

# Toxicity profiling and cellular effects of test compounds in a broad panel of HCA assays

Frosty Leochel, Jacob Fog, Ulla Henriksen, Betina K. Lundholt, Morten Præstegaard

## Abstract

A key limiting step in drug development is to predict adverse toxic effects of lead compounds. We have devised a series of cell-based high content analysis (HCA) assays for prediction of compound toxicity. These assays are based on combinations of cellular dyes, antibody staining and GFP-fusion proteins covering multiplexed readouts for general cytotoxicity, DNA damage, apoptosis, cell cycle, cytoskeletal rearrangements, and cell stress including ER and oxidative stress. Each of these primary readouts is supported by secondary HCA data substantiating the validity of each data point. A panel of 32 diverse toxic compounds with known inhibitory effects on cellular functions has been profiled in this tox assay panel using the Cellomics ArrayScan VTI high content imager. These data show that the HCA assay panel is capable of detecting cytotoxic compounds having a wide variety of modes of action. The assay activity profile can distinguish between compounds having quite similar mechanisms of toxicity, e.g. Cytochalasin A and Cytochalasin D. The PXR assay is the most effective at detecting a wide spectrum of toxic compounds, reflecting the role of the PXR protein as a physiological cellular sensor of foreign toxic substances. These data support our hypothesis that high content analysis methods carried out on a panel of relevant cell-based imaging assays hold great promise as a methodology to obtain predictive toxicity data.

## Introduction

High content cell-based assays have the potential to provide detailed information on the toxicity liabilities of test compounds. Measuring the effect of compounds on specific cellular targets that are linked to toxicity, as well as more general cytotoxic effects such as changes in cell morphology or proliferation, allows generation of a toxicity profile that can be used for prioritizing or deselecting drug candidates.

Multiple assay readouts from the same cells can provide information that would be more time-consuming and more expensive to obtain from running panels of traditional assays, each with a single readout. In addition, by simultaneously measuring the behavior of multiple targets in the same cell, there is the potential to discover effects that would be difficult to detect clearly in assays that query only one target effect at a time.

## Method

We have devised a series of cell-based high content analysis (HCA) assays for prediction of compound toxicity. These assays are based on combinations of cellular dyes, antibody staining, and GFP-fusion proteins covering multiplexed readouts for general cytotoxicity, DNA damage, apoptosis, cell cycle, cytoskeletal rearrangements, and cell stress including ER and oxidative stress. Each of these primary readouts is supported by secondary HCA data substantiating the validity of each data point.

## Compounds

A panel of 32 toxic compounds has been selected for this evaluation, covering a large range of cytotoxic profiles. The compounds fall into the following categories based on mode of action.

<b>DNA damage</b> Doxorubicin Camptothecin Actinomycin D Etoposide	<b>Cytoskeletal rearrangement</b> Nocodazole Linculin B Jaspilactonide Cytochalasin A and D	<b>Cell cycle modulators</b> Paclitaxel Aphidicolin
<b>Ion transport modulators</b> Valinomycin Thapsigargin	<b>Kinase inhibitors</b> LY294002 Staurosporine U0126 PMA Genistein	<b>Hepatotoxicity</b> Troglitazone Taurine
<b>Protein turnover</b> MG-132 Cycloheximide	<b>Mitochondrial function</b> CCCP Rotenone Oligomycin	<b>Phosphatase</b> Okadaic Acid
<b>Others</b> Leptomycin B Trichostatin A Tunicamycin Teniposide Cisplatin	<b>Stress</b> Anisomycin	

## Assays

The compounds were evaluated in a panel of high content assays including Redistribution assays, HCS Reagent Kits, and multiplex assays combining the two technologies.

**PXR Redistribution:** PXR is a steroid and xenobiotic receptor that functions in the regulation of endobiotic and xenobiotic metabolism. Ligand binding leads to upregulation of genes central to drug metabolism and drug efflux transporters. The PXR Redistribution assay makes it possible to evaluate PXR-mediated drug metabolism of test compounds at an early stage during drug discovery as well as being an early indicator of drug toxicity.

**Phospho-H2AX HCS Reagent Kit:** DNA damage induction by various agents leads to rapid phosphorylation of H2AX (core histone protein H2AX).

**p53/Mdm2 HCS Reagent Kit:** Measures response to stress from DNA damage, hypoxia, cytokines, metabolic changes, viral infection or oncogenes.

**ATF6/CHOP multiplex Kit:** Multiplex of the ATF6 Redistribution assay and the CHOP/GADD153 HCS Reagent Kit. Both proteins are involved in the response caused by ER stress and activation of the signaling network called the unfolded protein response (UPR).

**MK2 multiplex Kit:** Multiplex of the MK2 Redistribution assay and several HCS Reagent Kits (p38 and c-Jun/NFκB). The assay measures activation of the p38 pathway, JNK pathway and NFκB.

## Workflow

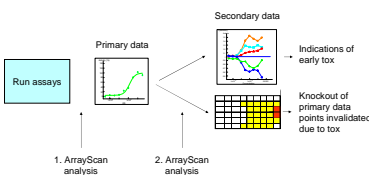


Figure 1: Workflow for the generation and analysis of primary and secondary readouts.

## Primary readouts

Compound	Description	EC50	Phospho-H2AX	p53/Mdm2	ATF6/CHOP	MK2 Multiplex
Actinomycin D	Actinomycin D	1.0	1.0	1.0	1.0	1.0
Camptothecin	Camptothecin	1.0	1.0	1.0	1.0	1.0
Cisplatin	Cisplatin	1.0	1.0	1.0	1.0	1.0
Cycloheximide	Cycloheximide	1.0	1.0	1.0	1.0	1.0
Doxorubicin	Doxorubicin	1.0	1.0	1.0	1.0	1.0
Etoposide	Etoposide	1.0	1.0	1.0	1.0	1.0
Genistein	Genistein	1.0	1.0	1.0	1.0	1.0
Leptomycin B	Leptomycin B	1.0	1.0	1.0	1.0	1.0
MG-132	MG-132	1.0	1.0	1.0	1.0	1.0
Nocodazole	Nocodazole	1.0	1.0	1.0	1.0	1.0
Paclitaxel	Paclitaxel	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Trichostatin A	Trichostatin A	1.0	1.0	1.0	1.0	1.0
U0126	U0126	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.0	1.0
CCCP	CCCP	1.0	1.0	1.0	1.0	1.0
Oligomycin	Oligomycin	1.0	1.0	1.0	1.0	1.0
Staurosporine	Staurosporine	1.0	1.0	1.0	1.0	1.0
Thapsigargin	Thapsigargin	1.0	1.0	1.0	1.0	1.0
Teniposide	Teniposide	1.0	1.0	1.0	1.0	1.0
Valinomycin	Valinomycin	1.0	1.0	1.0	1.0	1.0
Y-27632	Y-27632	1.0	1.0	1.0	1.0	1.0
Zinc Oxide	Zinc Oxide	1.0	1.0	1.0	1.0	1.0
Rotenone	Rotenone	1.0	1.0	1.0	1.0	1.0
Okadaic Acid	Okadaic Acid	1.0	1.0	1.0	1.	