

Chemical Compound Profiling of Cell-based Toxicity Assays Using Quantitative High- Throughput Screening

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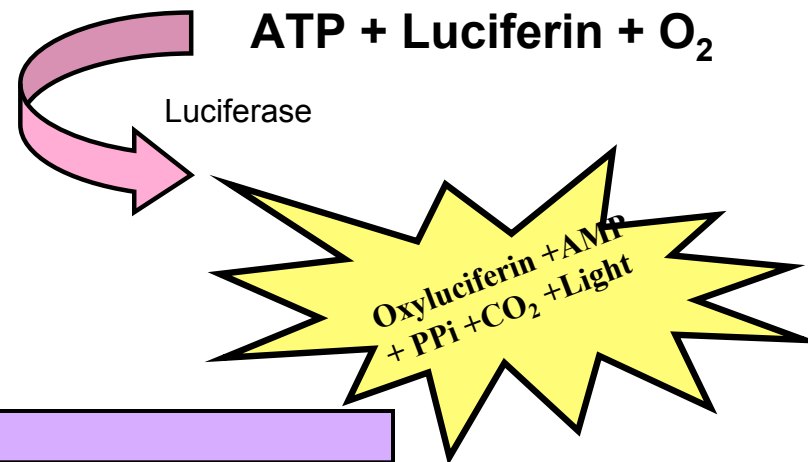
Abstract: Traditionally, toxicological profiles of drugs or chemicals rely on *in vivo* studies in laboratory animals. While these profiles provide useful information about the safety of chemical compounds, this approach is expensive, low-throughput, and inconsistently predictive of human biology and pathophysiology. To overcome these limitations, we have begun to develop a battery of cell-based assays to profile the toxicity of chemical compounds in a variety of cell types using quantitative high throughput screening (qHTS). The ultimate goal is to identify *in vitro* chemical signatures that could act as predictive surrogates for *in vivo* toxicity. Importantly, and distinct from conventional single concentration HTS screening, qHTS is capable of generating high-confidence positive and negative activity data, making it suitable for the comprehensive profiling of potentially toxic compounds. Thus far, the activity of 1,408 compounds from the National Toxicology Program (NTP) has been determined in assays for cytotoxicity and caspase 3/7 activity. Corresponding human and rodent cell lines derived from 6 tissues that are common targets of xenobiotic toxicity (liver, blood, kidney, brain, lung, and skin) were used in these assays. Data analysis has identified compounds that are toxic to all cell types at similar concentrations, as well as compounds that exhibit selective toxicity to particular cell types. In addition, some compounds appear to utilize the caspase 3/7 pathway as a mechanism for cytotoxicity, while others exhibit cytotoxicity independent of this pathway. These proof-of-principle data are being used to scale-up the profiling effort to incorporate more assay conditions, assay and cell types, and compounds. The resulting large data sets will provide a rich source of information for the development of *in vitro* toxicological signatures and the prediction of toxic effects of new chemical entities.

Cell-Based Approach to Predictive Toxicology

- Objective:
To develop cell-based high-throughput screening assays and to profile compounds from NTP/NIEHS toxicology program
- Chemical compounds (known and potential toxins/toxicants):
 - 1408 compounds selected by NTP/NIEHS
 - chemicals tested in Salmonella (Ames test)
 - natural products of interest to NTP
- Assay types:
 - Cytotoxicity assay: measurement of cellular ATP content
 - Caspase 3/7 assay: measurement of caspase 3/7 activity
 - Membrane integrity assay: measurement of LDH release
 - ACEA's RT-CES assay: time-course of toxic effect in cells – kinetic signature
 - Future assays
- Assay format (qHTS):
 - 14 concentration dilutions
 - Concentration range from 0.5 nM to 92 uM in 5uL/well
- Types of Cells:
 - Human and rodent primary cells and cell lines derived from 6 tissues (liver, blood, kidney, brain, lung, and skin) that are common targets of xenobiotic toxicity

CellTiter-Glo Luminescent Cell Viability Assay

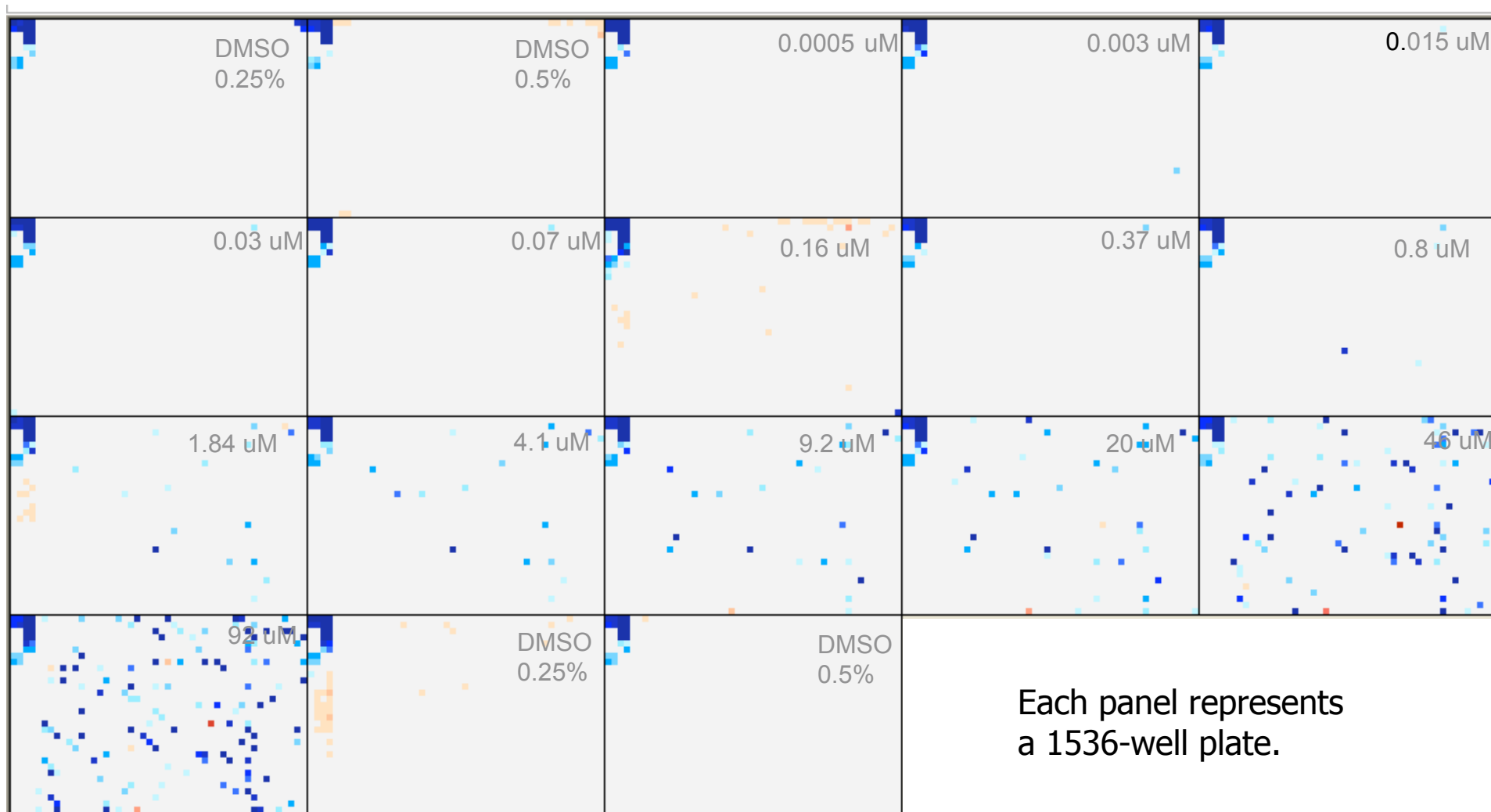
- Description
 - Method of measuring number of viable cells
 - Based on quantitation of ATP, an indicator of metabolic activity
 - Luminescent signal proportional to amount of ATP present
- Applications
 - Cell proliferation
 - Cytotoxicity
 - Cell viability



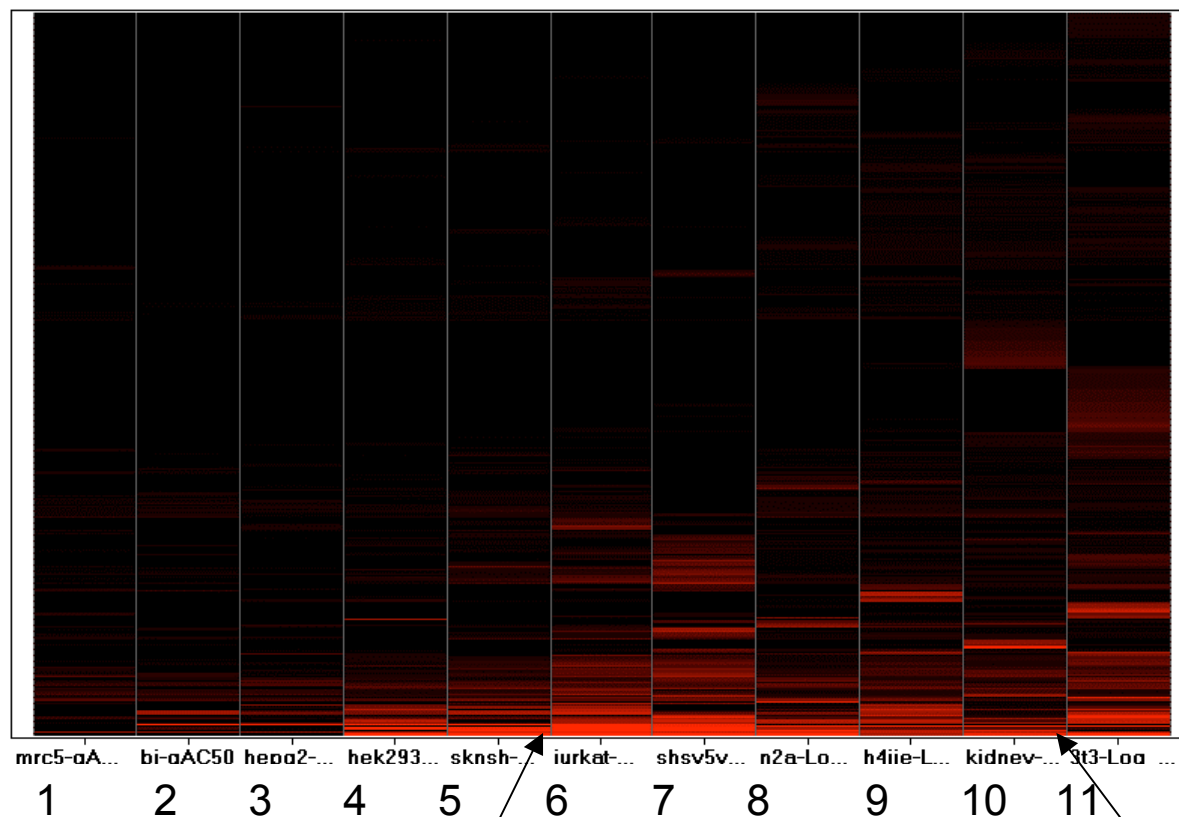
1536 well plate format			
Sequence	Parameter	Value	Description
1	Reagent	5 ul	1-2k cells/well
2	Time	3-5hr	37°C incubation
3	Compounds	23 nl	0.59 nM -92 _ M
4	Time	40 hr	37°C incubation
5	Reagent	5 ul	CellTiter Glo reagent
6	Time	20 min	Room Temperature
7	Detection	Luminescence	Viewlux plate reader

qHTS results map for HepG2 cells

Cell Viability Assay

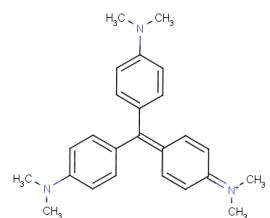


Compound Toxicity Across Cell lines

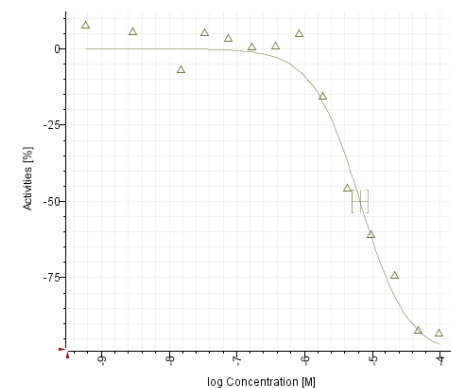


Cell Viability Assay

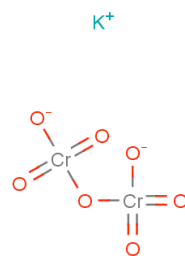
- 1 MRC-5
- 2 BJ
- 3 HepG2
- 4 HEK293
- 5 SK-N-SH
- 6 Jurkat
- 7 SH-SY5Y
- 8 N2a
- 9 H-4-IIE
- 10 Rat Kidney cells
- 11 NIH 3T3



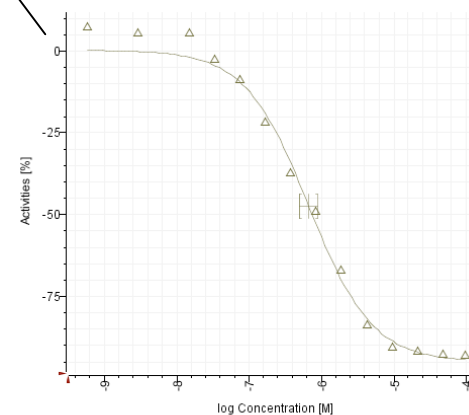
Hexamethyl-p-rosaniline chloride
(Gentian violet)



AB07044785-1

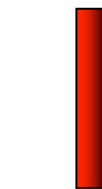


Potassium Chromate



AB02540527-1

Active



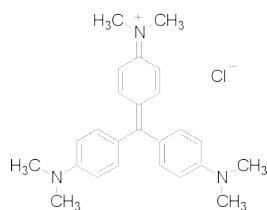
Inactive

Toxicological Profiles of Compounds

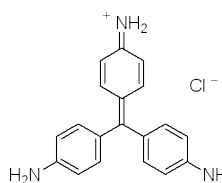
- Toxicity based on the cell types
 - Some of compounds were active in all the cell types
 - Some of compounds were active in the certain types of cells
- Toxicity based on the assays
 - Cell viability assay
 - Caspase 3 assay
- Toxicity based on the time course
 - ACEA real time cell detection system

Chemicals Decreased Cell Viability

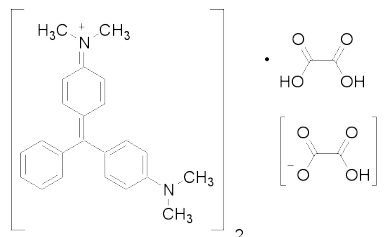
Compound names	NIH 3T3	H-4-IIE	N2a	BJ	Hek 293	HepG2	Jurkat	MRC-5	Sk-N-SH	SH-SY5Y	Kidney cells
tetra-N-Octylammonium bromide	3.2E-06	1.3E-05	3.2E-06	1.4E-05	7.2E-06	4.9E-06	7.9E-06	1.0E-05	9.3E-06	7.9E-06	1.0E-05
Hexamethyl-p-rostaniline chloride (Gentian violet)	1.0E-06	2.0E-06	7.9E-07	9.3E-06	5.1E-06	8.5E-06	3.8E-06	4.1E-06	1.8E-06	1.6E-06	1.3E-05
Pararosaniline hydrochloride	3.2E-06	3.2E-05	4.0E-06	2.4E-05	1.9E-05	1.9E-05	1.5E-05	7.6E-05	4.4E-05	2.5E-06	1.6E-05
Malachite Green Oxalate	2.0E-07	7.9E-08	3.2E-08	3.5E-07	2.5E-07	4.9E-07	2.7E-08	4.6E-07	5.2E-08	1.0E-07	2.0E-07
Digitonin	3.2E-05	2.0E-05	3.2E-05	2.1E-05	2.8E-05	1.3E-05	8.7E-07	2.5E-05	2.8E-05	4.0E-05	7.9E-06
a-Solanine	3.2E-05	3.2E-05	3.2E-05	4.3E-05	3.9E-05	2.3E-05	3.3E-05	4.5E-05	2.5E-05	7.9E-05	5.0E-05
Sodium dichromate dihydrate (VI)	1.6E-05	1.6E-05	3.2E-05	2.0E-05	3.1E-05	4.7E-05	1.6E-06	4.2E-06	1.9E-05	4.0E-07	2.5E-06
Potassium dichromate	1.3E-05	1.6E-05	2.5E-05	8.7E-06	1.9E-05	7.6E-06	6.6E-07	9.8E-06	3.9E-06	4.0E-06	5.0E-07
Sodium Dichromate Dihydrate	3.2E-05	3.2E-05	4.0E-05	1.5E-05	3.2E-05	1.5E-05	2.6E-06	1.1E-05	1.7E-05	1.3E-05	7.9E-06



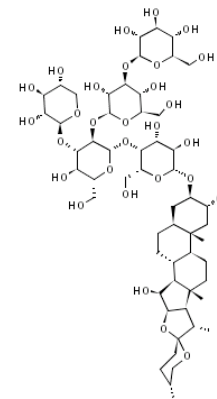
Hexamethyl-p-rostaniline chloride
(Gentian violet)



Pararosaniline hydrochloride



Malachite Green Oxalate



Digitonin

Compound Toxicity Across Assays

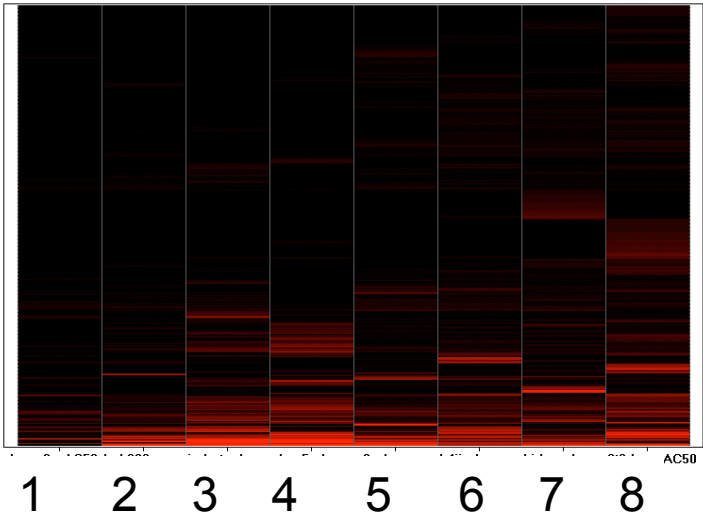
1	HepG2
2	HEK293
3	Jurkat
4	SH-SY5Y
5	N2a
6	H-4-IIE
7	Rat Kidney
8	NIH 3T3

Active

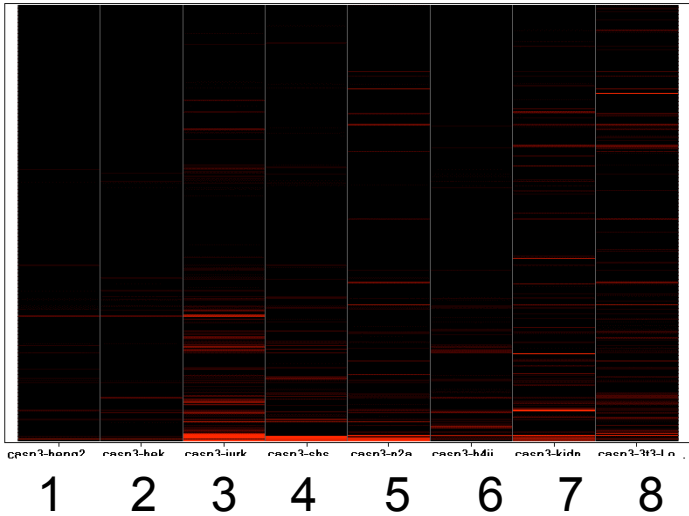


Inactive

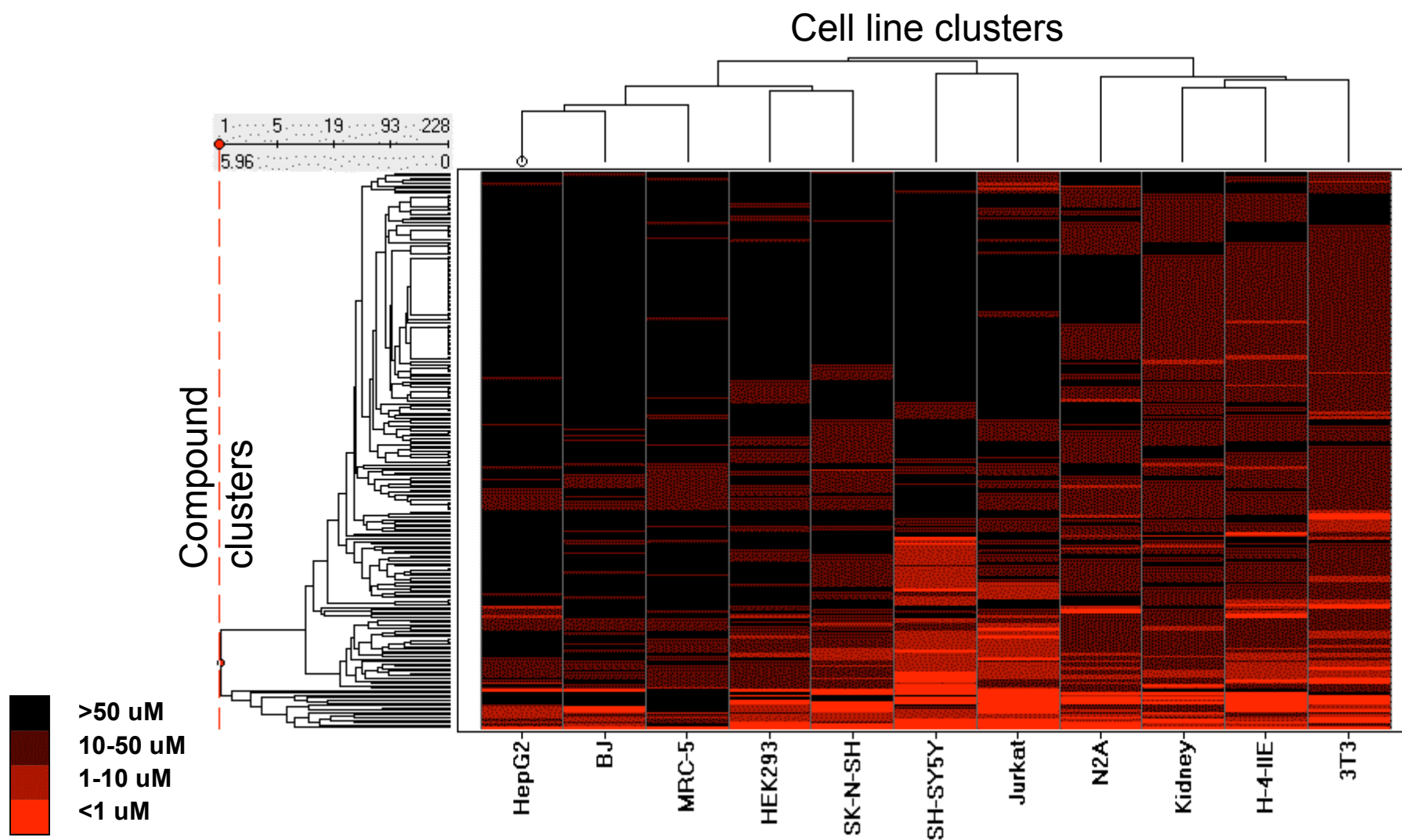
Cell Viability



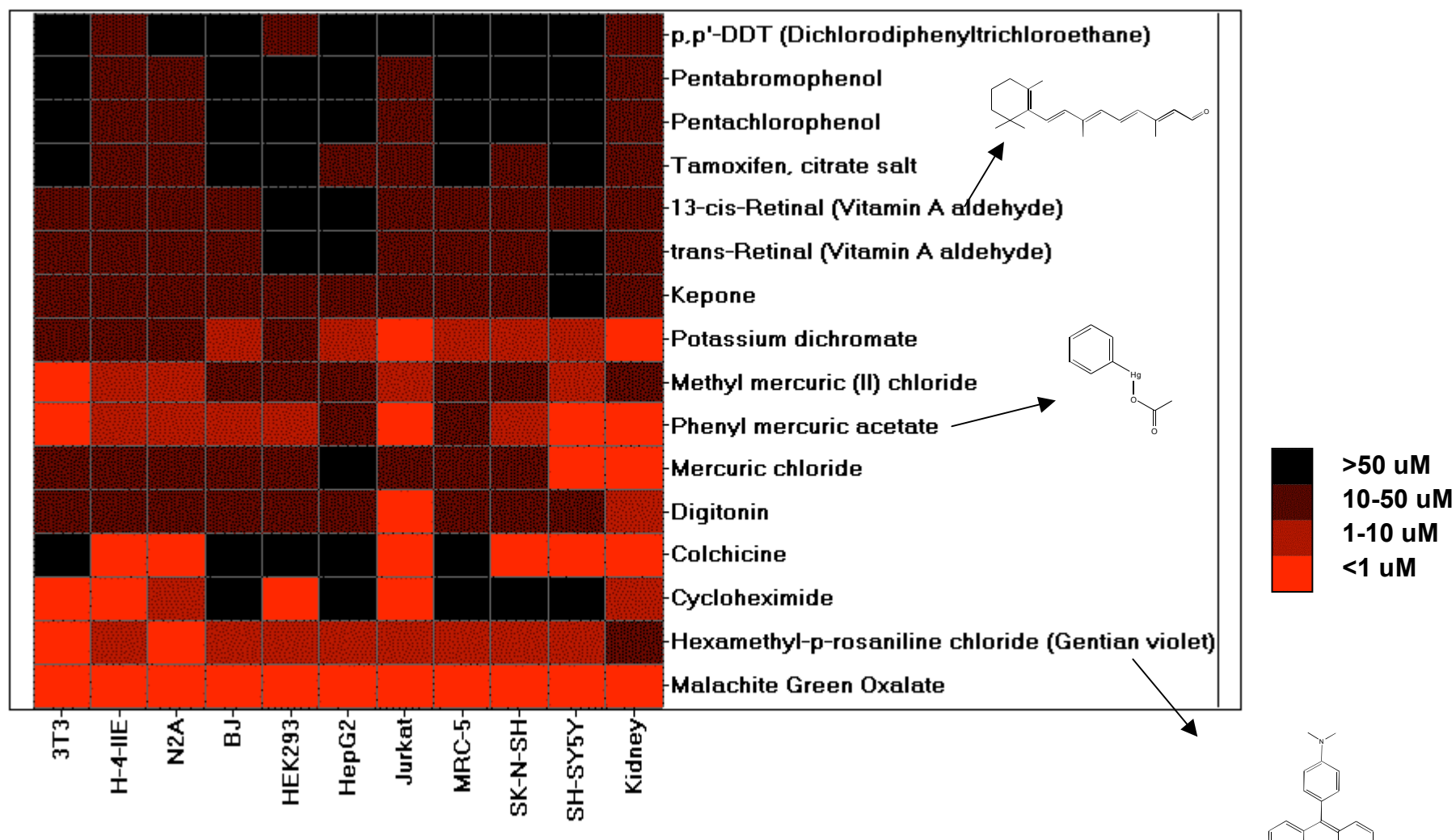
Caspase-3



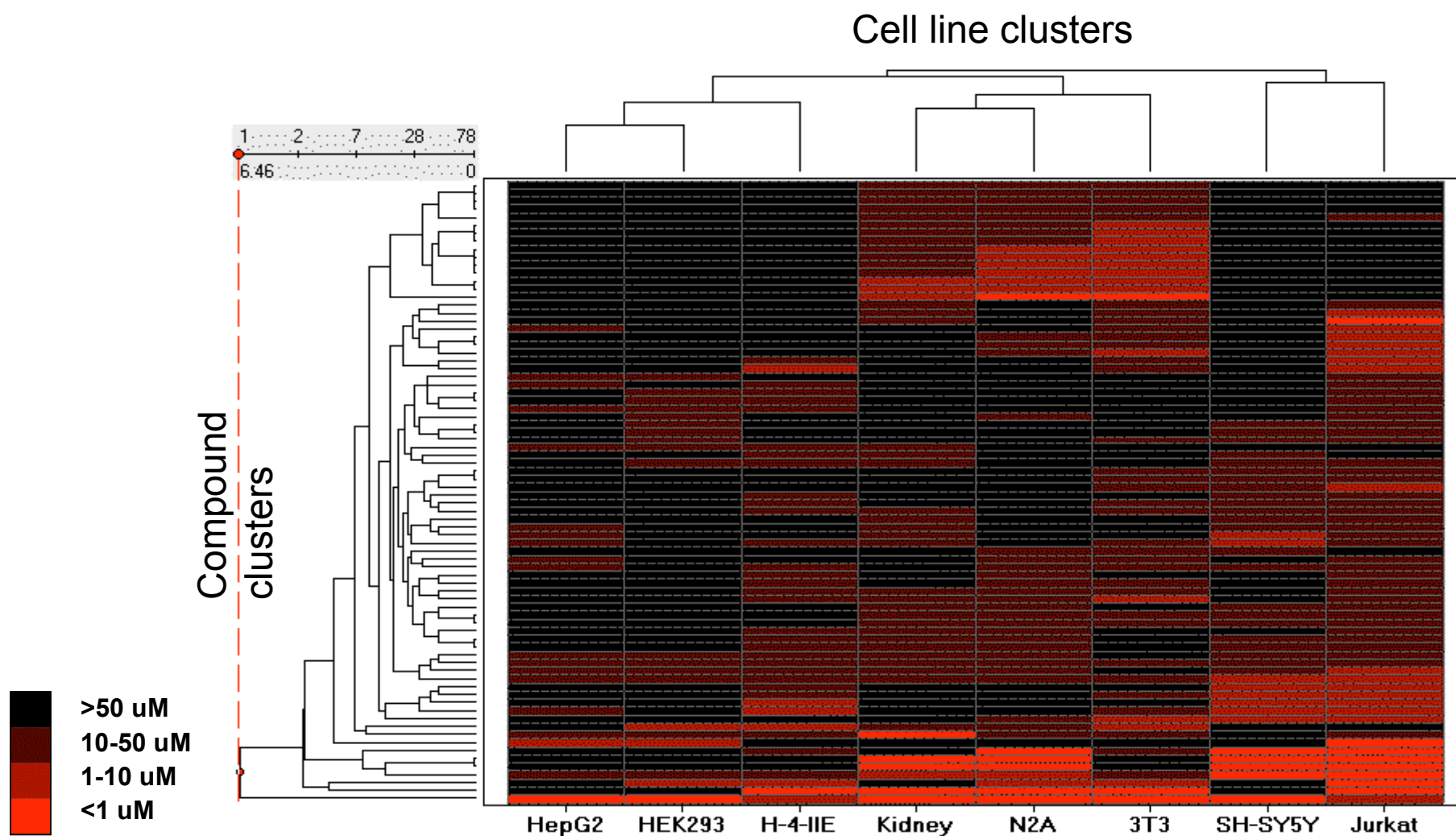
Compound Profile in Cell Viability Assay



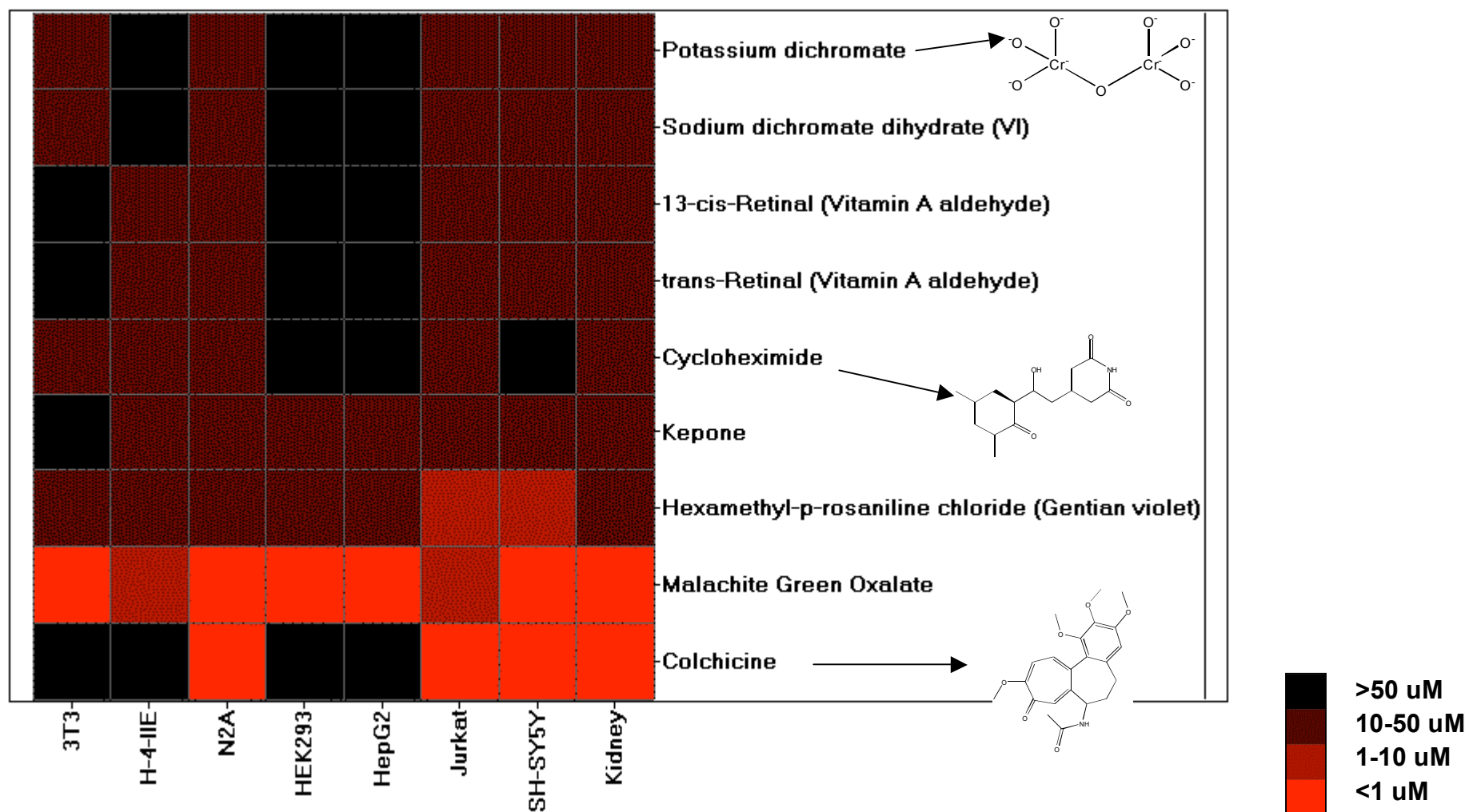
Compound Pattern in Cell Viability Assay



Compound Profile in Caspase-3 Assay



Compound Pattern in Caspase-3 Assay



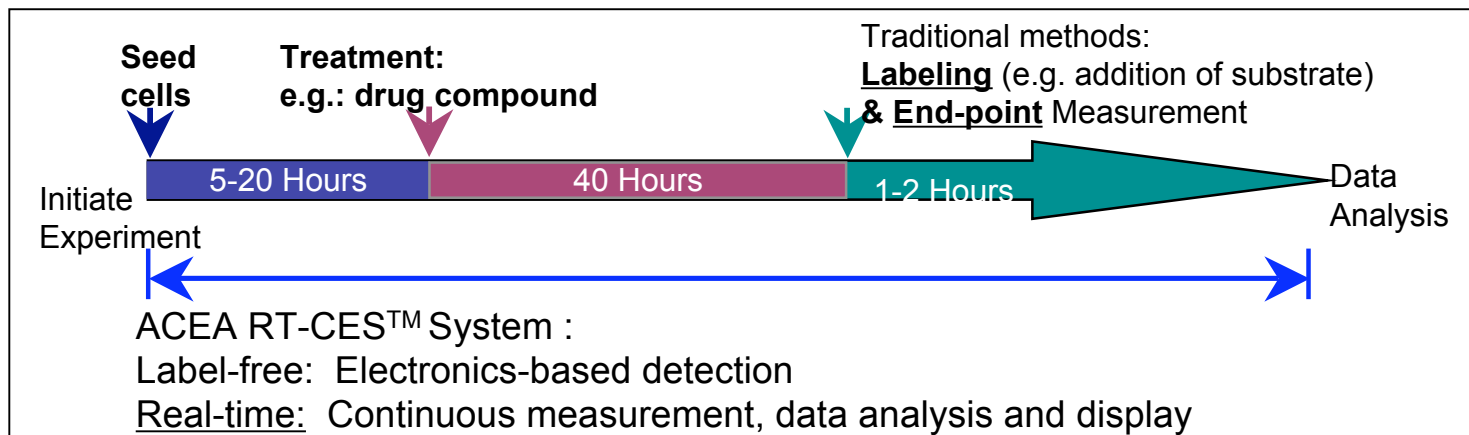
Acea technology: RT-CES™ System Label-free Detection

E-Plate 16™

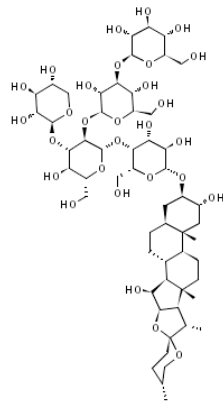
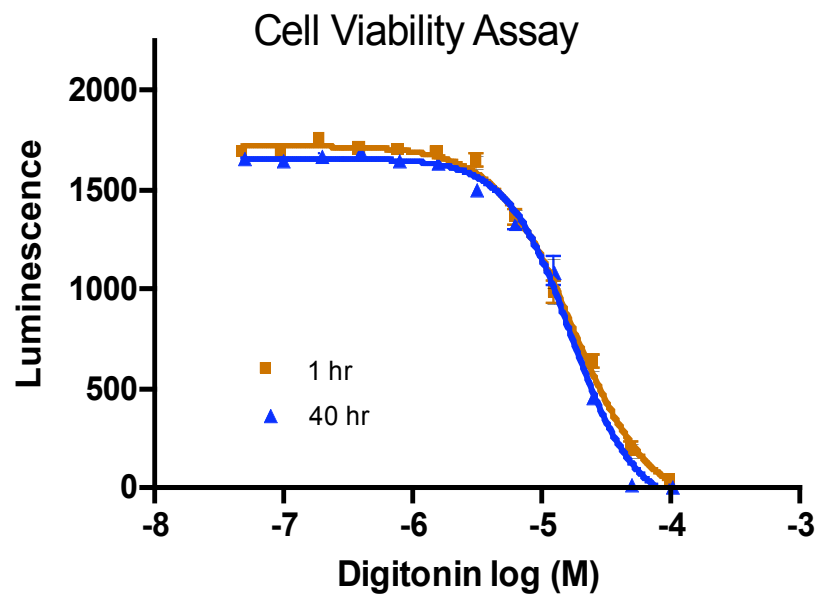
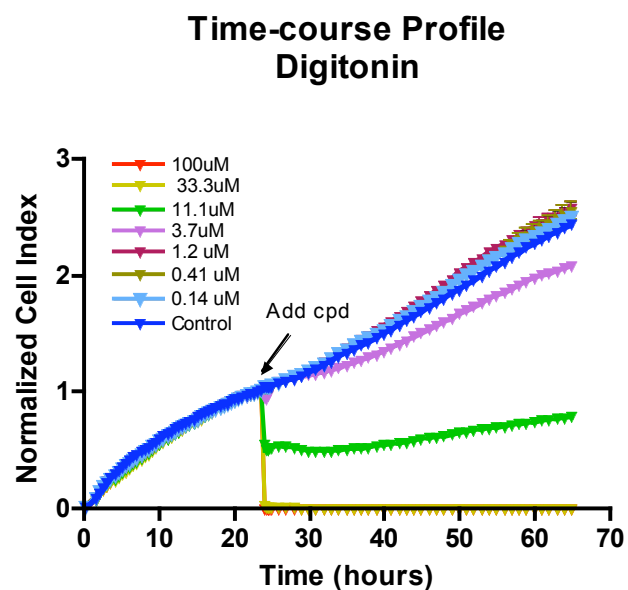


16x Device Station

RT-CES: real time cell electronic sensing

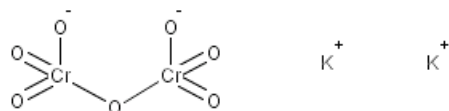
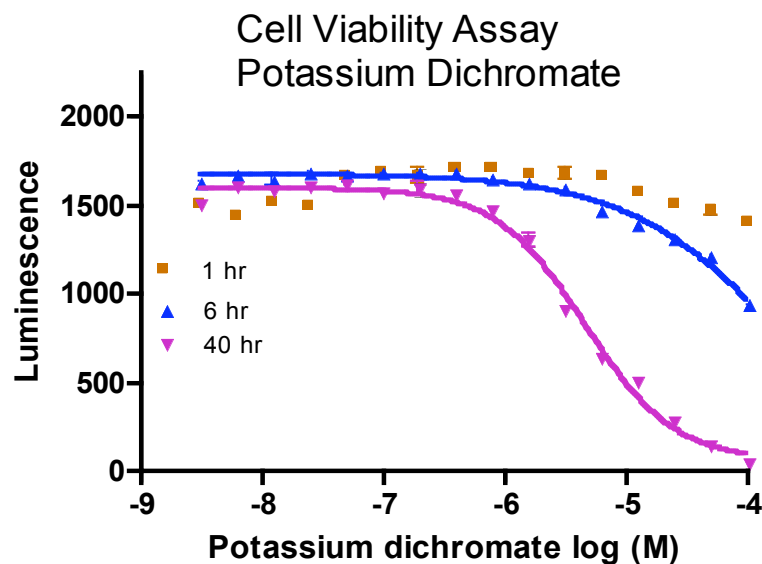
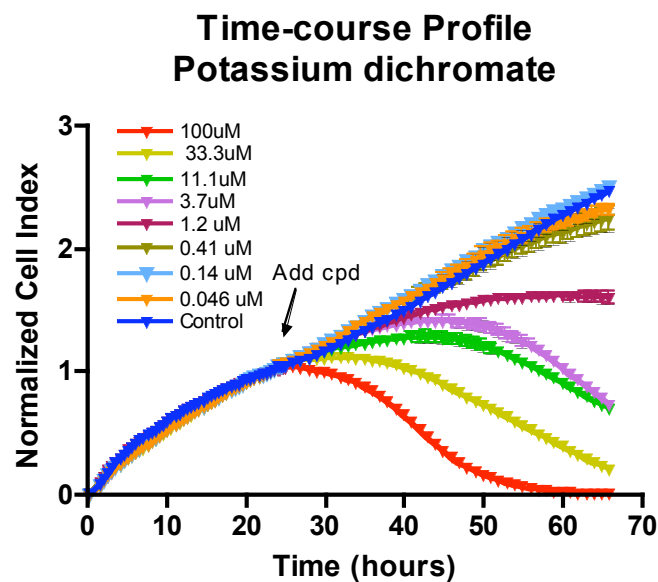


Chemical Signature of Digitonin in HepG2 Cells

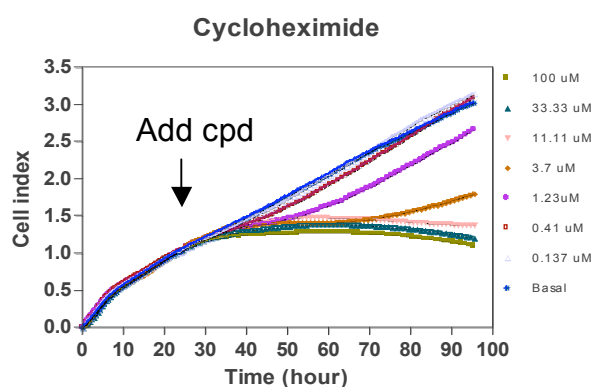


Note: Digitonin is a mild nonionic detergent

Chemical Signature of DNA Damage in HepG2 cells

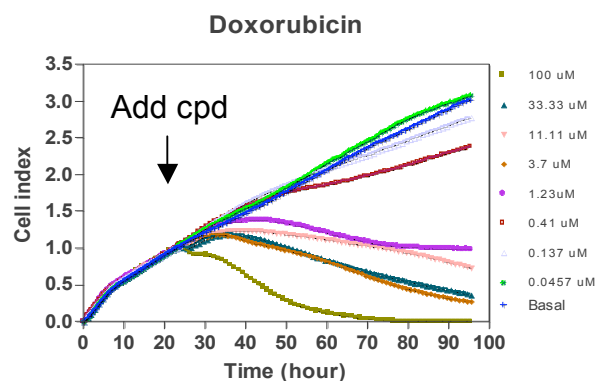
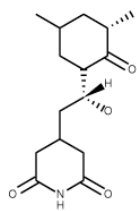


Chemical Signatures of Cytotoxicity Determined by ACEA's RT-CES System in HepG2 Cells



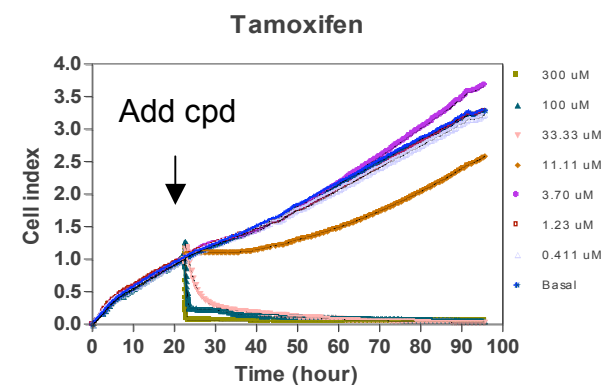
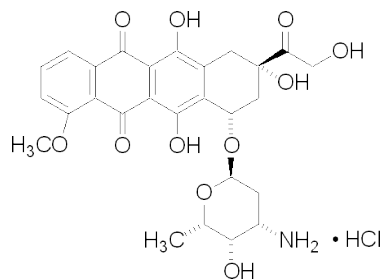
Protein synthesis inhibition

IC₅₀ = 0.94 uM at 40% inhibition (40 hr)



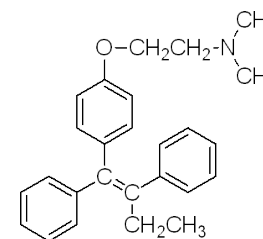
DNA damaging

IC₅₀ = 1.42 uM (40 hr)



Apoptosis, Ion channel, Kinases

IC₅₀ = 12.43 uM (40 hr)



Summary

- NIH Chemical Genomics Center in collaborating with NIEHS/NTP is exploring the new methods to predict toxicity of chemicals
- 1408 chemicals and potential toxins used environmentally were selected for testing in *in vitro* cell-based assays
- Ten human and rodent cell lines derived from six tissues and four types of assays have been used to determine the toxic effects of this collection
- *In vitro* data will be further analyzed and compared with *in vivo* data
- More cell types and assays including high content screening will be applied for the toxic compound screening