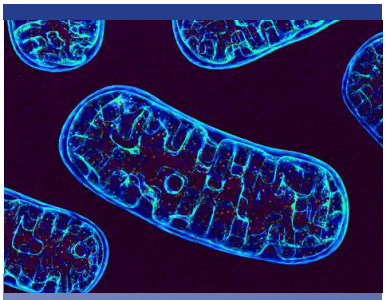
cyprotex AN EVOTEC COMPANY

In Vitro **Toxicology**

Mitochondrial Oxidative Stress

Background Information



'ROS trafficking between mitochondria could constitute a positive-feedback mechanism resulting in an elevated production of ROS that could be propagated throughout the cell and may cause perceptible mitochondrial and cellular injury.'

[1]Zorov DB *et al.* (2014) *Physiol Rev* **94(3)**; 909-950

- Mitochondria consume the majority of cellular oxygen and regulate redox-signalling[1].
- Toxic drugs can induce mitochondrial reactive oxygen species (mROS) causing mitochondrial/cellular damage which has been linked to the pathology of many diseases[2].
- The Cyprotex mitochondrial oxidative stress assay detects selective mROS production caused by the toxicity of novel compounds.

Protocol

Cell Line
HepG2 cell line; other cell types on request

Analysis Platform
Cellomics ArrayScan® CX7, XTI and VTI (Thermo Scientific)

Test Compound Concentrations
8 point dose response curve with top concentration based on 100x C_{max} or solubility limit*

Compound Requirements
50 µL of a solution to achieve 100x C_{max} (200x top concentration to maintain 0.5% DMSO) or equivalent amount in solid compound

Number of Replicates
3 replicates per concentration*

Time Points
24 hour*

Quality Controls
Negative control: 0.5% DMSO (vehicle)
Positive controls: 2 known mROS-inducing compounds

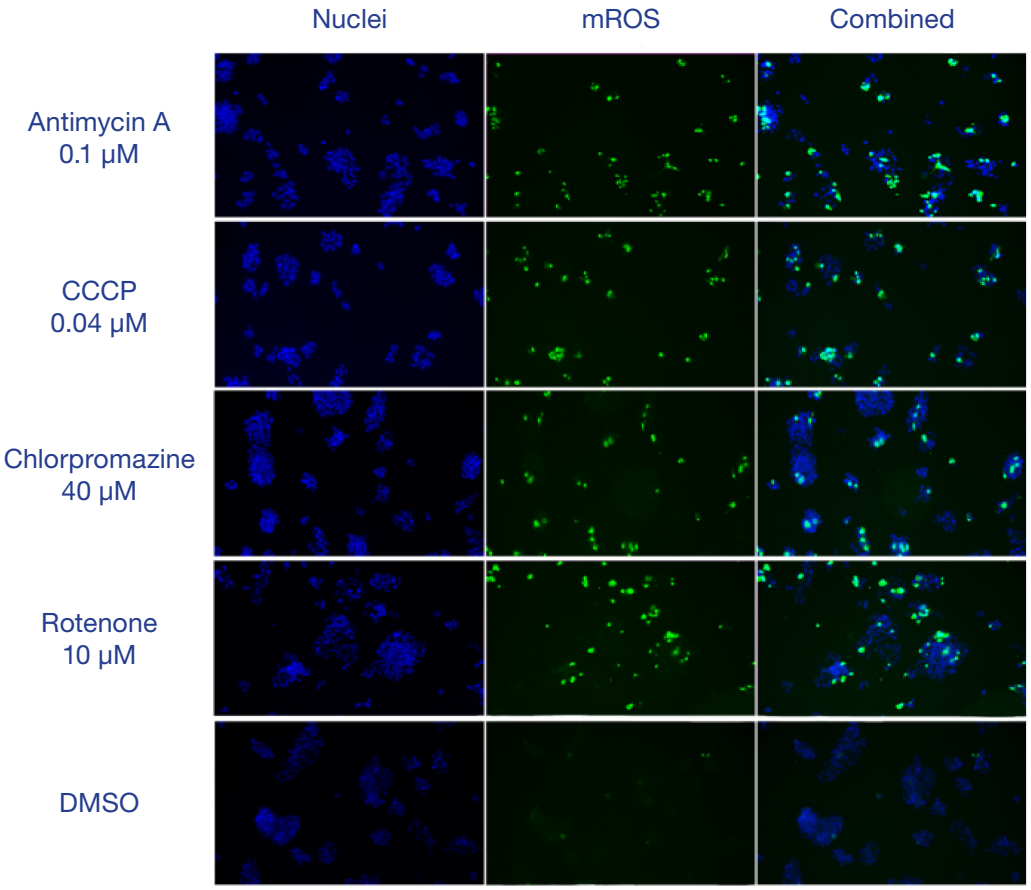
Data Delivery
Minimum effective concentration (MEC) and AC_{50} value for each measured parameter, mitochondrial oxidative stress (mROS), and cell health (cell count, nuclear size, DNA structure and cellular ATP)

*Other options available on request

To find out more contact **enquiries@cyprotex.com**

Figure 1

Representative HCS images of four mROS inducing test compounds alongside vehicle control; nuclei (blue), mROS (green).

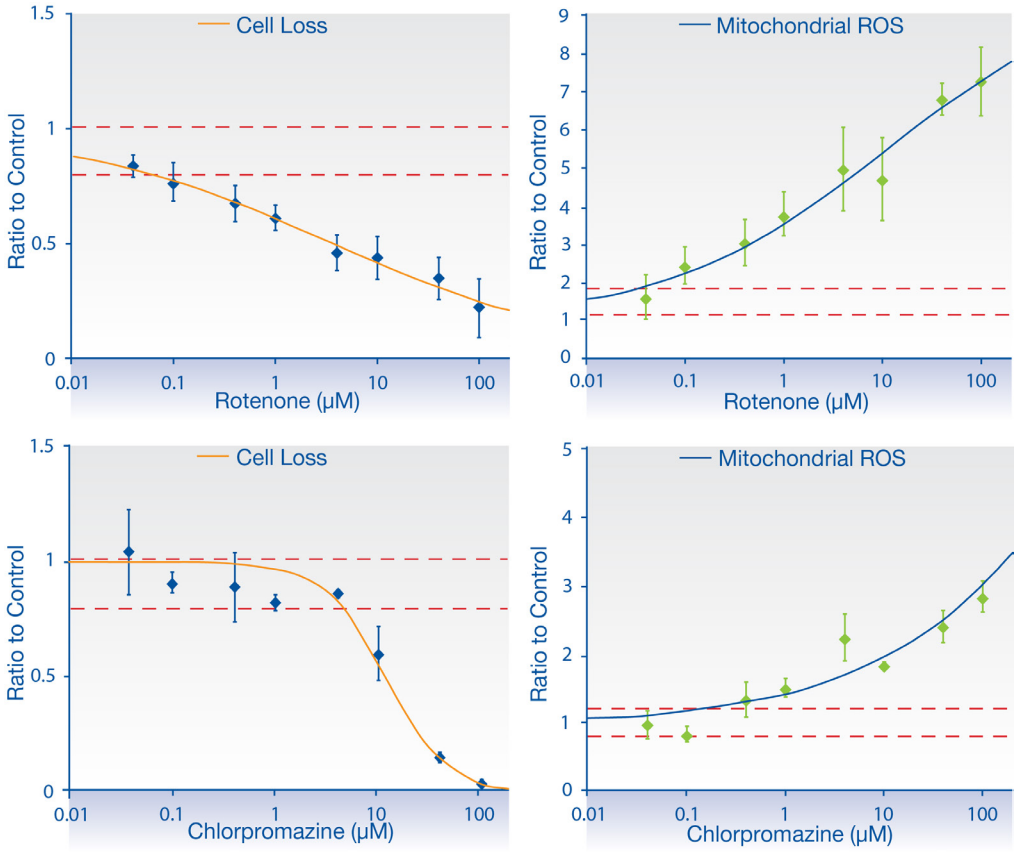


HepG2 cells were plated on tissue culture treated black walled clear bottomed polystyrene plates. The cells were dosed with test compound at a range of concentrations.

At the end of the incubation period (24 hours), the cells were labelled with Hoechst (nuclei) and mitoSox® (mROS) then imaged using an automated fluorescent cellular imager, CellInsight CX7 High-Content Screening (HCS) Platform (Thermo Scientific Cellomics).

Figure 2

Representative dose-response curves for rotenone and chlorpromazine displaying increased mROS formation.



References

[1] Zorov DB *et al.*, (2014) Mitochondrial Reactive Oxygen Species (ROS) and ROS-Induced ROS Release. *Physiol Rev* **94(3)**; 909-950

[2] Ježek P *et al.*, (2020) Redox Signaling from Mitochondria: Signal Propagation and its Targets. *Biomolecules* **10(1)**; 93