Supporting Information for:

A systematic study of mitochondrial toxicity of environmental chemicals using quantitative high throughput screening

Matias S. Attene-Ramos†, Ruili Huang†, Srilatha Sakamuru†, Kristine L. Witt‡, Gyda C. Beeson§, Louie Shou†, Rick G. Schnellmann§, Craig C. Beeson§,‖, Raymond R. Tice‡, Christopher P. Austin†, and Menghang Xia†

†National Center for Advancing Translational Sciences, National Institutes of Health, Bethesda, MD 20892

‡National Institute of Environmental Health Sciences, Division of the National Toxicology Program, Research Triangle Park, NC 27709

§Department of Drug Discovery and Biomedical Sciences, Medical University of South Carolina, Charleston, SC 29425

‖MitoHealth, Charleston SC 29403

Address correspondence to:

Menghang Xia, Ph.D.

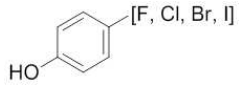
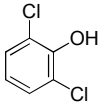
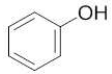
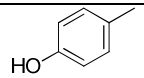
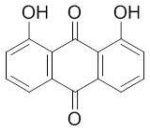
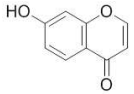
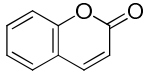
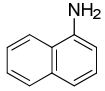
National Institutes of Health, National Center for Advancing Translational Sciences, NIH Chemical Genomics Center

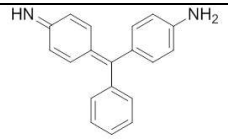
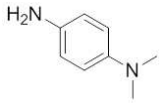
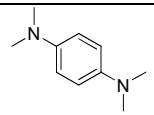
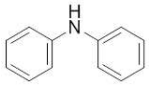
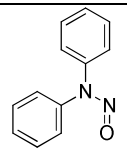
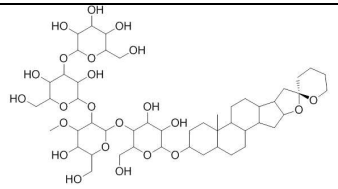
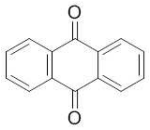
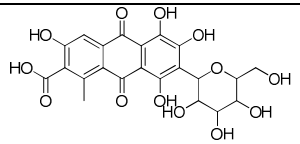
9800 Medical Center Drive, Bethesda, MD 20892-3370

Phone: 301-217-5718

Email: mxia@mail.nih.gov

Table S1 A description of the chemical clusters generated by the 76 active compounds in the MMP assay for both the 1 and 5 h time points. The 76 active compounds for 1 and 5 h were clustered based on structural similarity [Hierarchical (agglomerative) nesting] using the Leadscope software (Leadscope Inc., Columbus, OH, USA), resulting in 11 structural clusters and 23 singletons. Some inactive compound (both in screening and follow up MMP studies) that has a close structure to the members of each cluster has listed as an example.

Cluster	Scaffold	Number of compounds in cluster	Inactive compound
Singletons		23	
1		12	 2,6-Dichlorophenol
2		10	 Cresol (m-/p- mixture)
3		6	
4		5	 Coumarin
5		7	

			1-Naphthylamine
6		3	
7		2	 N,N,N',N'-Tetramethyl-p-phenylenediamine
8		2	 N-Nitrosodiphenylamine
9		2	
10		2	 Carminic acid

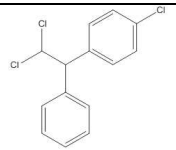
11		2	

Table S2 A summary of previously published screening data (compound potency (half maximal activity concentration, µM)) for the 38 compounds. Activation of caspase 9 and 3/7 was measured at 5 and 16 h of treatment, respectively, while cytotoxicity based on ATP content was measured at 40 h of treatment [22, 34]. Compounds were defined as active (curves 1.1, 1.2 and 2.1), inconclusive (curves 1.3, 1.4, 2.2, 2.3, 2.4 and 3) and inactives (curves 4) according to the type of concentration response curve observed [21, 26].

Sample Name	Caspase 9, 5 h	Caspase 3, 16h	Cytotoxicity (based on ATP content), 40 h
1,5-Naphthalenediamine	Inactive	Inactive	Inactive
1,8-Dihydroxy-4,5-dinitroanthraquinone	Inactive	Inactive	29.33
2,2'-Methylenebis-(4-chlorophenol)	Inactive	Inconclusive	26.59
2,2'-Thiobis(4-chlorophenol)	Inactive	Inactive	27.16
2-Aminoanthraquinone	Inconclusive	Inactive	Inconclusive
3,4,5-Trichlorophenol	Inactive	Inactive	50.12
3,4-Dichlorophenyl isocyanate	Inactive	Inactive	38.70
4,4-Thiobis(6-t-butyl-m-cresol)	Inconclusive	70.79	31.62
4-Cumylphenol	Inactive	Inconclusive	Inactive
4-Hydroxyphenyl retinamide	Inconclusive	31.62	25.12
Apigenin	Inactive	Inactive	41.43

Basic red 9 (p-Rosaniline HCl)	Inactive	Inactive	7.17
Captan	Inactive	Inactive	15.27
Curcumin	Inactive	Inactive	43.42
Diethylstilbestrol [DES]	Inactive	Inactive	Inactive
Digitonin	Inactive	Inactive	12.24
Diphenylurea	Inactive	Inactive	Inconclusive
Emodin	Inconclusive	Inactive	46.49
Formulated fenaminosulf (Dexon)	Inactive	Inactive	4.48
Genistein (4',5,7-Trihydroxyisoflavone)	Inconclusive	Inactive	Inconclusive
Hexachlorophene	Inactive	Inactive	6.50
Hexamethyl-p-rosaniline chloride (Gentian violet)	70.79	28.18	2.61
Kaempferol	Inactive	Inactive	Inactive
Kepone	Inconclusive	Inconclusive	29.33
Malachite green oxalate	Inconclusive	0.63	1.65
Nitazoxanide	Inactive	Inactive	9.36
N-Phenyl-2-naphthylamine	Inactive	Inactive	Inactive
o,p'-DDD (Mitotane)	Inactive	Inactive	Inactive

o-Benzyl-p-chlorophenol	Inactive	Inactive	Inactive
p -n -Nonylphenol	Inconclusive	Inconclusive	Inconclusive
p-Aminophenol	Inactive	Inactive	63.90
Phenmedipham	Inactive	Inactive	Inconclusive
Phenyl mercuric acetate	Inactive	Inactive	5.88
Resveratrol	Inconclusive	Inactive	Inconclusive
Riboflavin	Inactive	Inactive	Inactive
Silibinin	Inactive	Inactive	Inactive
tetra-N-Octylammonium bromide	Inconclusive	14.13	4.18
Zearalenone	Inactive	Inactive	29.91

Figure S1 qHTS MMP assay experimental design: a) A detailed protocol of the MMP screen, and b) a plate map of the control plate used for the screen. Columns 1 to 3 have different concentrations of the control FCCP. Column 4 has DMSO. Columns 5 to 48 are empty (in the assay plates will receive compounds from the library (compound) plates.

a

Step	Parameter	Value	Description
1	Plate cells	2000 cells/5uL	Plate Cells in black clear bottom 1536 well plates, using 8 tip dispense (Multidrop)
2	Incubation time	Overnight	Incubate at 37° C, 5% CO_2 .
3	Compound Addition	23nL	Pintool transfer of control (1-4 columns) and compound library (5-48 columns).
4	Incubation time	1 hr/5h	Incubate at 37° C, 5% CO_2.
5	Reagent	5uL	Addition of MMP dye solution (Bioraptr)
6	Incubation time	30 min	Incubate at 37° C, 5% CO_2
7	Readout	Envision	Protocol name: Mitochondrial FITC Bottom, at Ex: FITC 485; Em: FITC 535

b