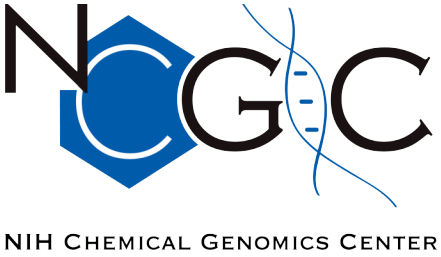
Chemical Compound Profiling of Cell-based Toxicity Assays Using Quantitative High-Throughput Screening

Menghang Xia, Kristine Witt*, Noel Southall, Ming-Hsuang Cho, Ajit Jadhav, Ruili Huang, Cynthia Smith*, Jennifer Fostel*, Raymond Tice*, Jim Inglese, and Chris Austin

NIH Chemical Genomics Center, NIH/NHGRI,
* NTP/NIEHS



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

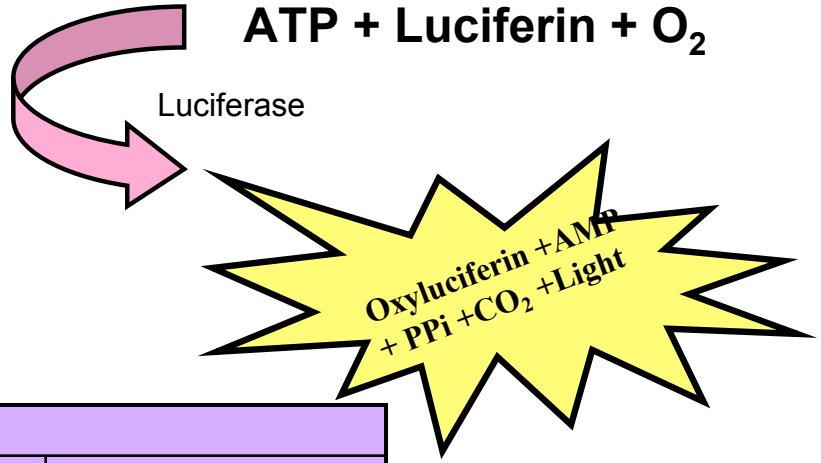
- Assay types:
  - Cytotoxicity assay: measurement of cellular ATP content
  - Caspase 3/7 assay: measurement of caspase 3/7 activity
  - Membrane integrity assay: measurement of LDH release
  - ACEA's RT-CES assay: time-course of toxic effect in cells – kinetic signature
  - Future assays

- 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

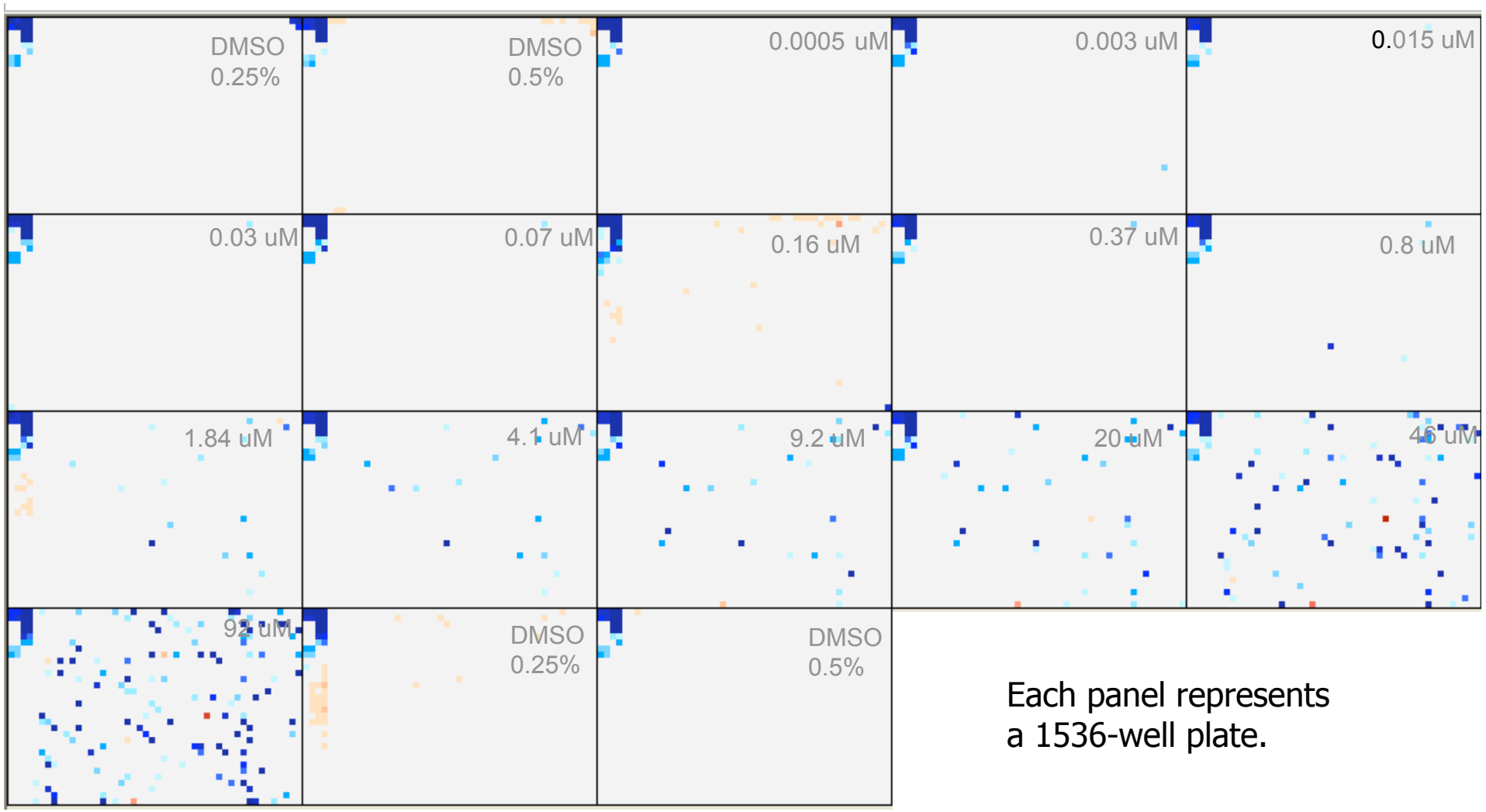
- Description
  - Method of measuring number of viable cells
  - Based on quantitation of ATP, an indicator of metabolic activity
  - Luminescent signal proportional to amount of ATP present
- Applications
  - Cell proliferation
  - Cytotoxicity
  - Cell viability



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

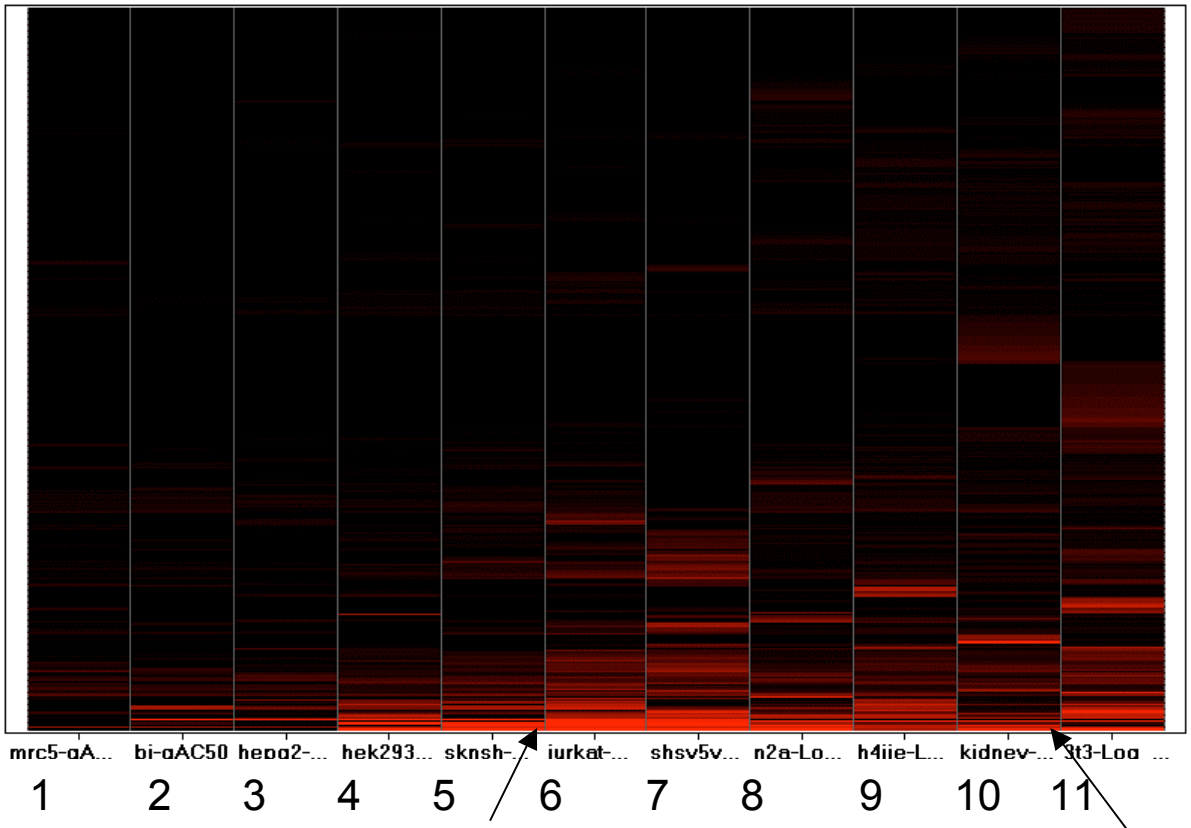
qHTS results map for HepG2 cells

Cell Viability Assay



Each panel represents
a 1536-well plate.

Compound Toxicity Across Cell lines

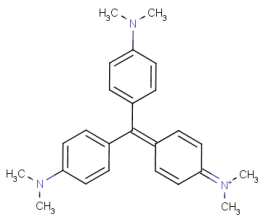


Cell Viability Assay

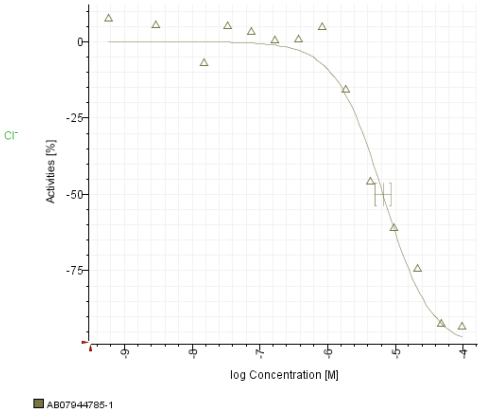
1. MRC-5
2. BJ
3. HepG2
4. HEK293
5. SK-N-SH
6. Jurkat
7. SH-SY5Y
8. N2a
9. H-4-IIE
10. Rat Kidney cells
11. NIH 3T3

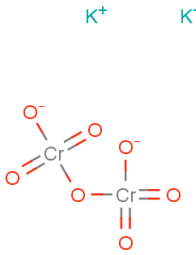
Active

Inactive



Hexamethyl-p-rosaniline chloride (Gentian violet)





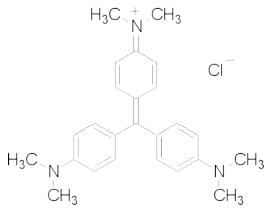
Potassium Chromate

Toxicological Profiles of Compounds

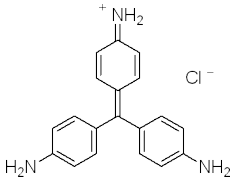
- Toxicity based on the cell types
  - Some of compounds were active in all the cell types
  - Some of compounds were active in the certain types of cells
- Toxicity based on the assays
  - Cell viability assay
  - Caspase 3 assay
- Toxicity based on the time course
  - ACEA real time cell detection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

Chemicals Decreased Cell Viability

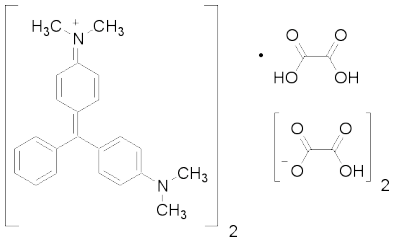
Compound names	NIH 3T3	H-4-IIE	N2a	BJ	Hek 293	HepG2	Jurkat	MRC-5	Sk-N-SH	SH-SY5Y	Kidney cells
tetra-N-Octylammonium bromide	3.2E-06	1.3E-05	3.2E-06	1.4E-05	7.2E-06	4.9E-06	7.9E-06	1.0E-05	9.3E-06	7.9E-06	1.0E-05
Hexamethyl-p-rosaniline chloride (Gentian violet)	1.0E-06	2.0E-06	7.9E-07	9.3E-06	5.1E-06	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



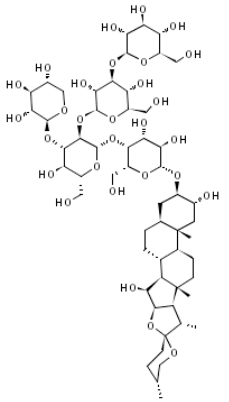
Hexamethyl-p-rosaniline chloride
(Gentian violet)



Pararosaniline hydrochloride



Malachite Green Oxalate



Digitonin

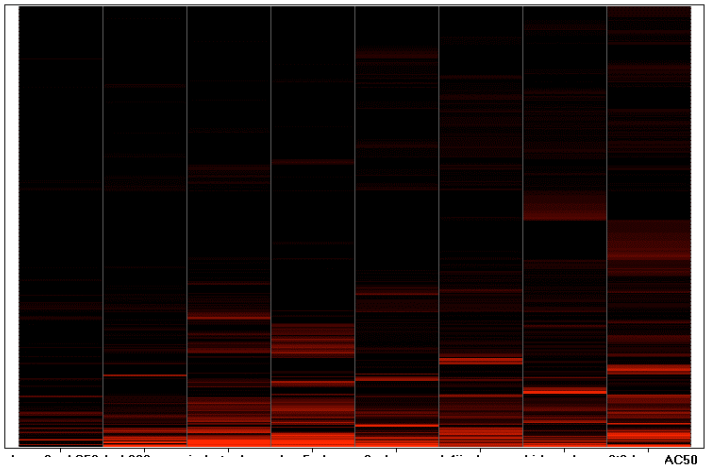
Compound Toxicity Across Assays

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

Active

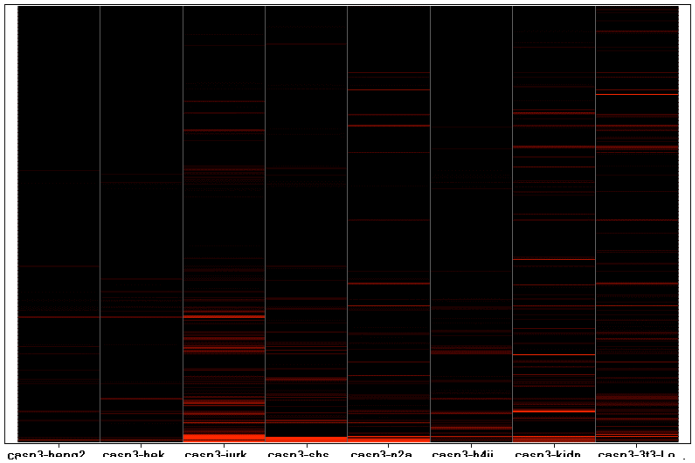
Inactive

Cell Viability



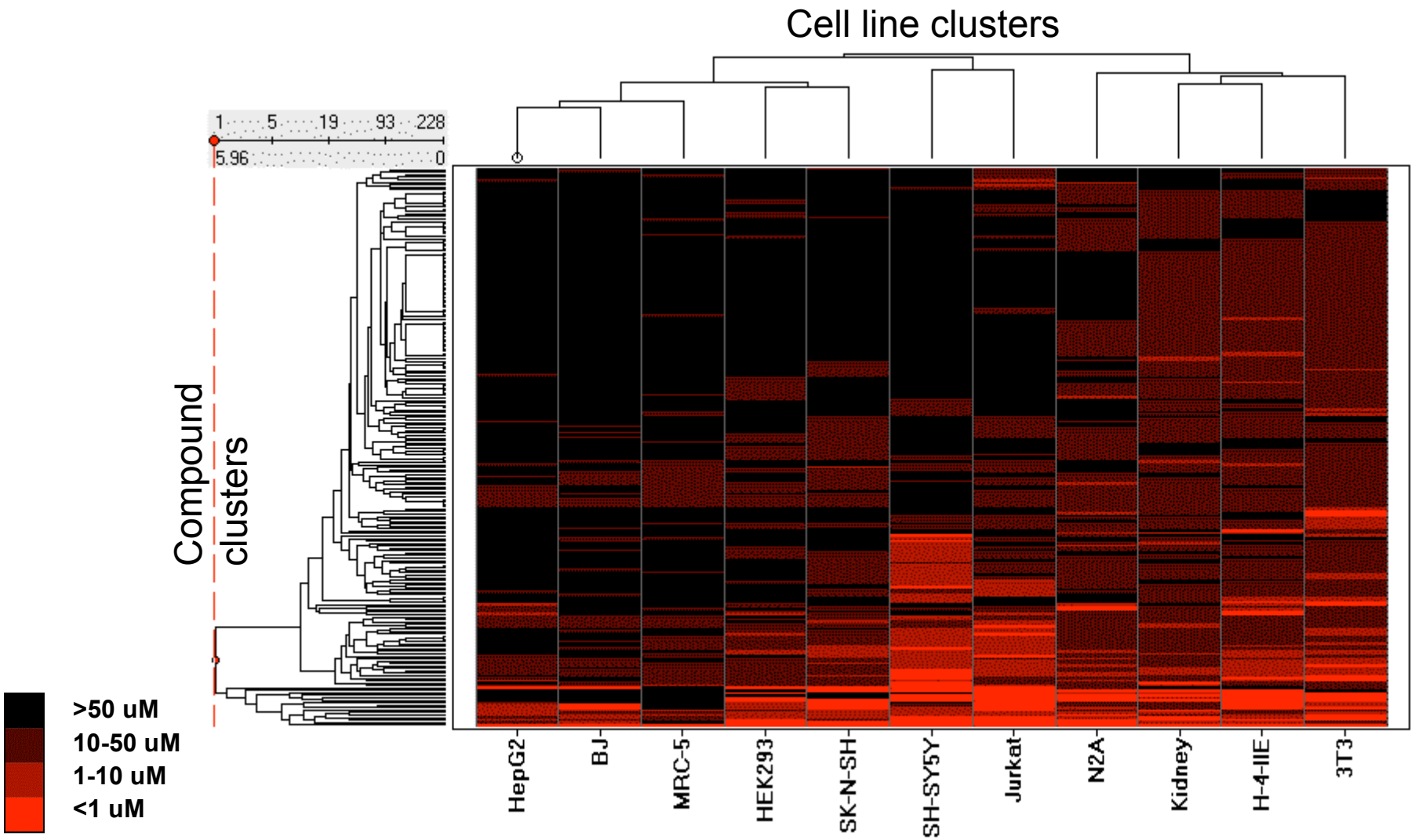
1 2 3 4 5 6 7 8

Caspase-3

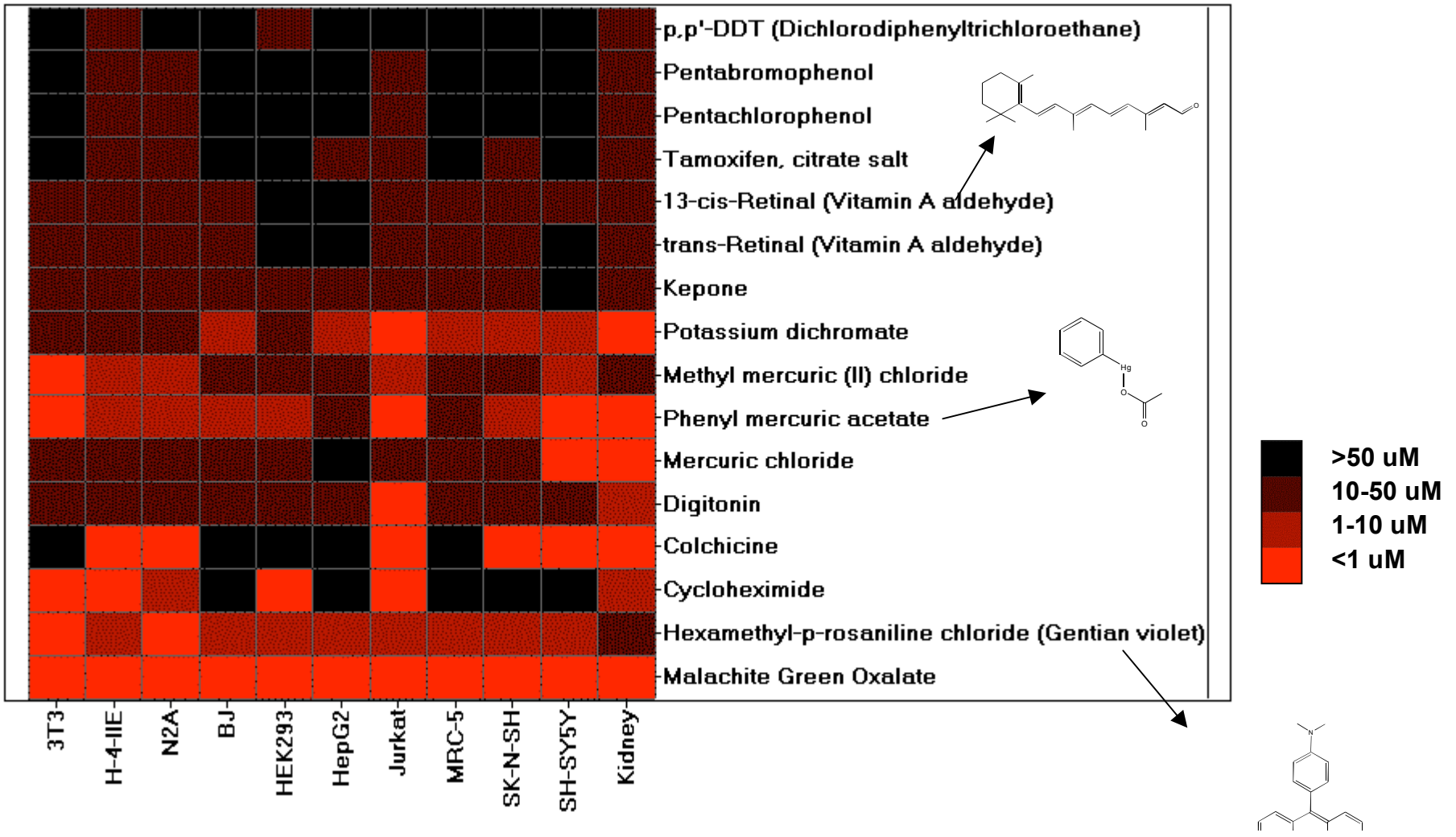


1 2 3 4 5 6 7 8

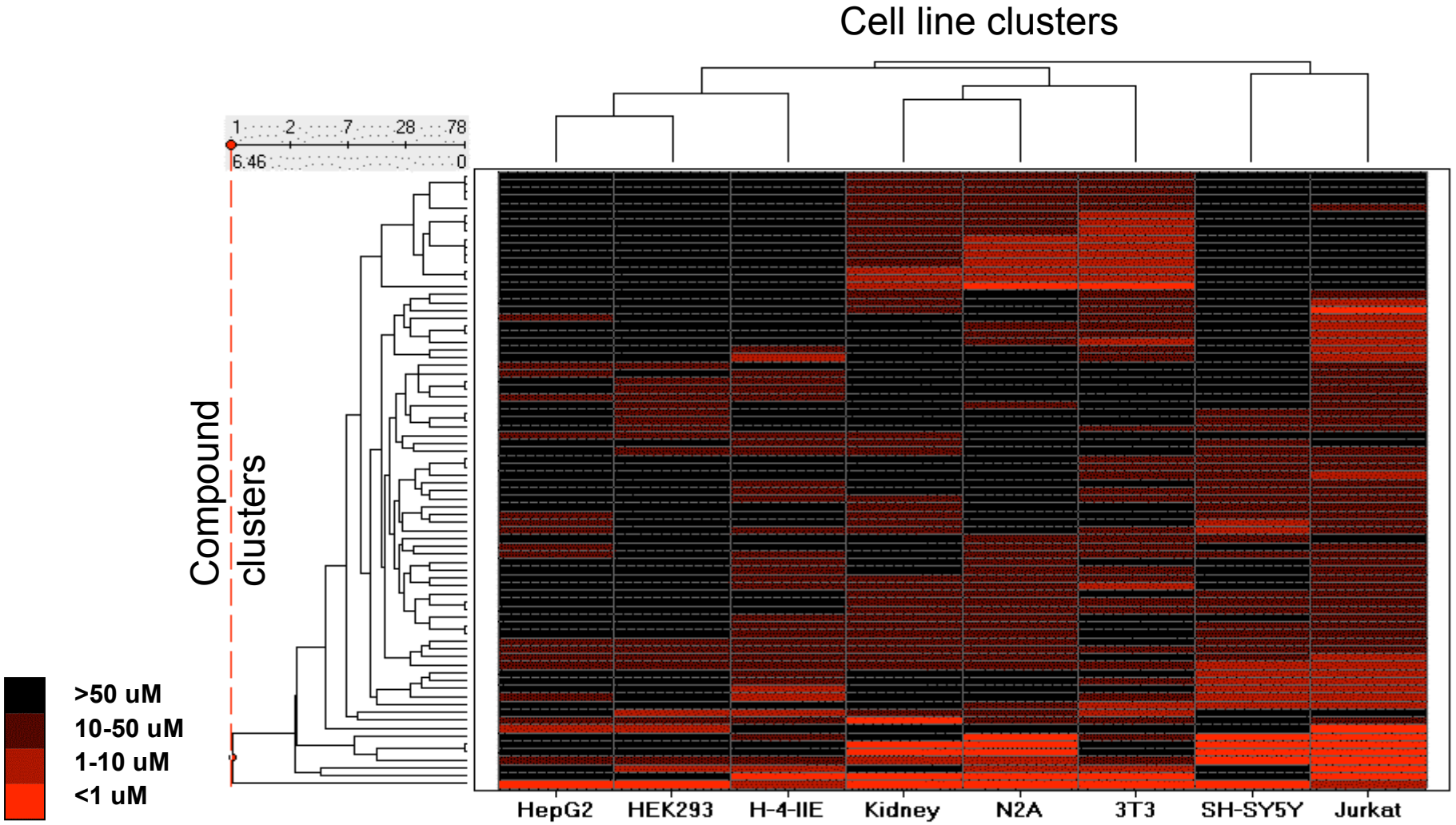
Compound Profile in Cell Viability Assay



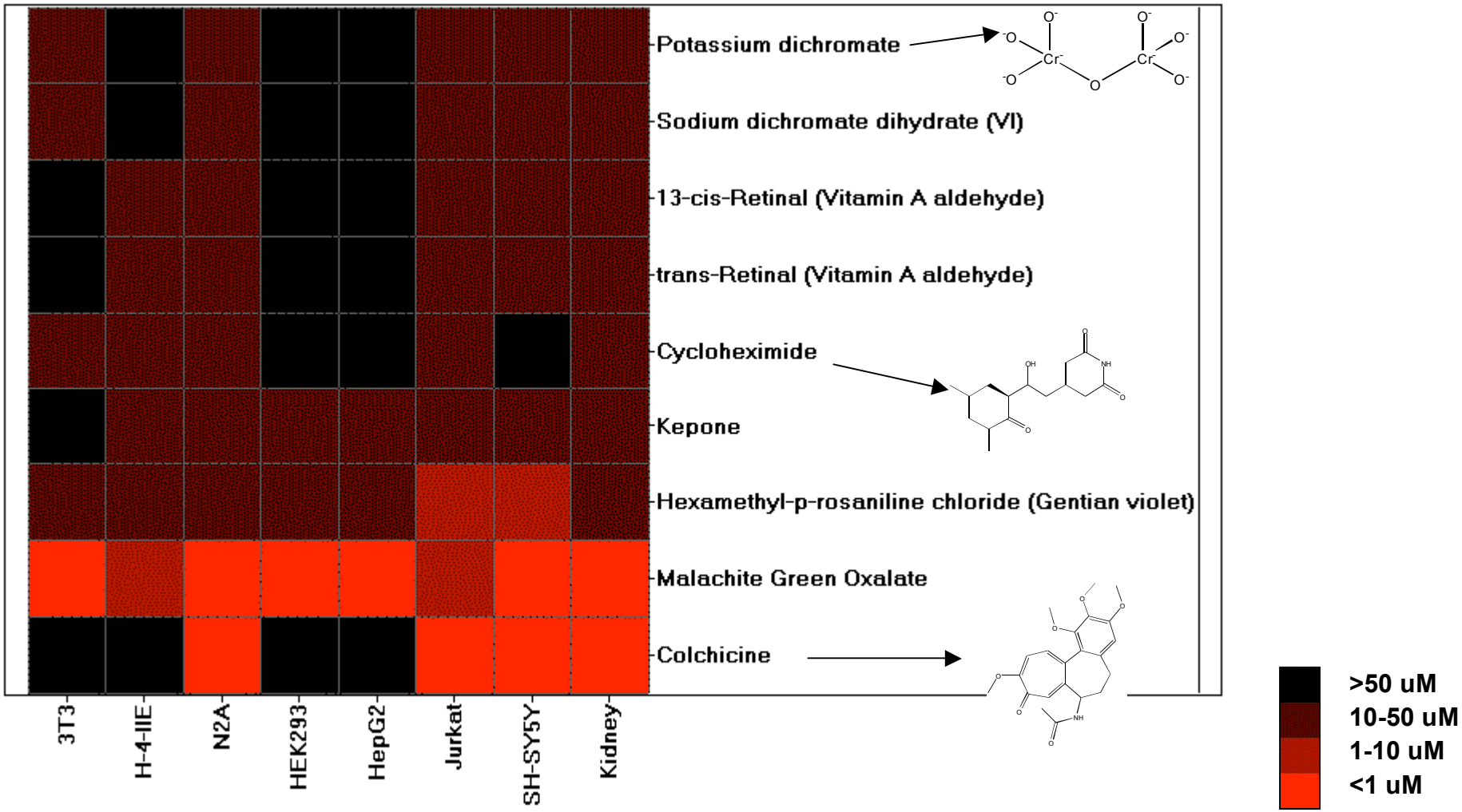
Compound Pattern in Cell Viability Assay



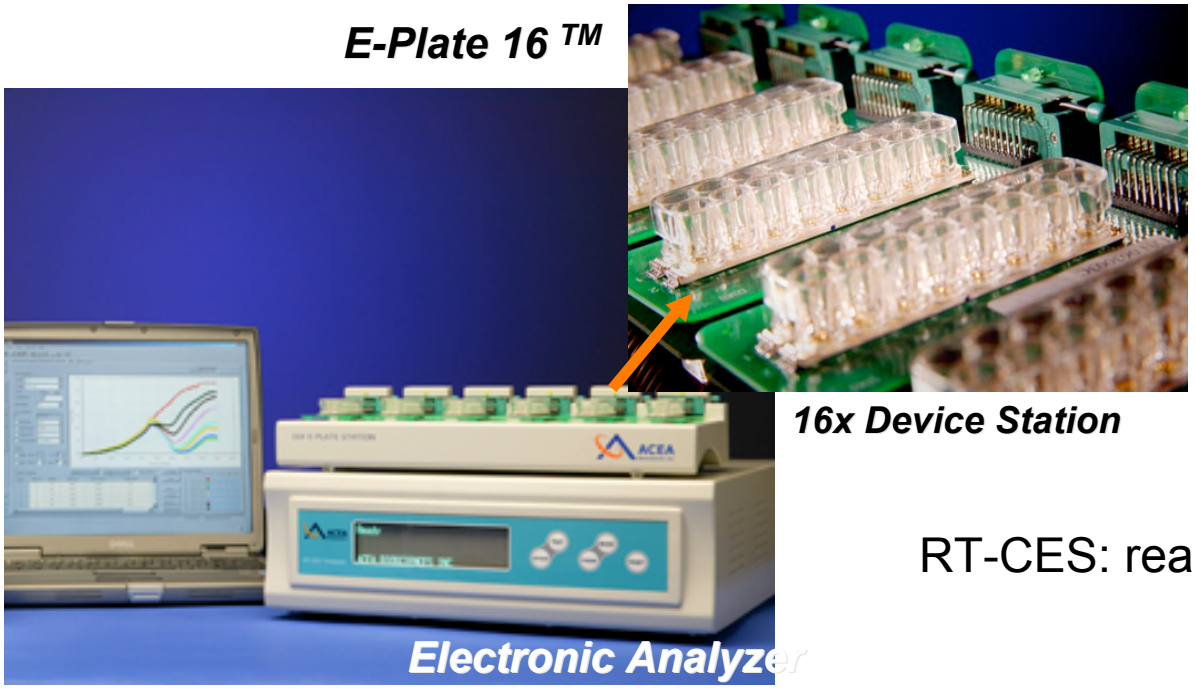
Compound Profile in Caspase-3 Assay



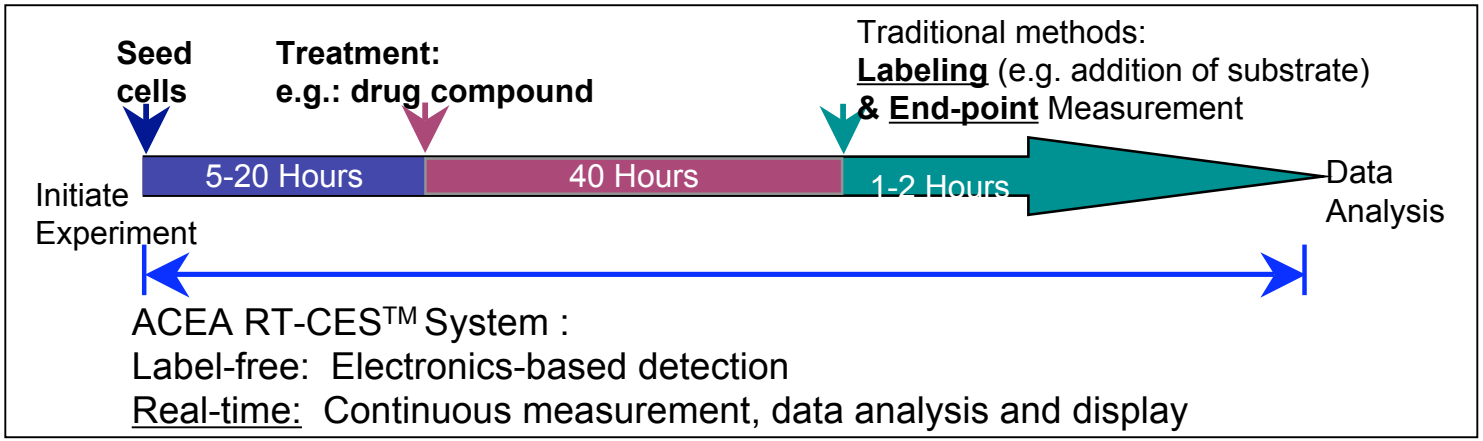
Compound Pattern in Caspase-3 Assay



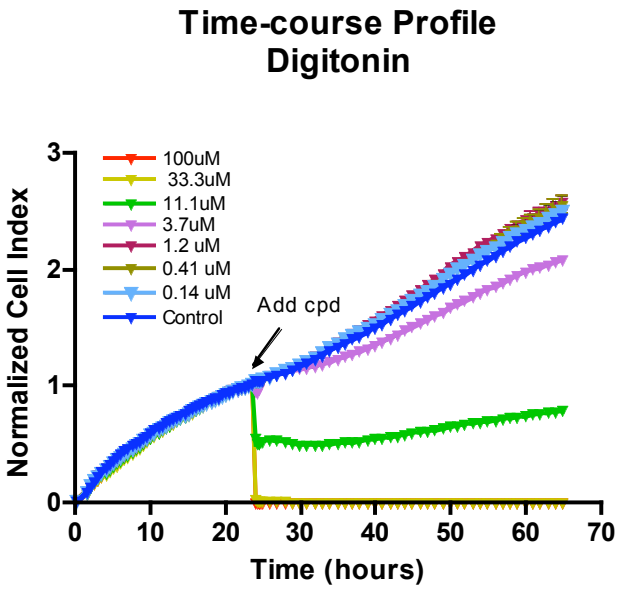
Acea technology: RT-CES™ System Label-free Detection

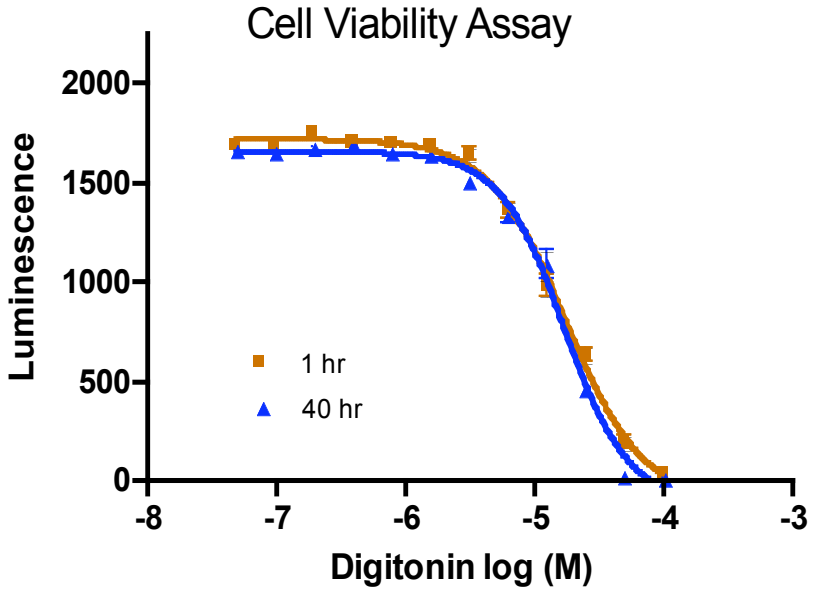


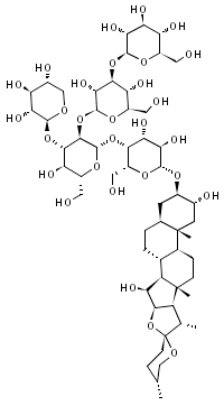
RT-CES: real time cell electronic sensing



Chemical Signature of Digitonin in HepG2 Cells

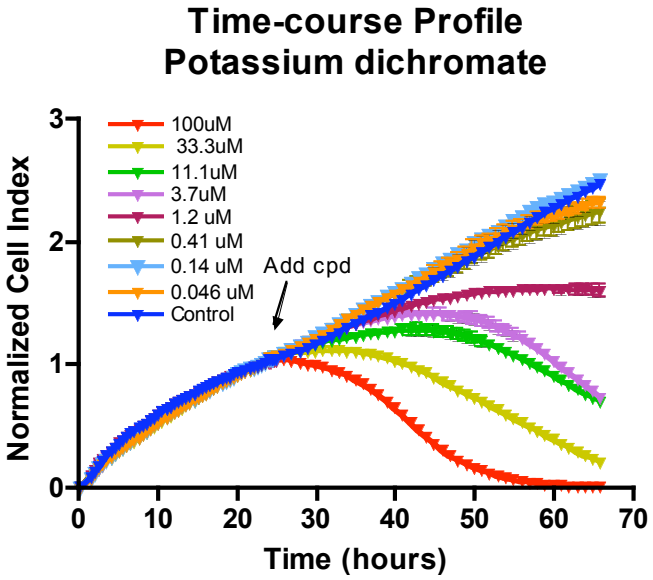


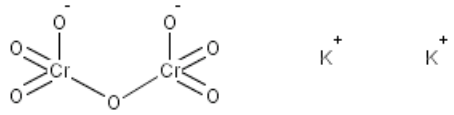


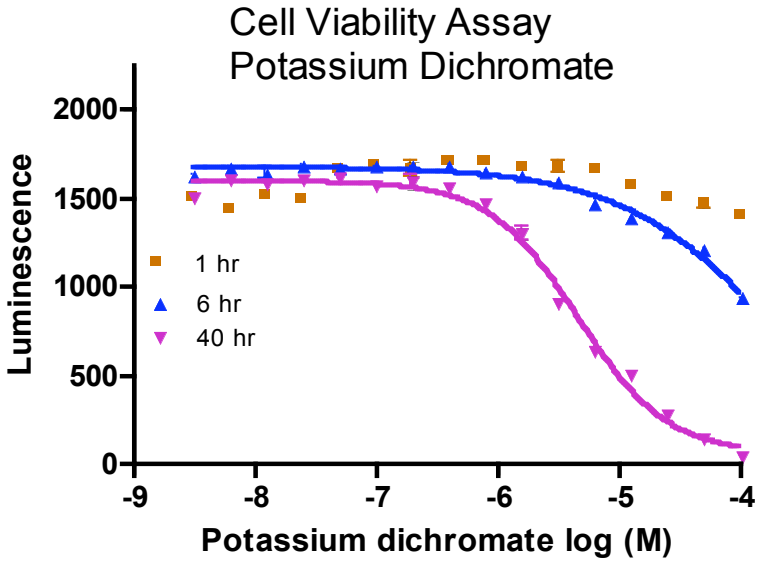


Note: Digitonin is a mild nonionic detergent

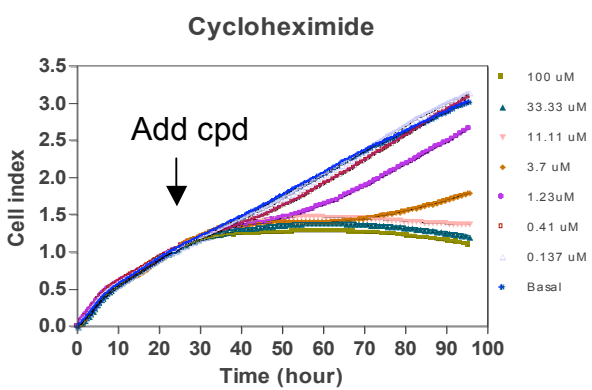
Chemical Signature of DNA Damage in HepG2 cells

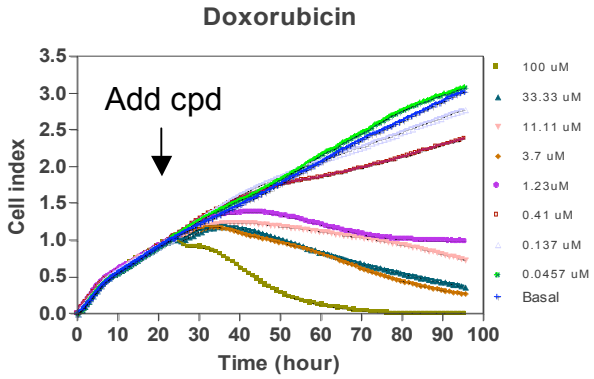


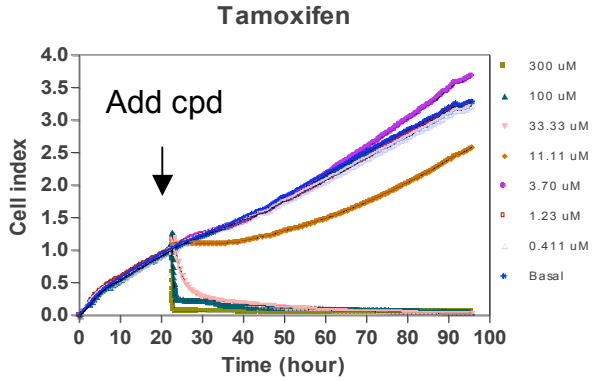




Chemical Signatures of Cytotoxicity Determined by ACEA's RT-CES System in HepG2 Cells

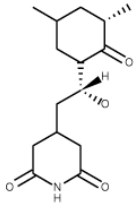






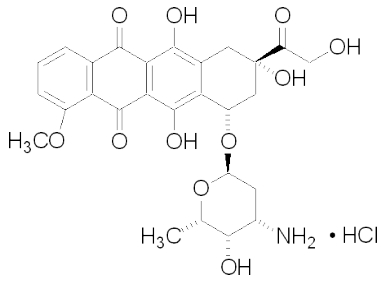
Protein synthesis inhibition

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



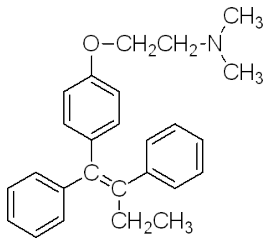
DNA damaging

IC50 = 1.42uM (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