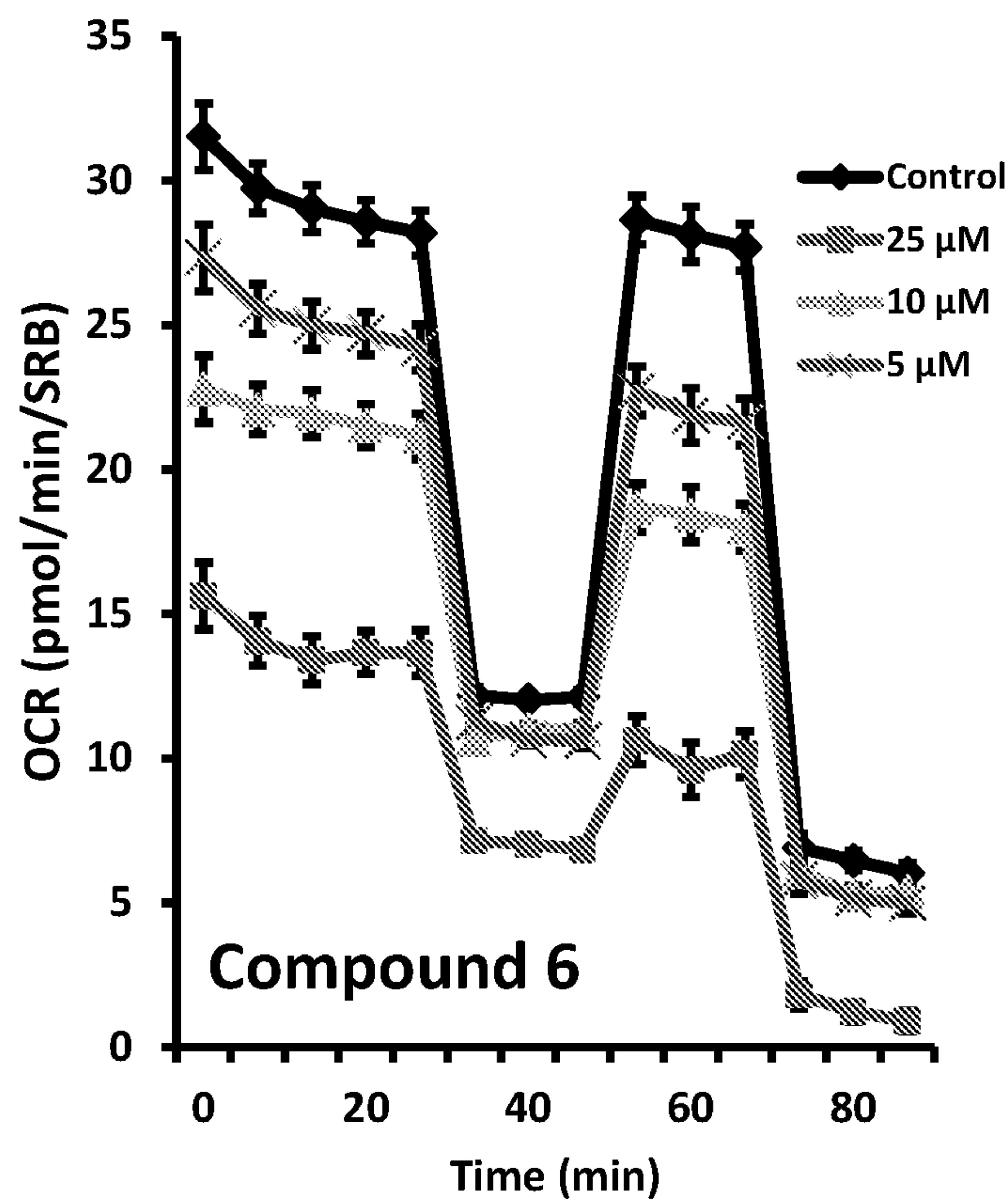


FIG. 4C

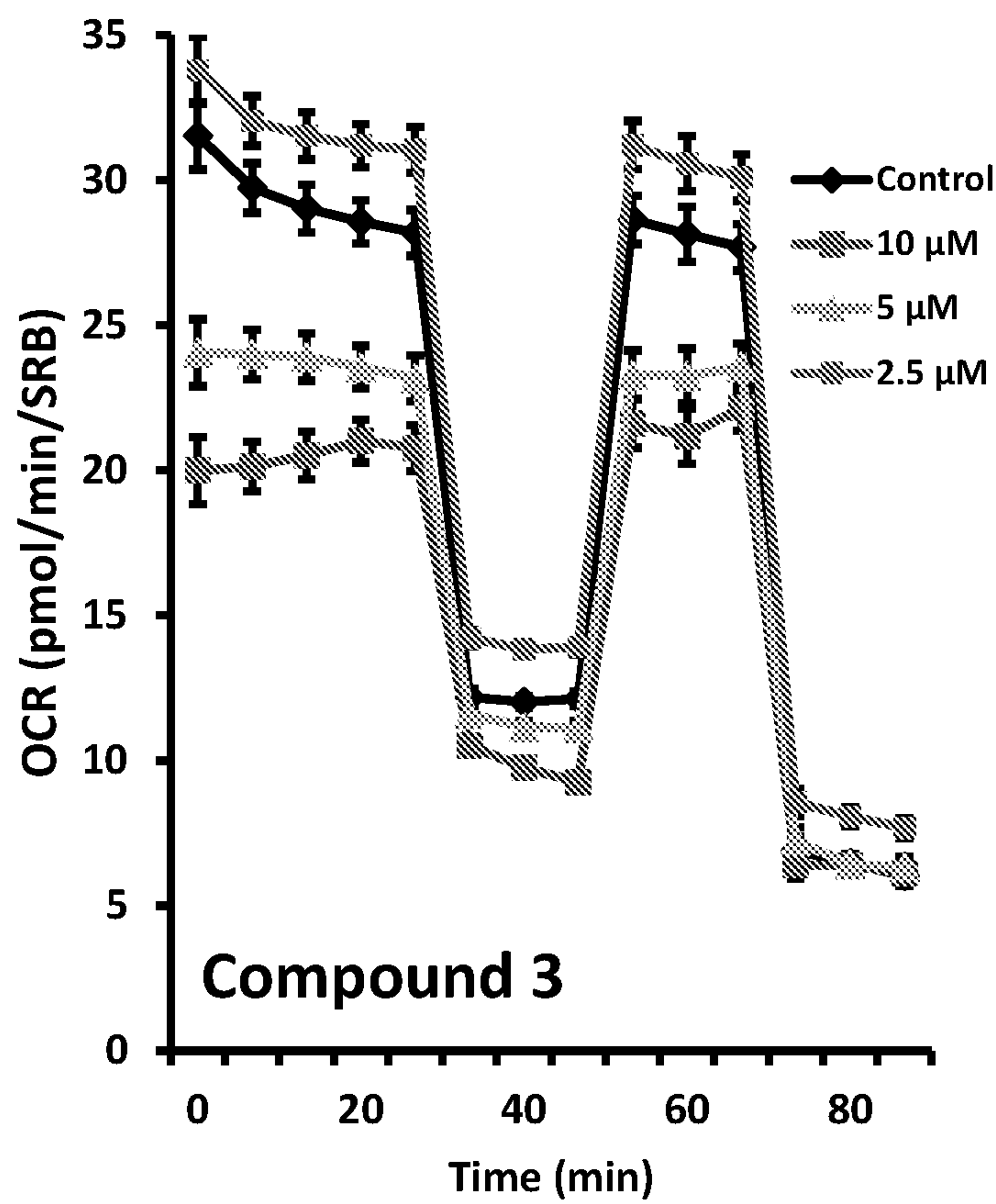
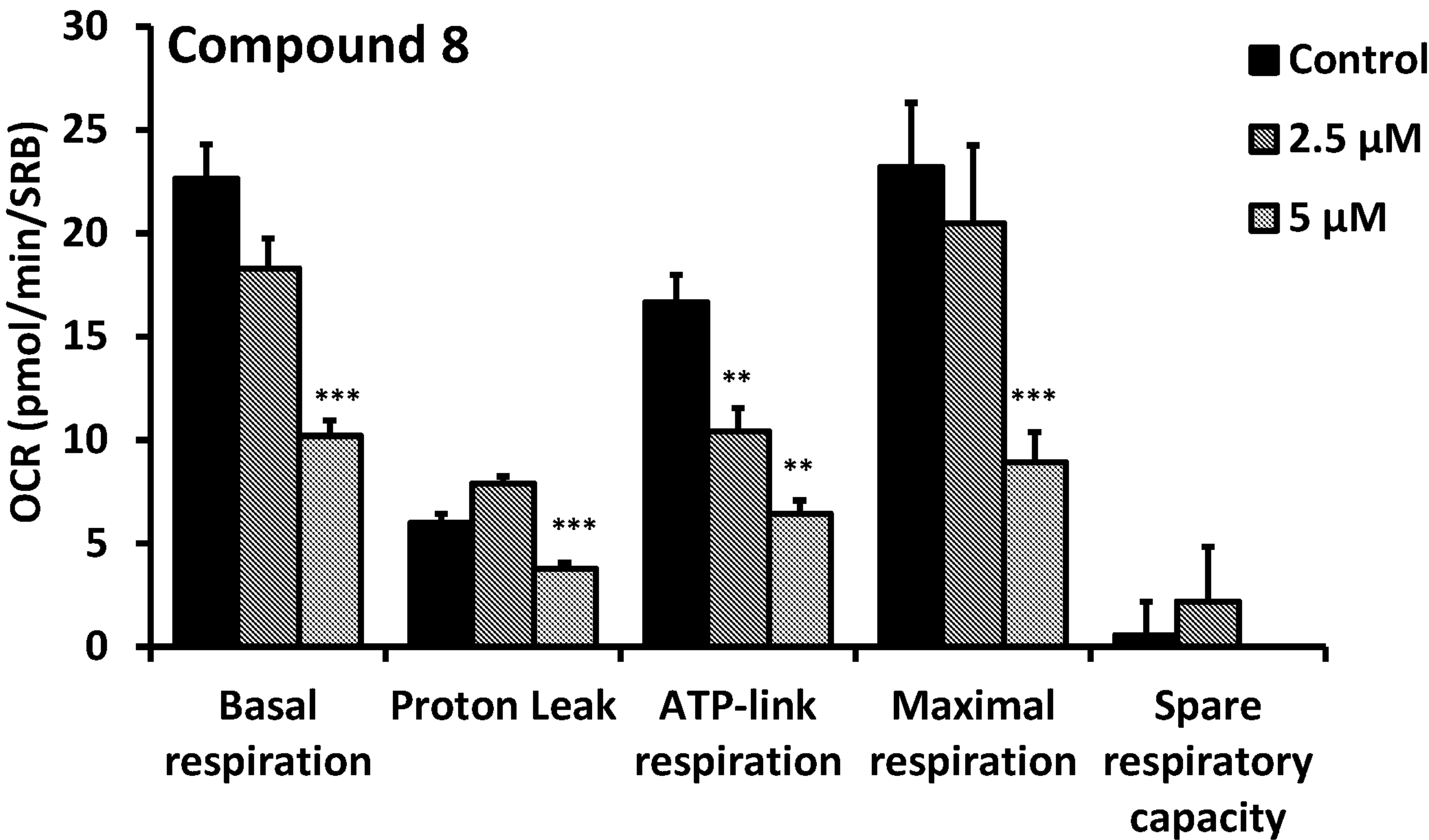
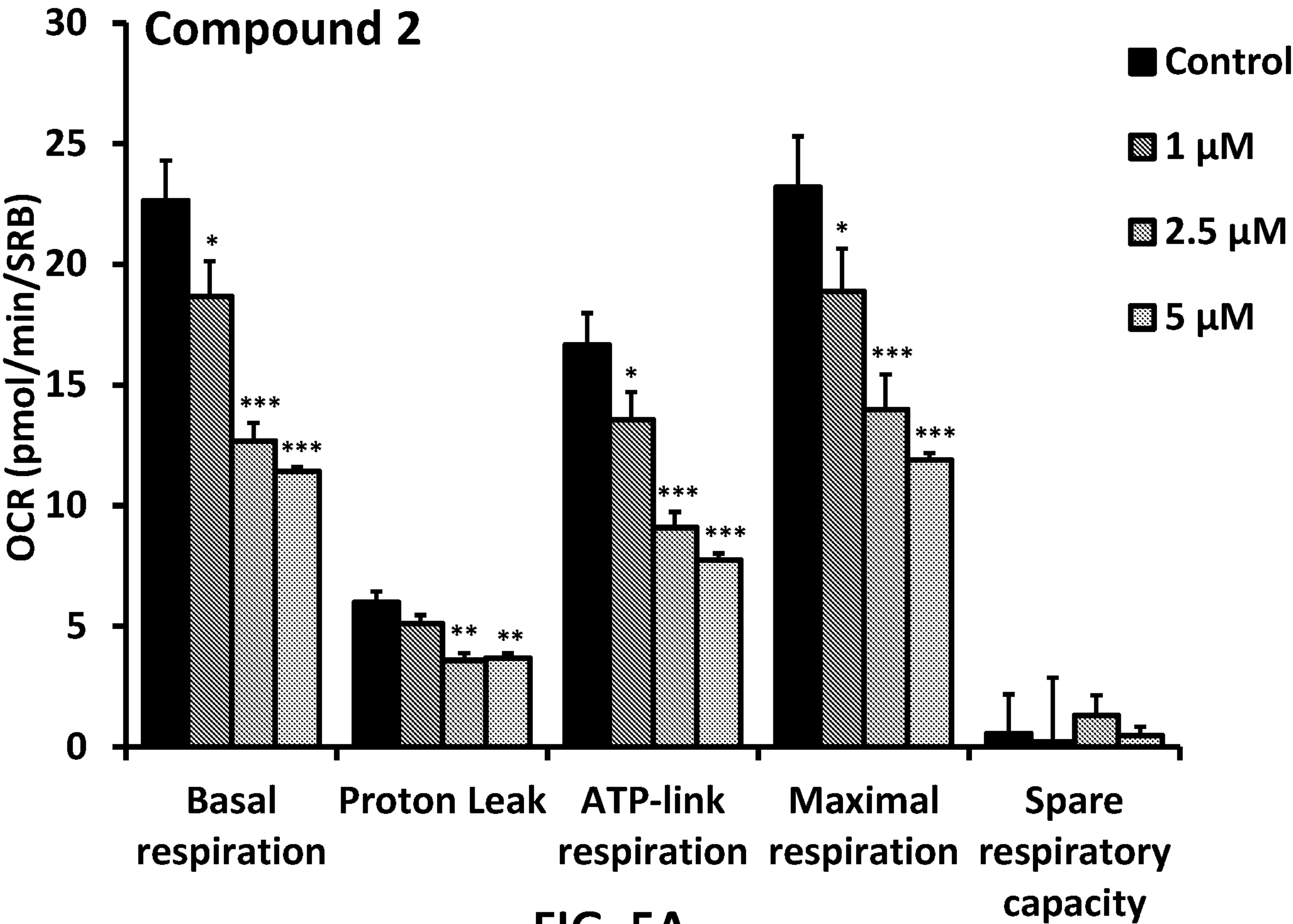


FIG. 4D



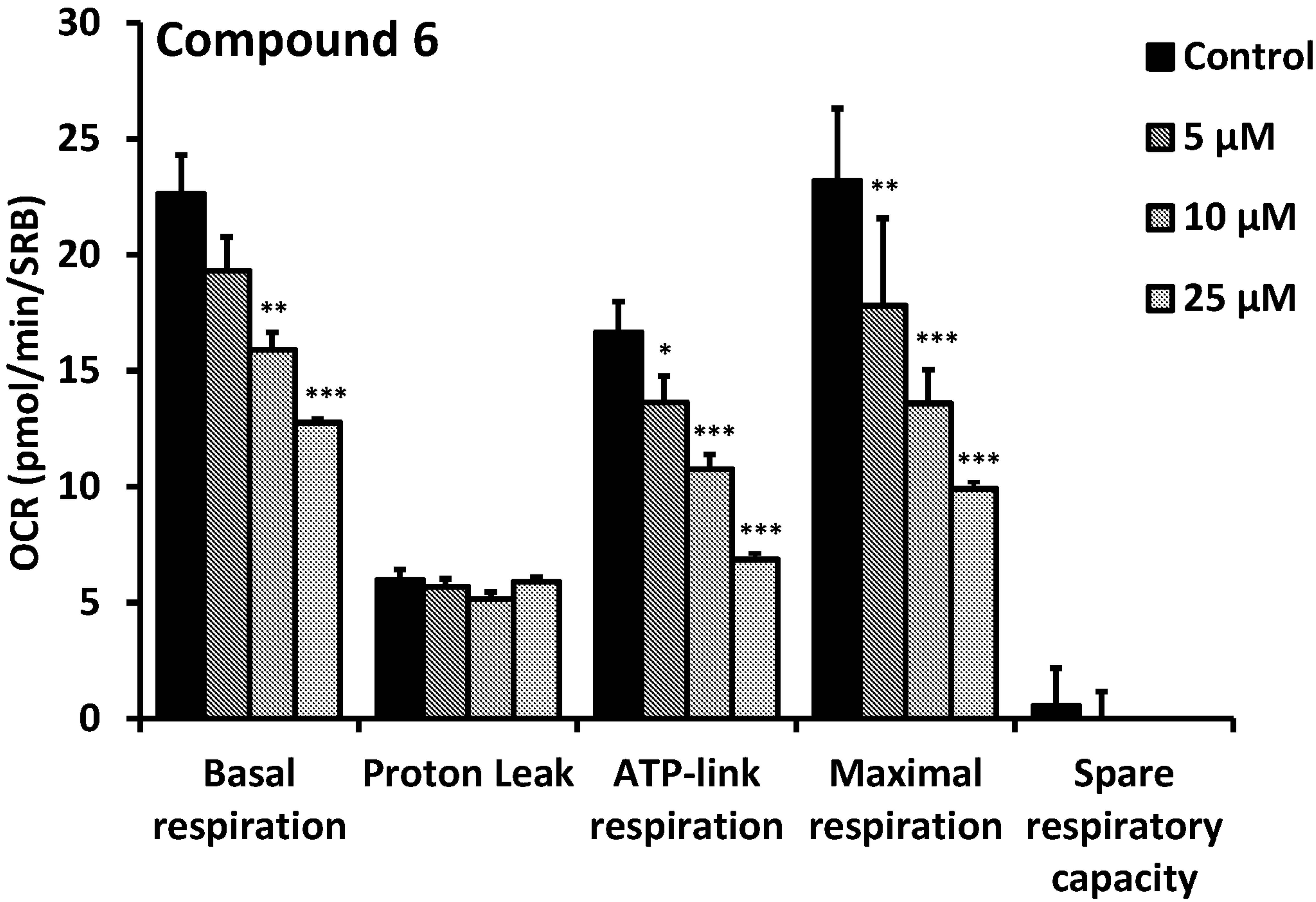


FIG. 6A

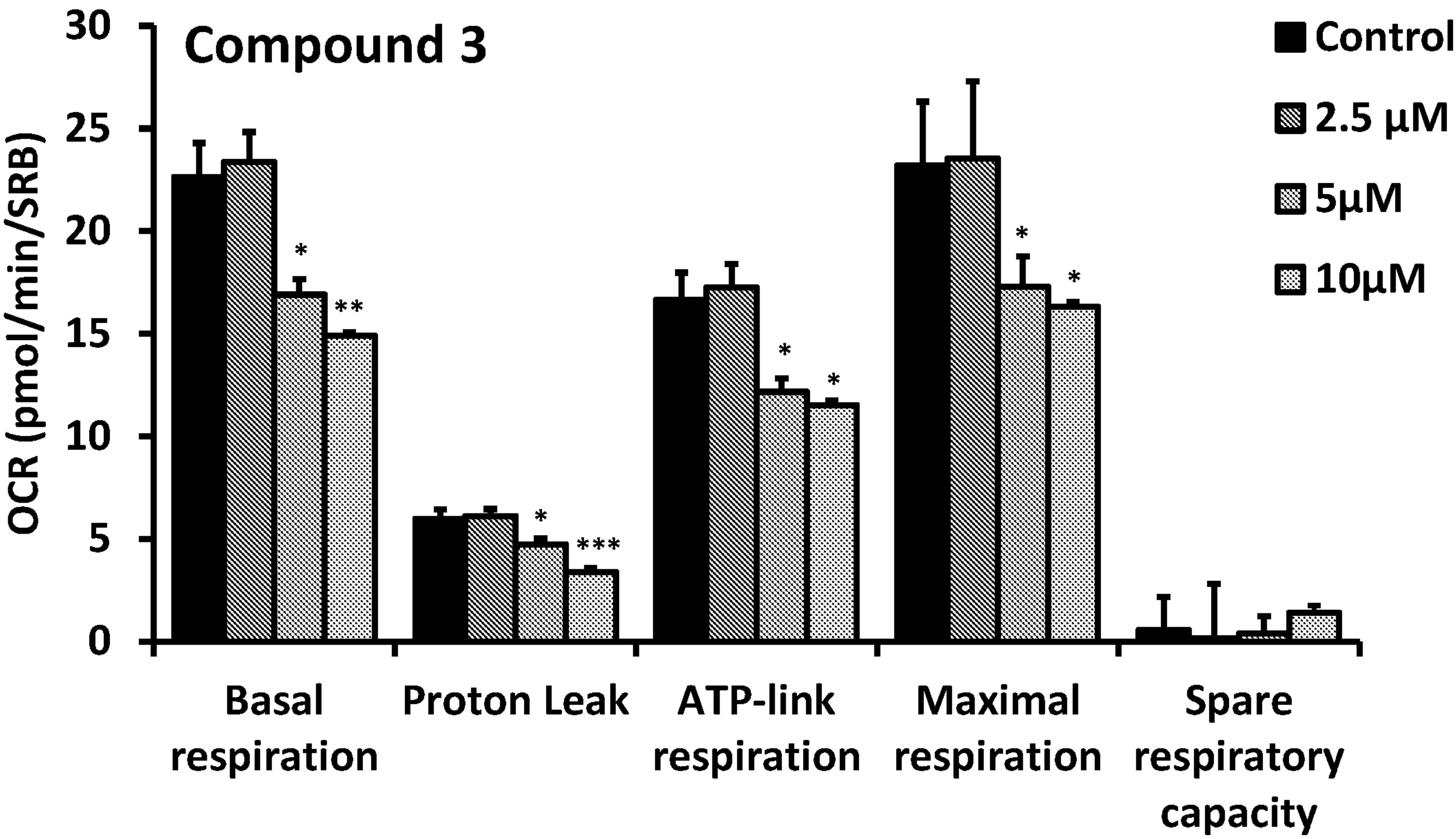


FIG. 6B

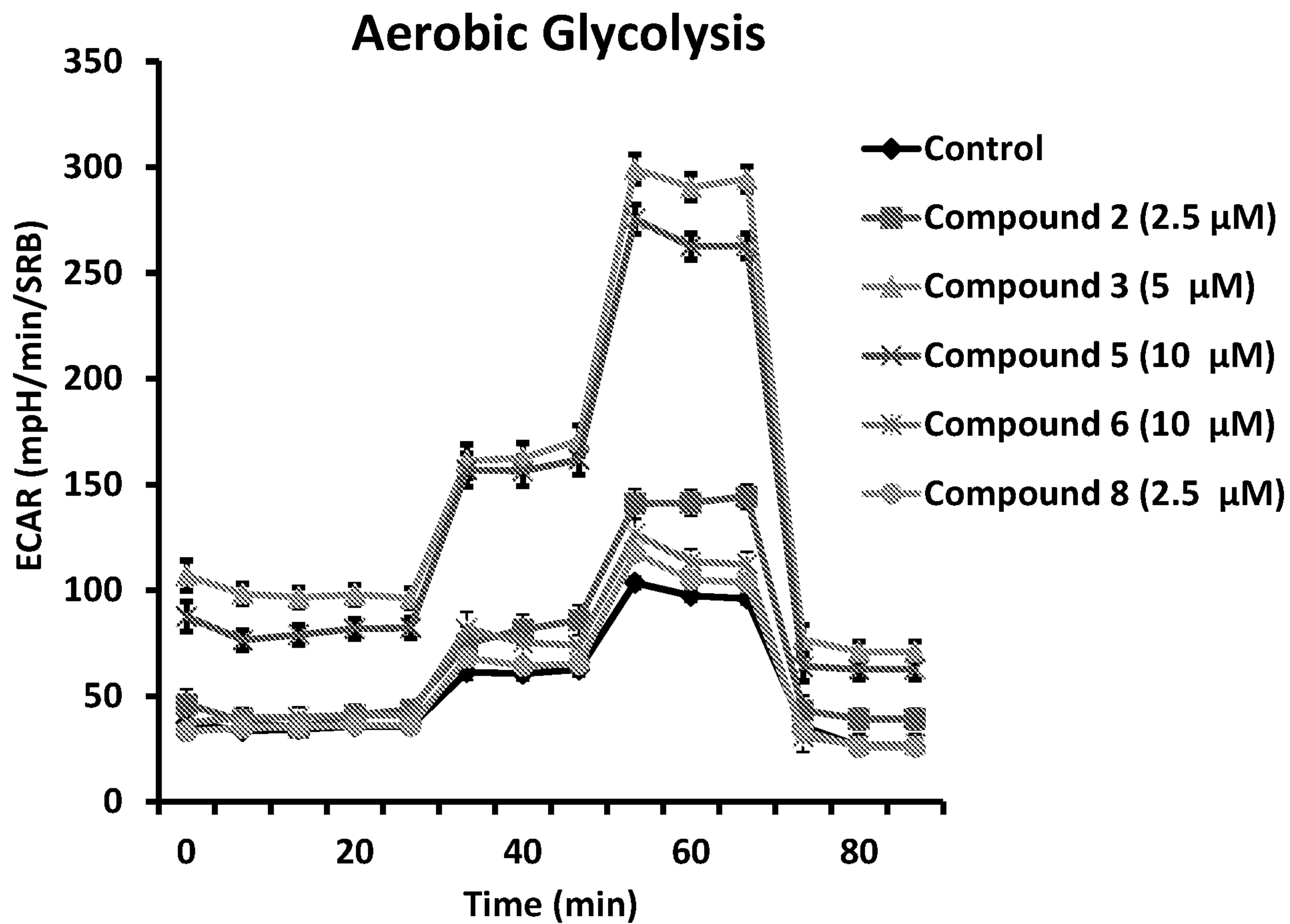


FIG. 7A

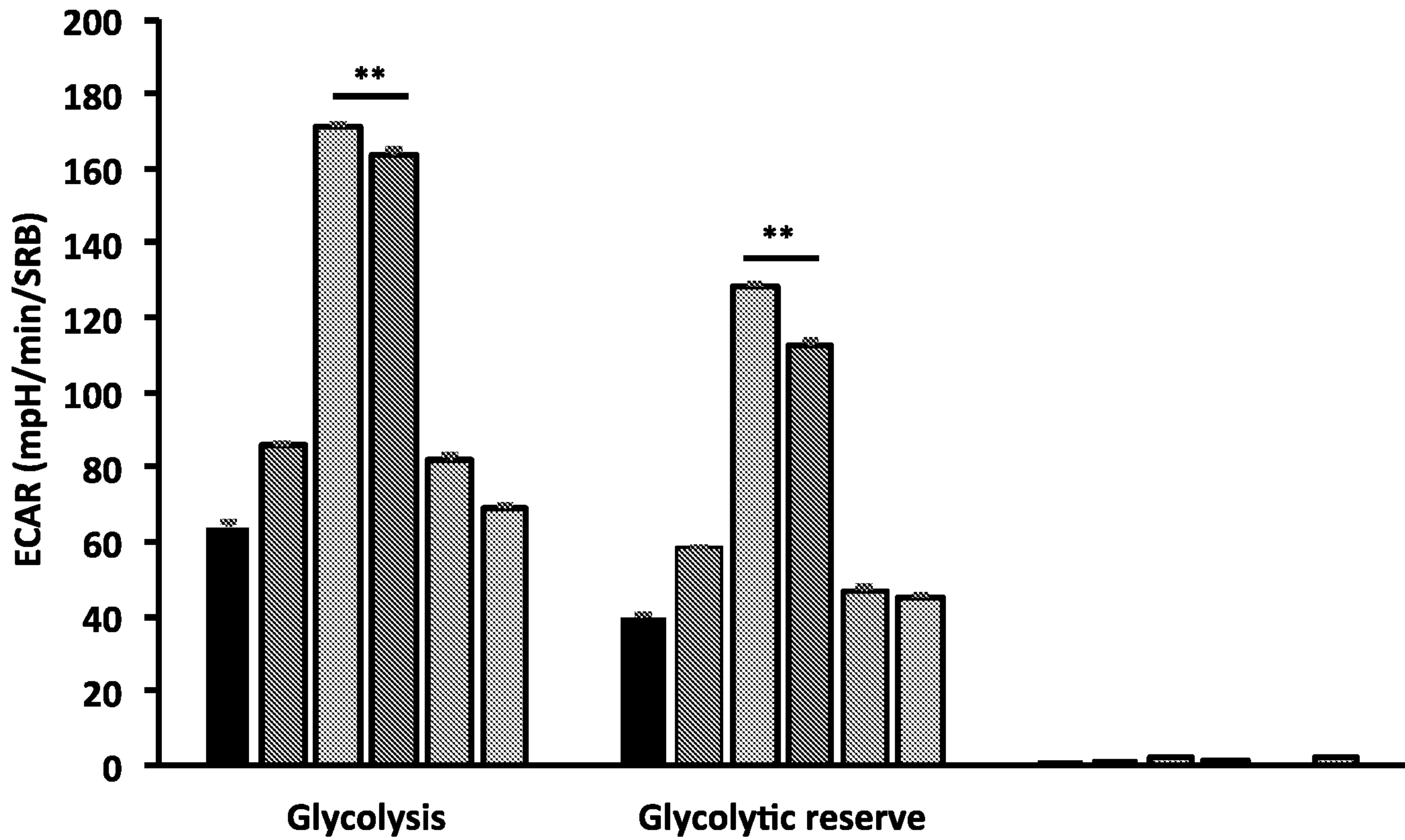


FIG. 7B

Compound 2

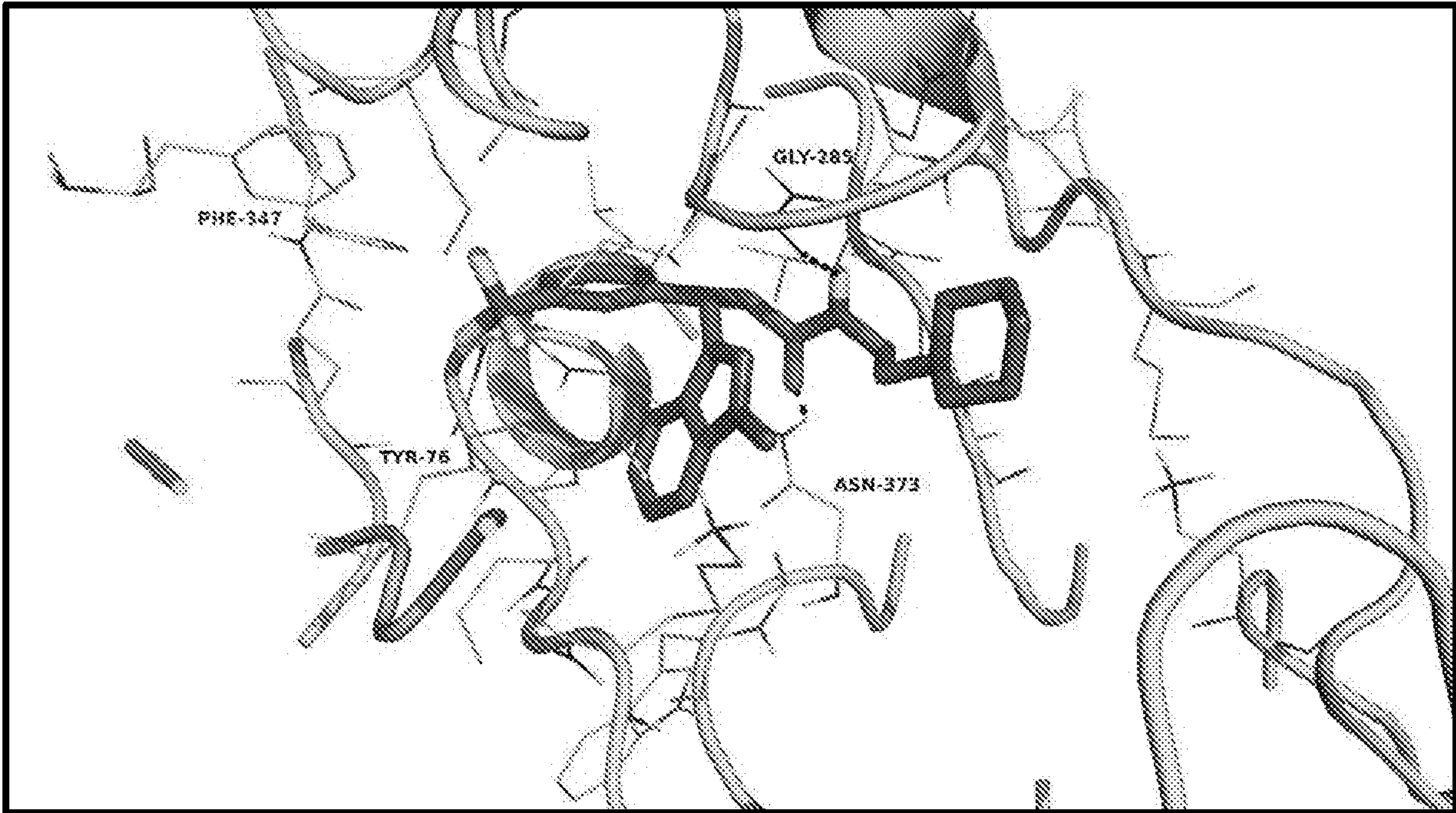


FIG. 8A

Compound 8

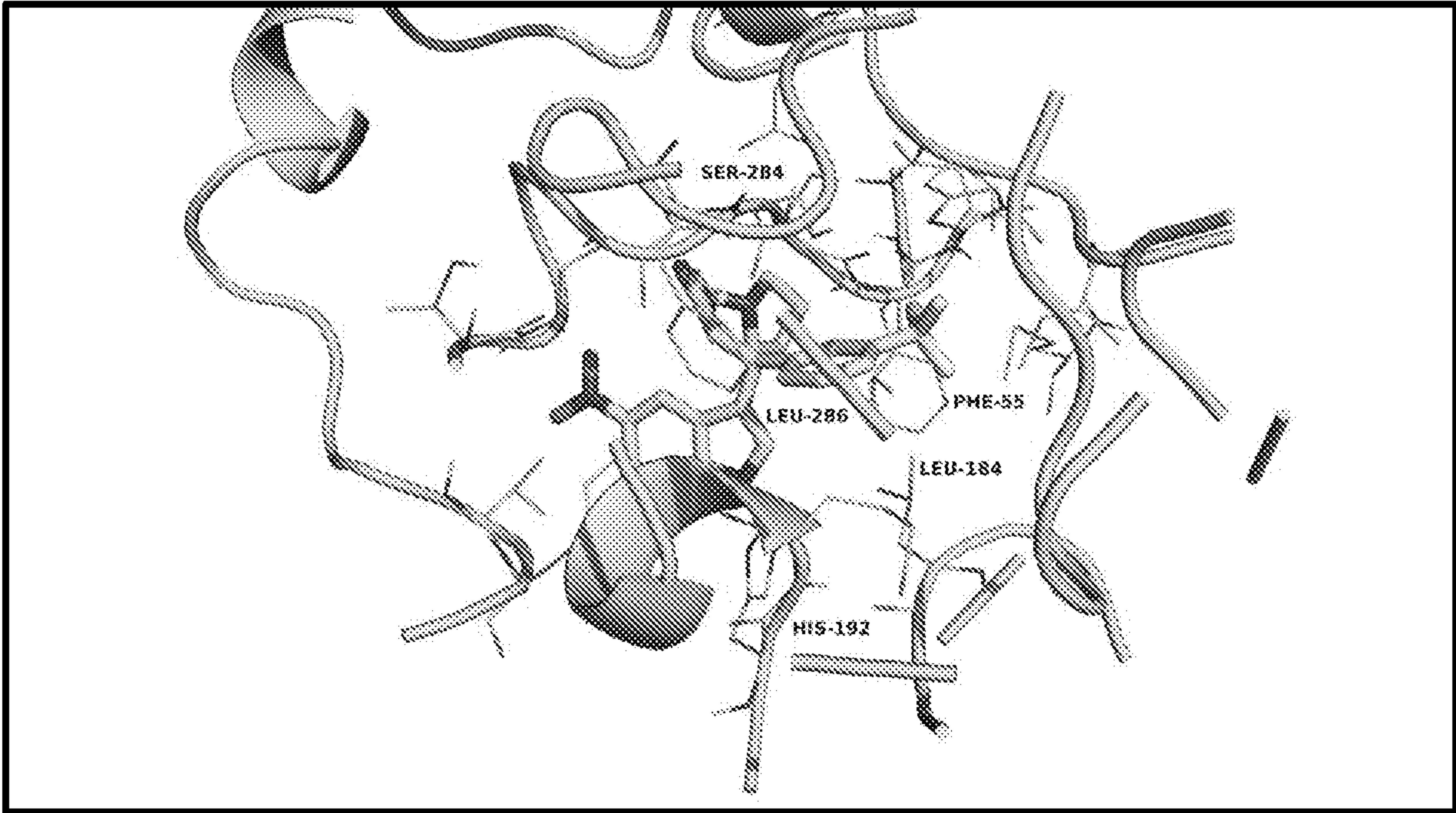


FIG. 8B

Compound 3

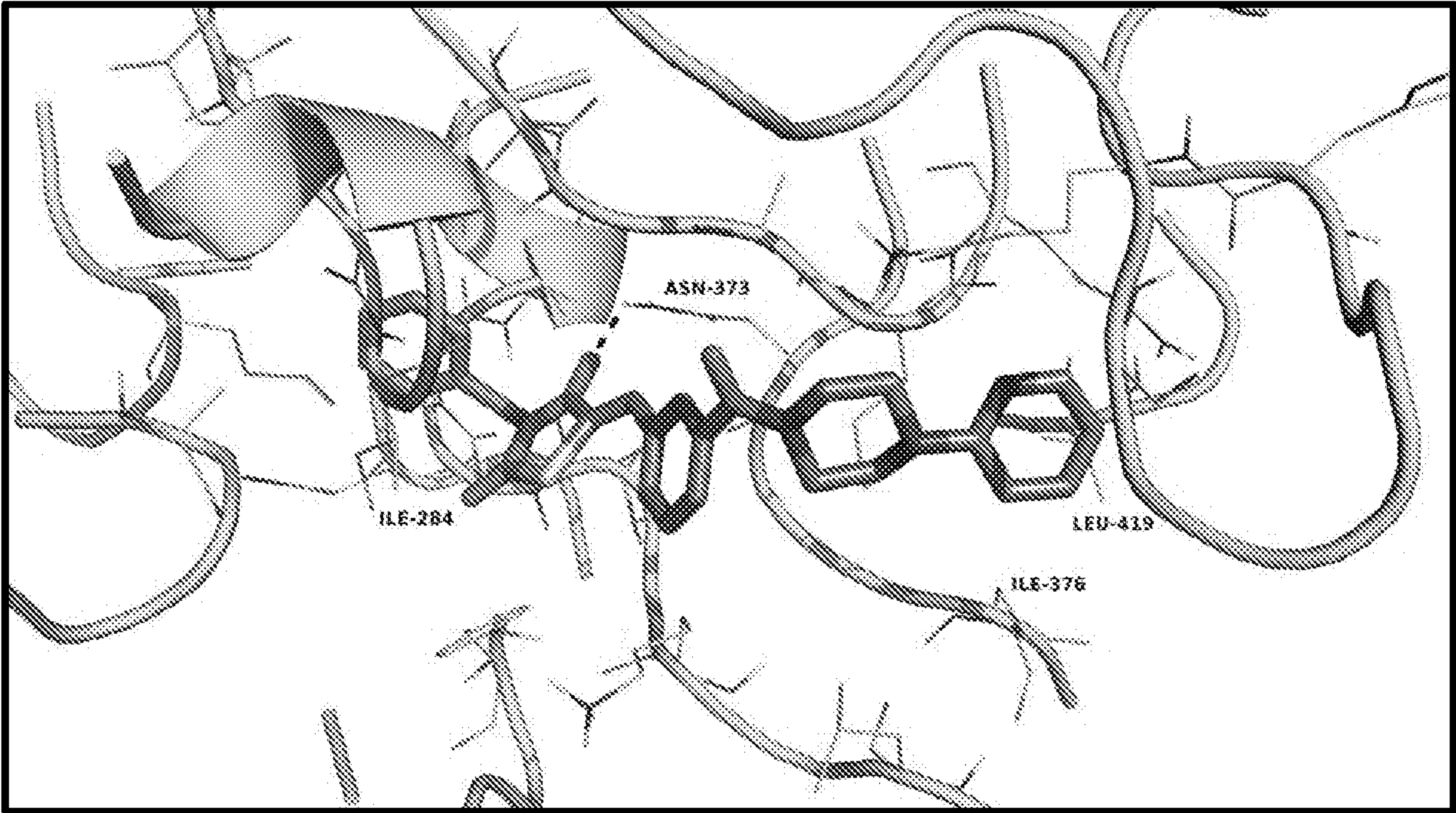


FIG. 8C

Compound 6

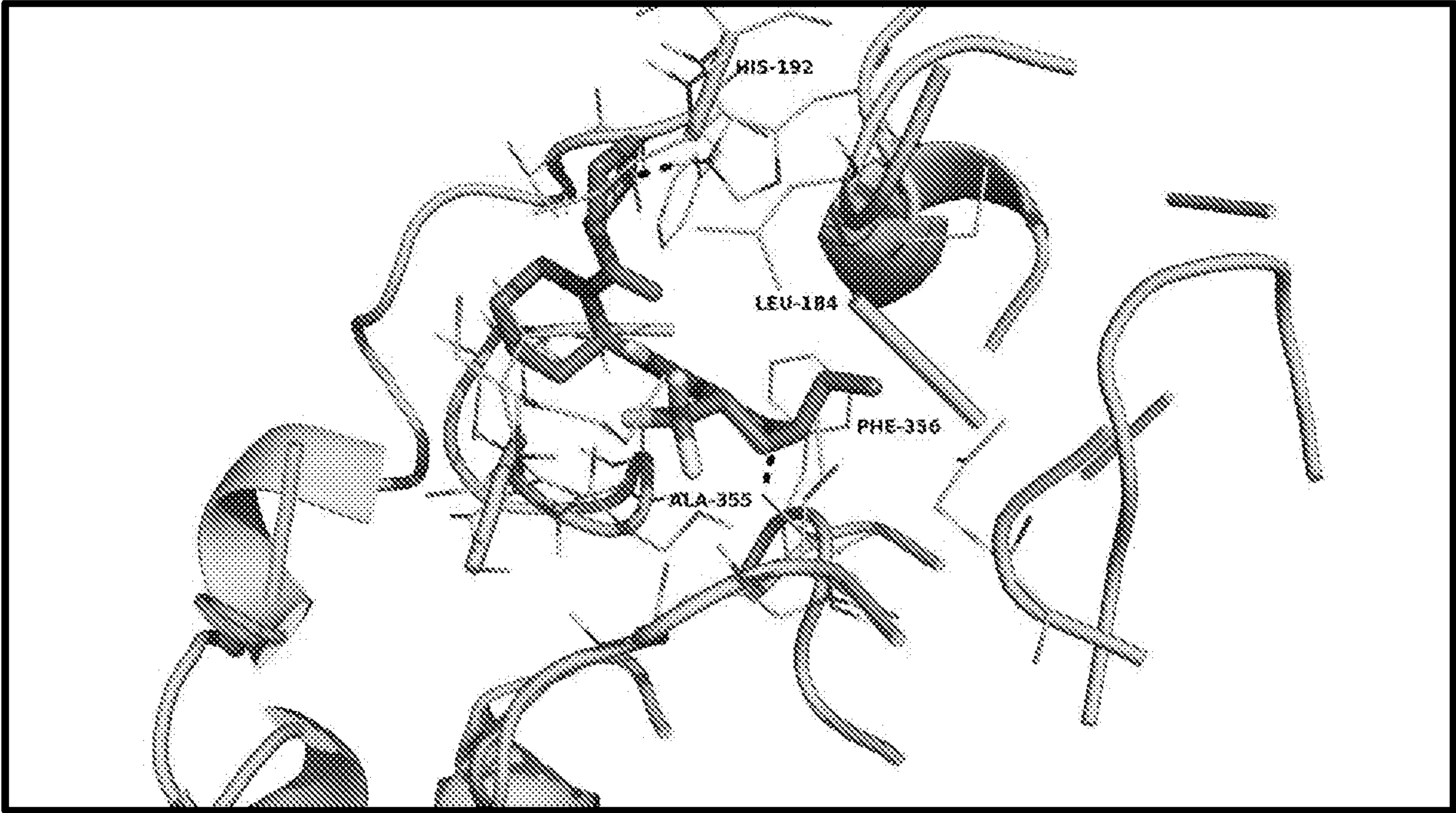


FIG. 8D

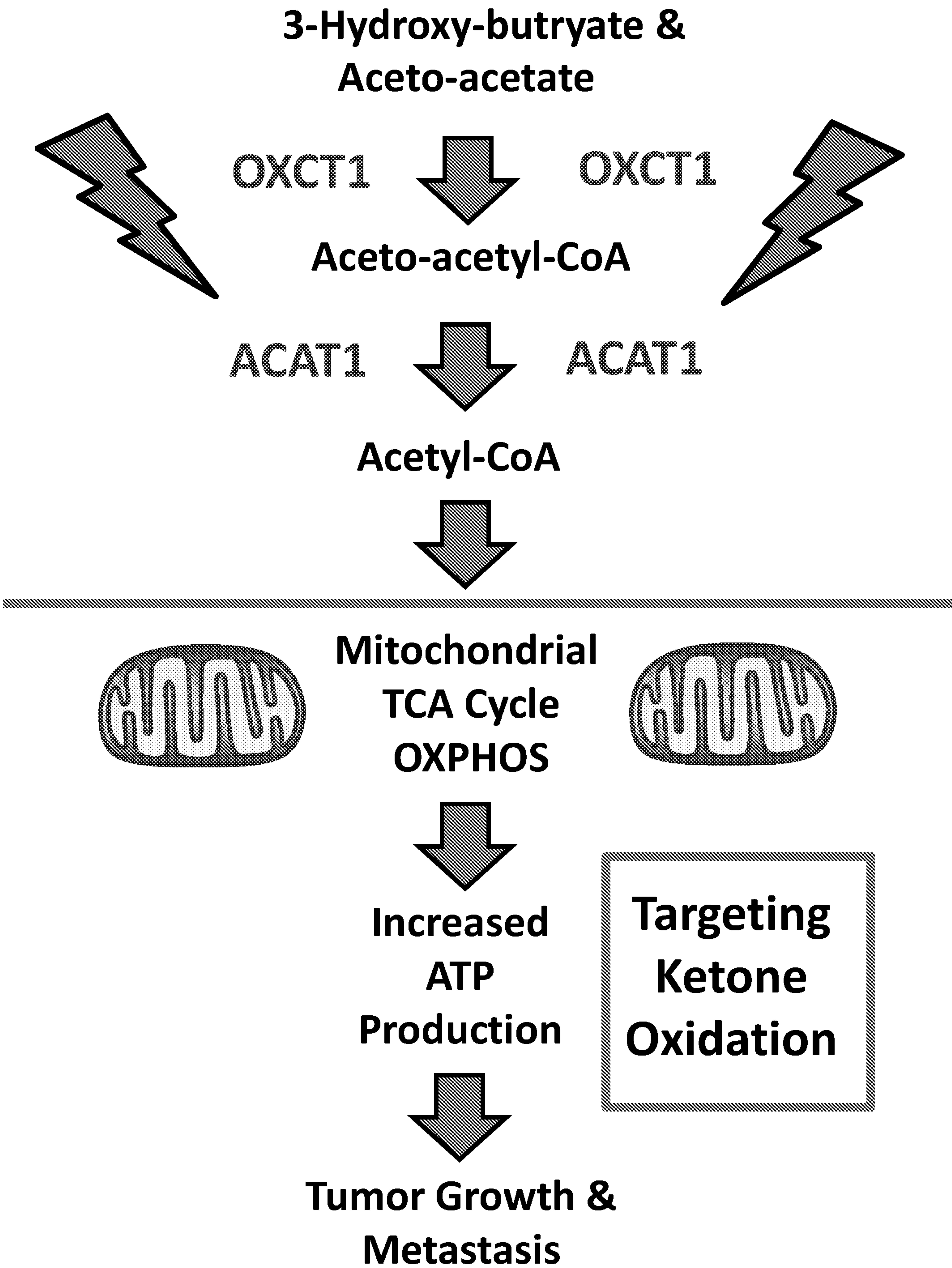
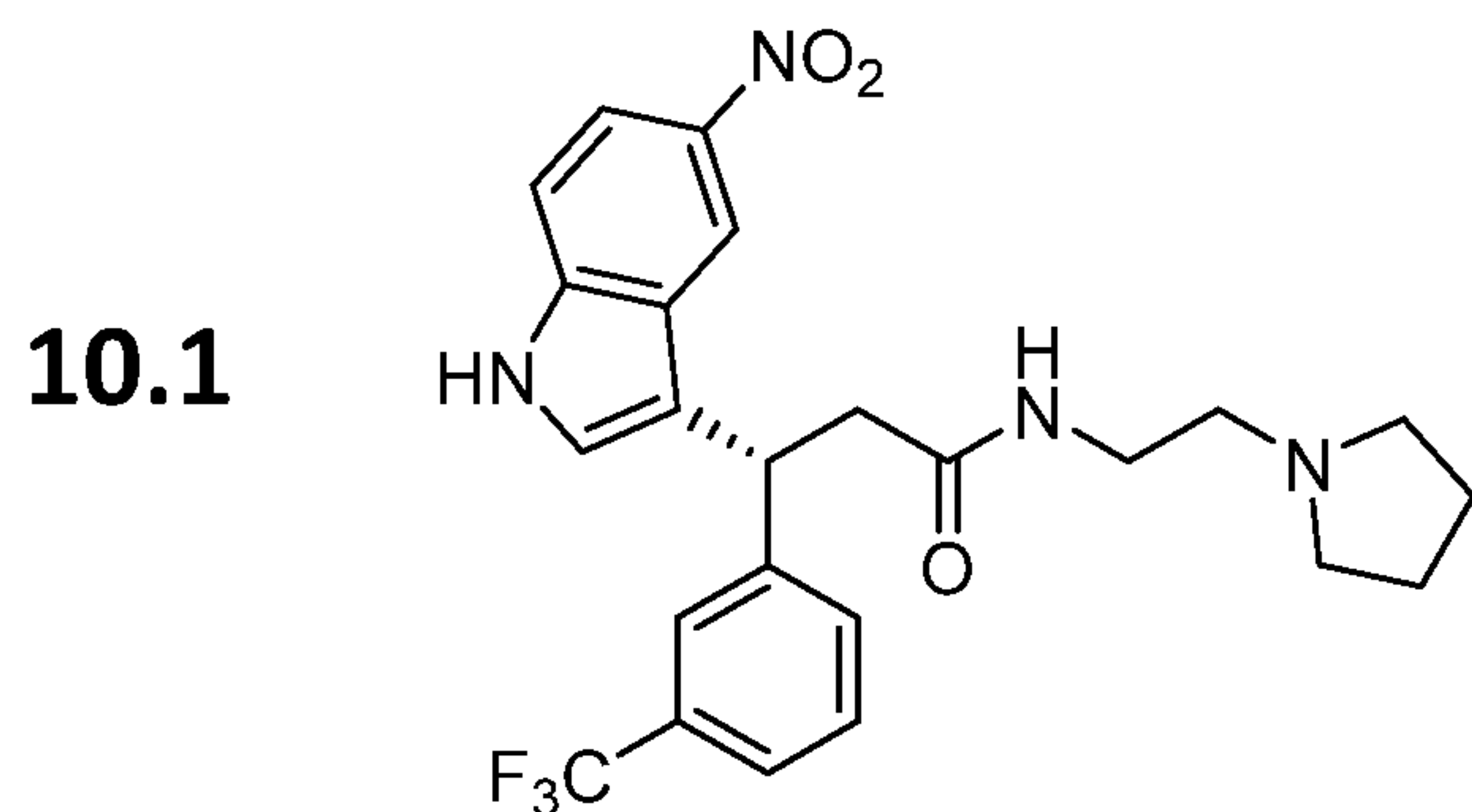
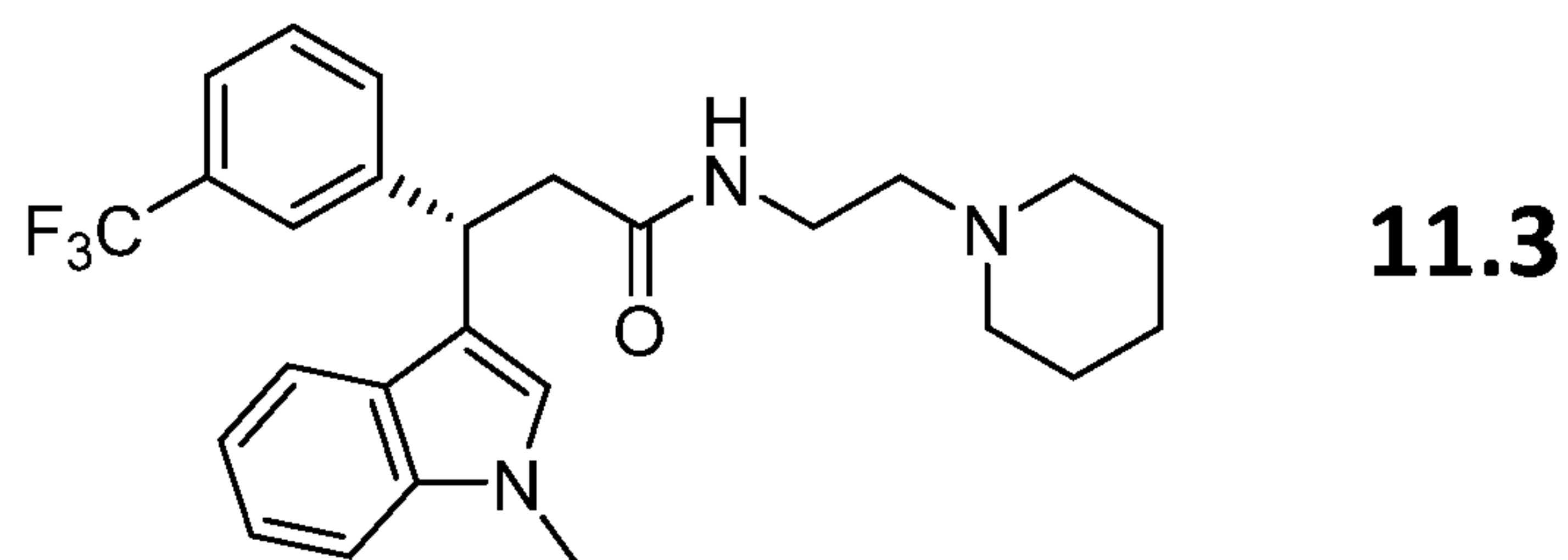
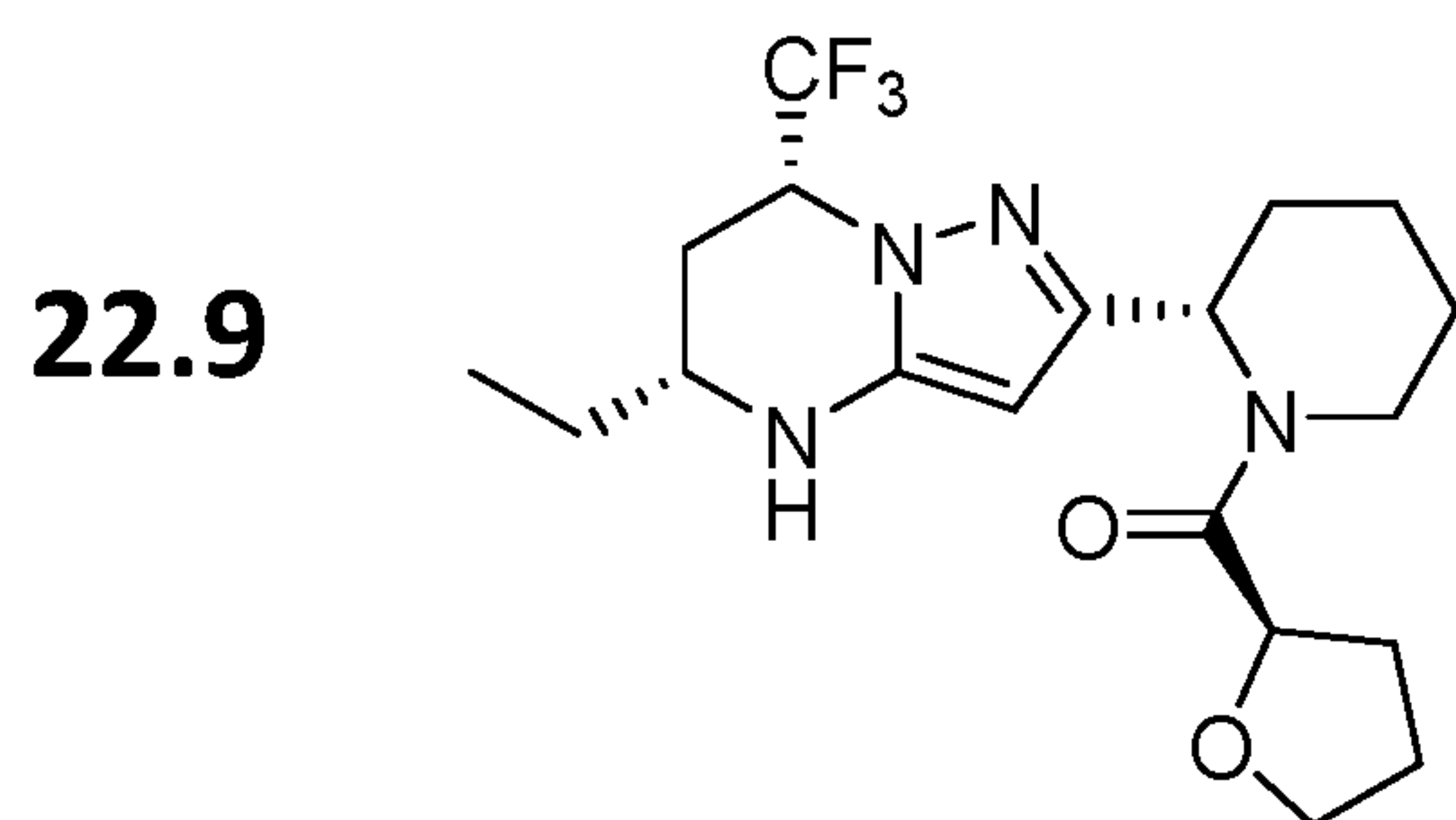
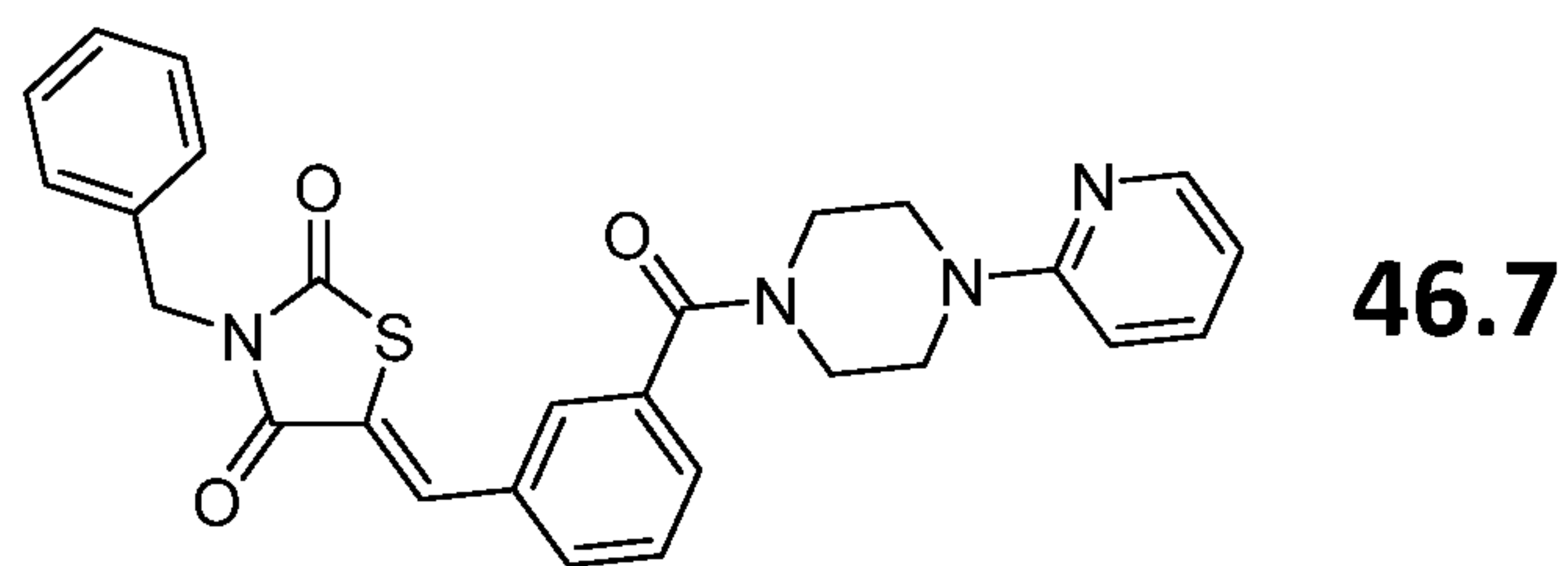
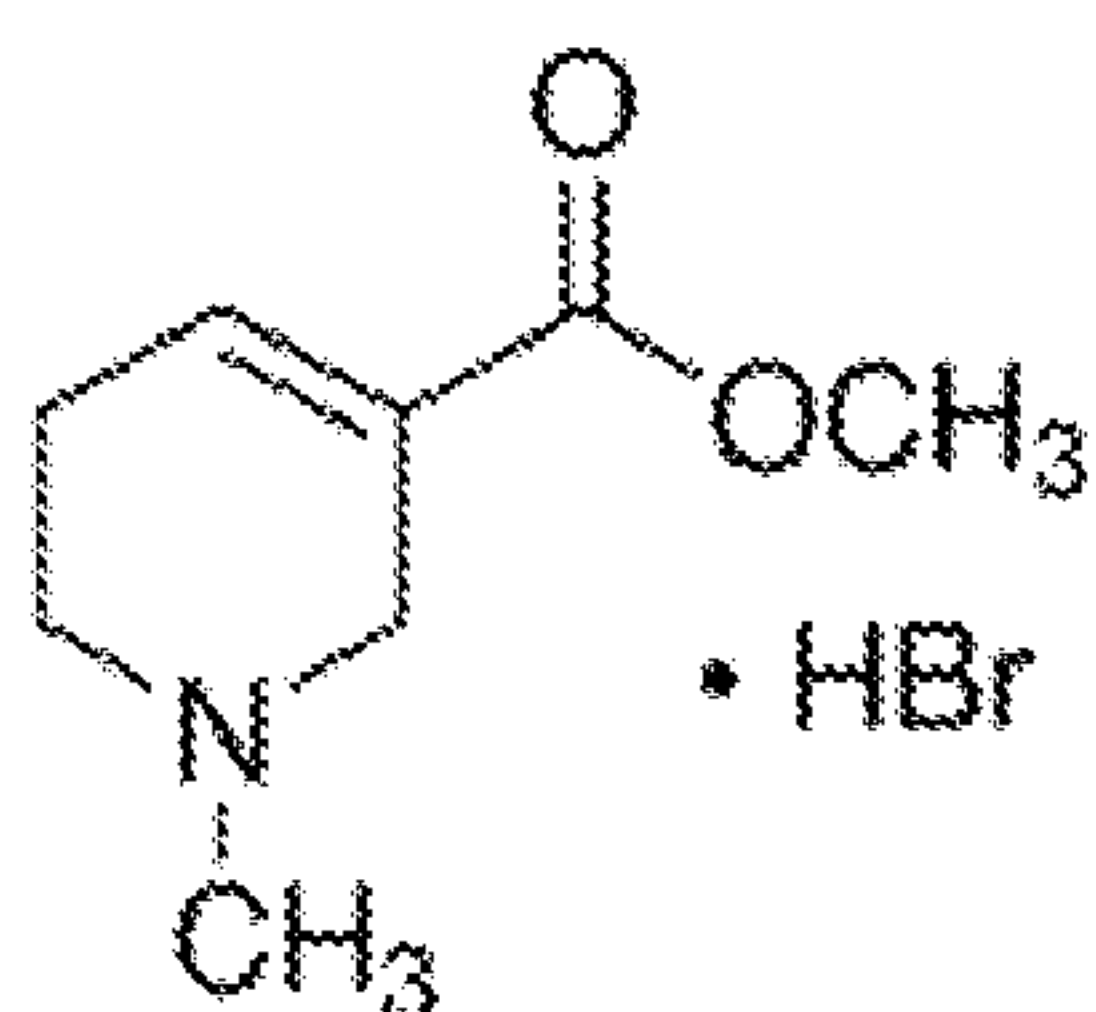


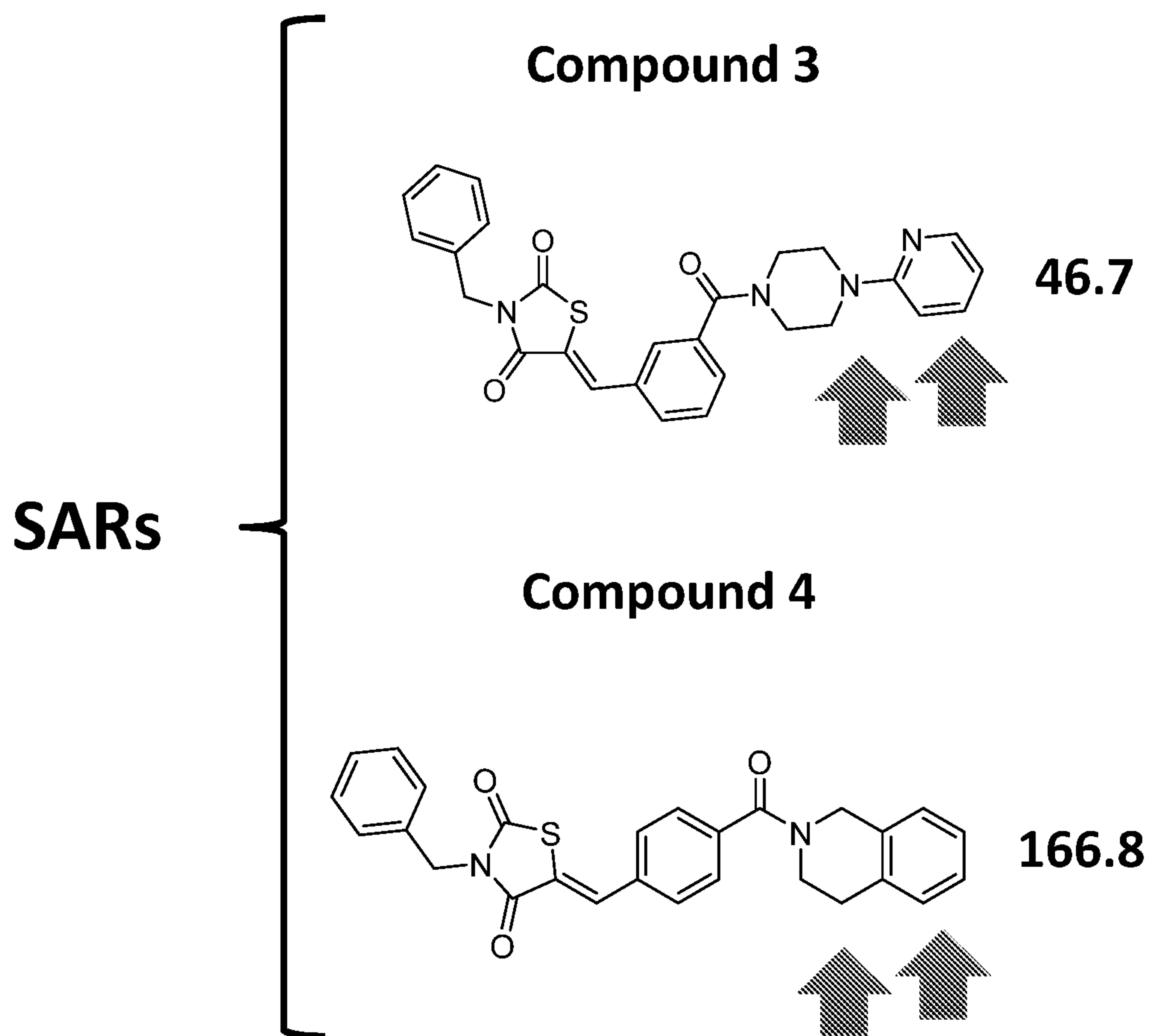
FIG. 9

Summary of the Mitoketoscins

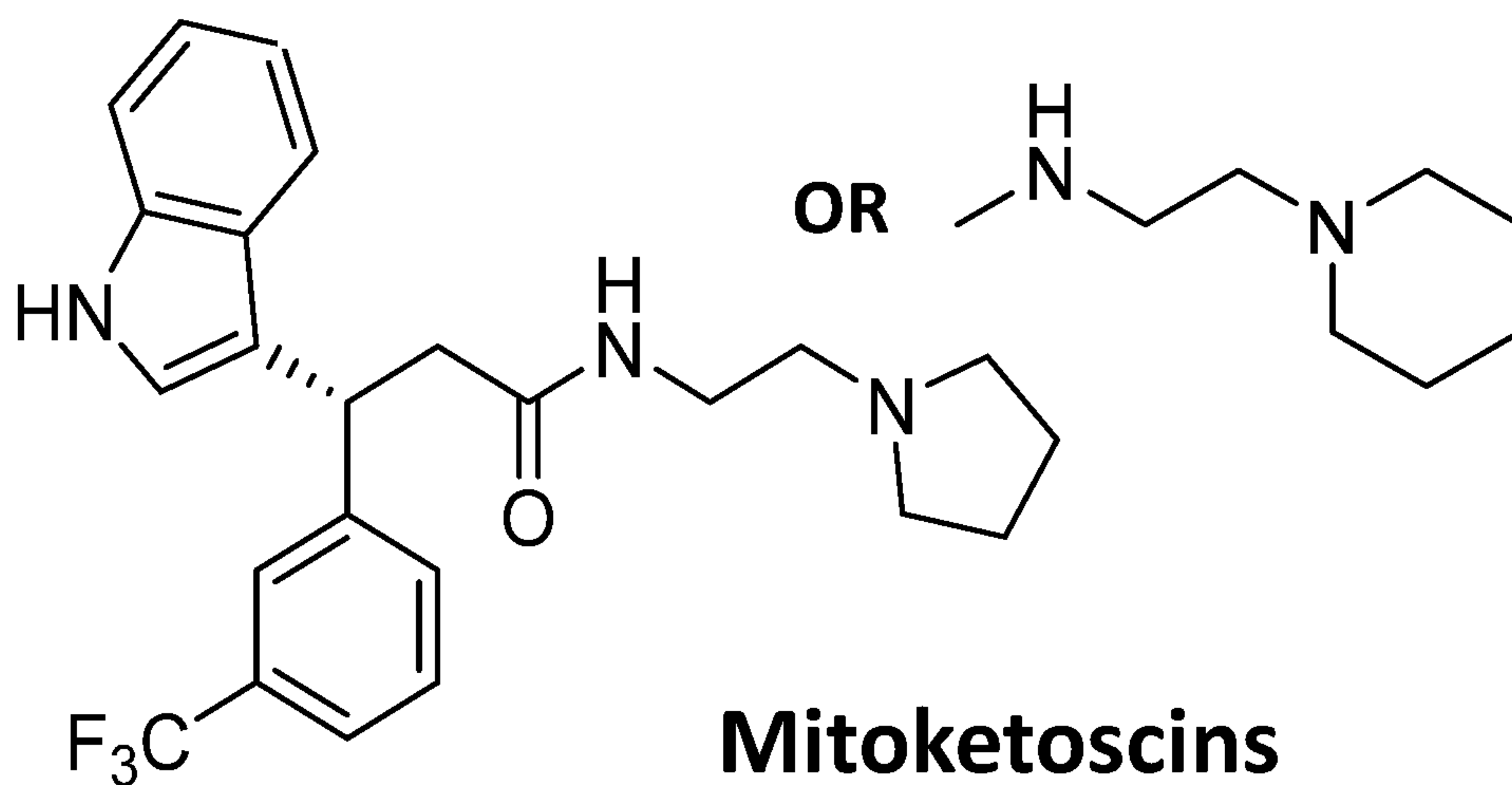
Compound 8**Compound 2****Compound 6****Compound 3****FIG. 10A**

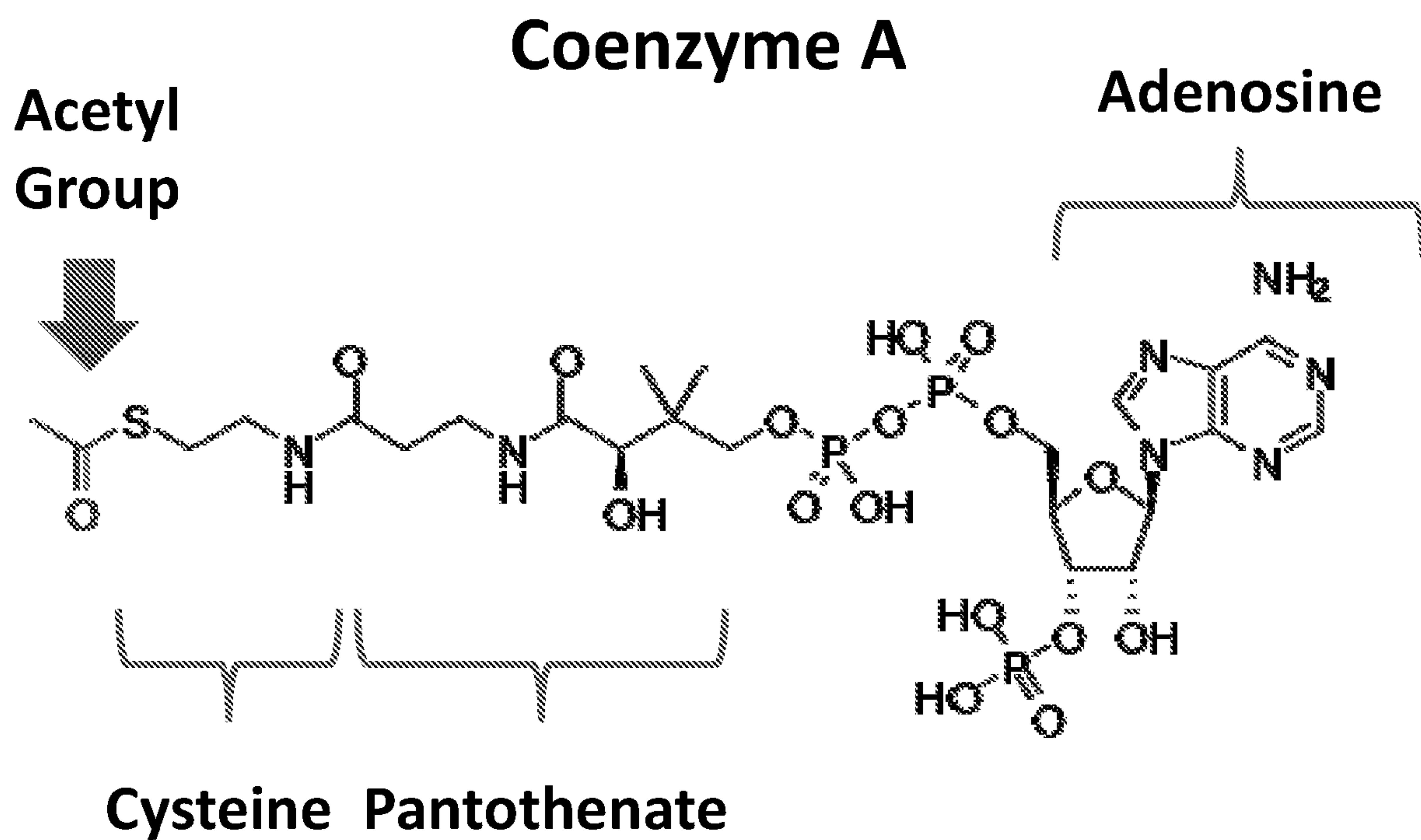
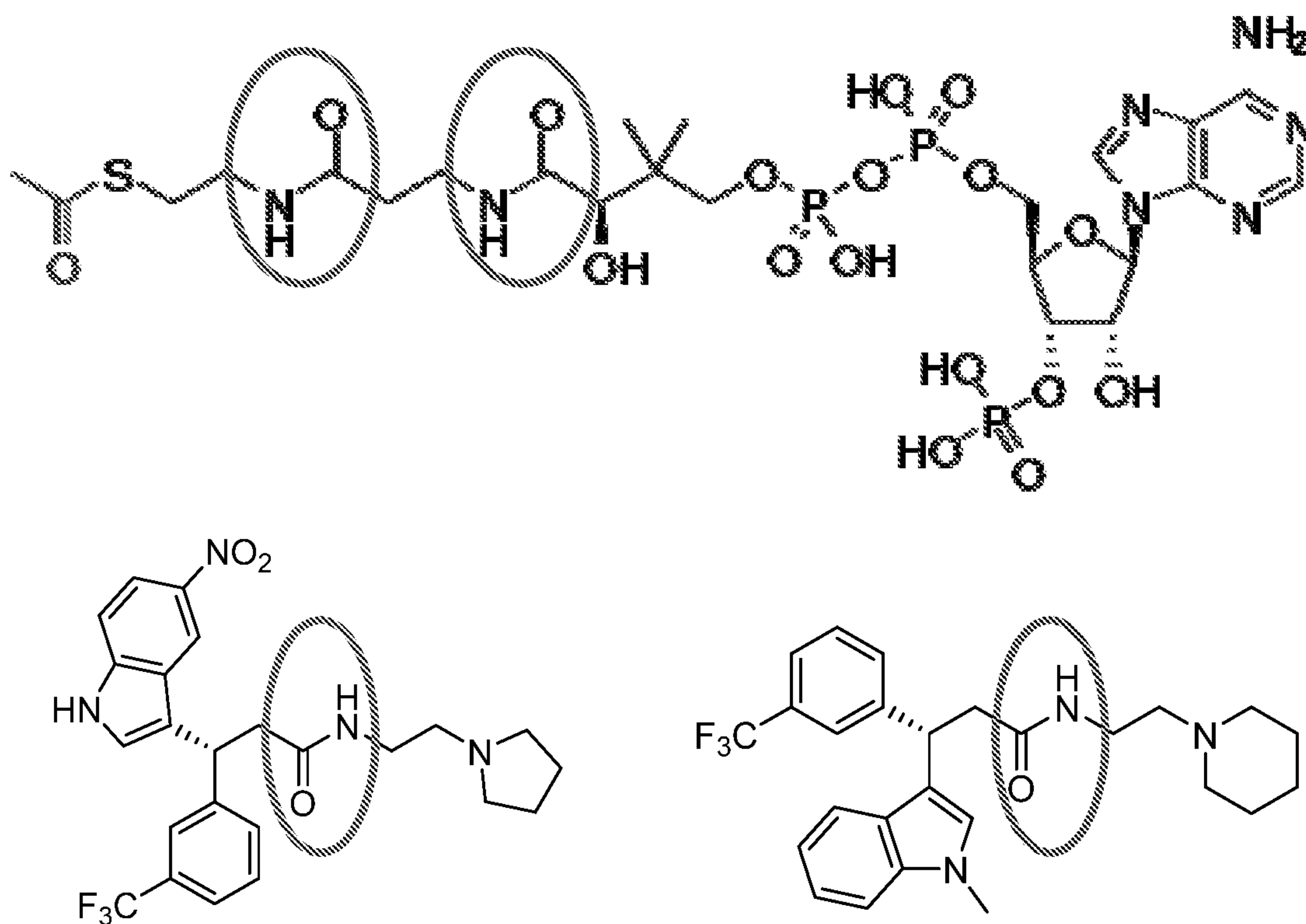
Arecoline

**FIG. 10B**

**FIG. 11A**

Pharmacophore: for Compounds 2 & 8

**FIG. 11B**

**FIG. 12A**

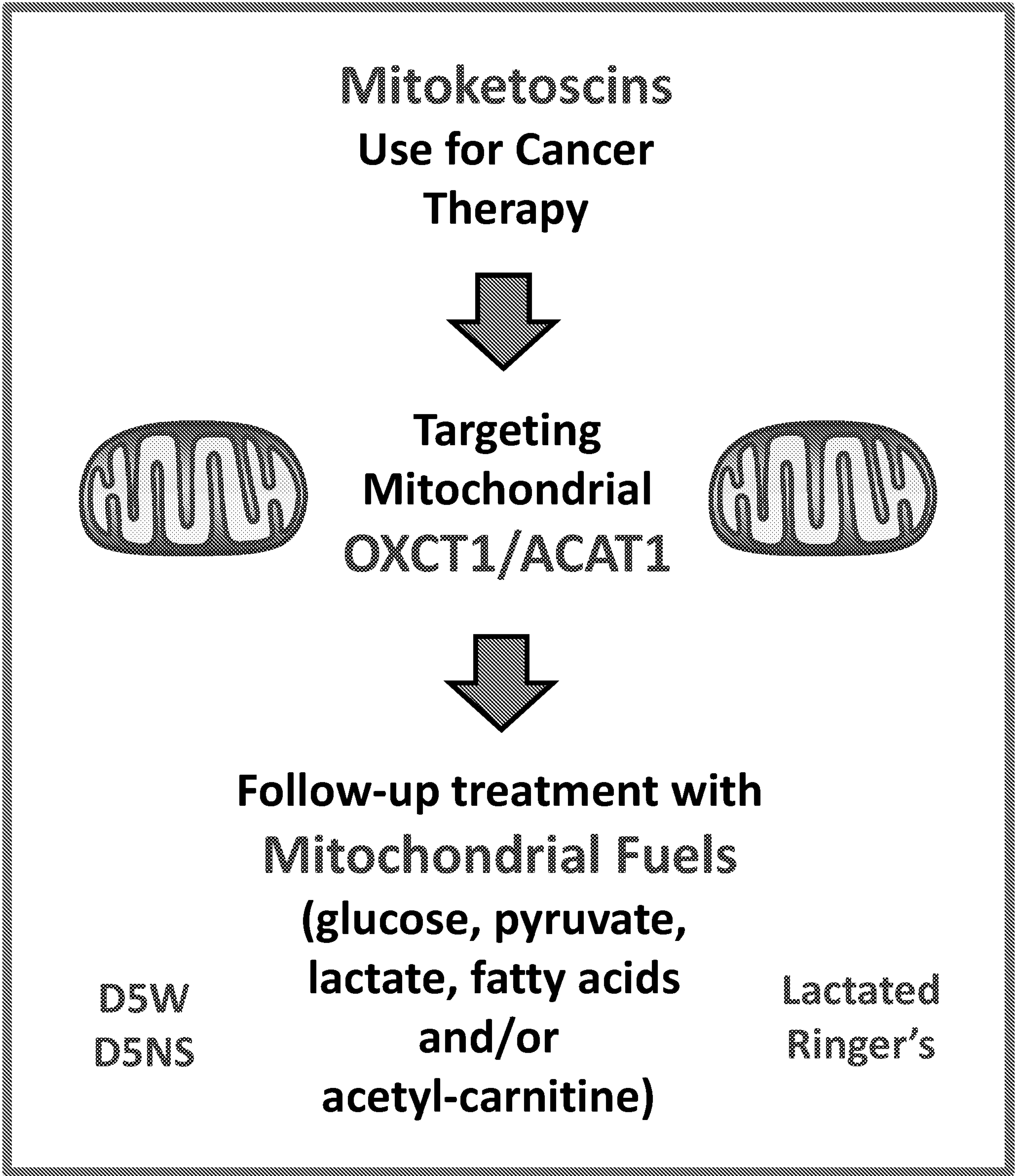


FIG. 13