High-throughput phenotypic profiling for grouping of 68 conazoles

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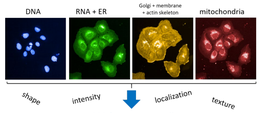
Introduction

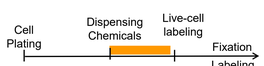
What is phenotypic profiling?



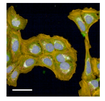
- Image-based phenotypic profiling with the Cell Painting assay is a chemical screening method that measures a large variety of morphological features of individual cells in *in vitro* cultures.
- No requirement for *a priori* knowledge of molecular targets.
- **Is used as an efficient and cost-effective method for evaluating chemical bioactivity.**

Methods

Experiment



U-2 OS osteosarcoma cells

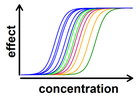


Fluorescent labels
DNA: H-33342
RNA: SYTO14
ER: Concanavalin A-488
Actin: Phalloidin-568
Golgi + Membrane: wheat germ agglutinin (WGA) -555
Mitochondria: MitoTracker

Data processing

1. **Segmentation** Outline of cell nuclei and cytoplasm is traced.
2. **Feature extraction** Profiling of cell compartments and fluorescent channels for different feature groups, such as intensity and texture.
3. **Data reduction & normalization** Cell-level data is aggregated to the well-level and normalized

profile →



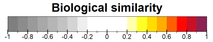
see
- Nyffeler et al. 2020 PMID: 31899216
- Nyffeler et al. 2021 PMID: 32862757

Estimation of a phenotype altering concentration (PAC).

Calculation of biological similarity

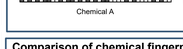
1. **Feature Selection** Features with low reproducibility or high correlation to another feature were removed. 289/1300 features were retained.
2. **Selection of conditions** The lowest three concentrations above the PAC are selected.
3. **Generation of signatures** replacing |values| < 1.5 with 0
4. **Comparison of signatures** Compute Kendall correlation of the 289 selected features.
5. **Aggregate data by chemical** Retain for each chemical pair the highest correlation value

Biological similarity



Calculation of structural similarity

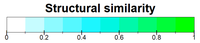
1. **Feature Selection:** 729 ToxPrint fingerprints



2. **Comparison of chemical fingerprints** Jaccard (Tanimoto) similarity:

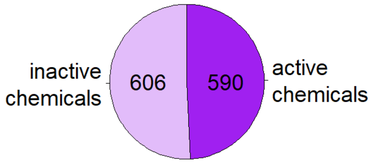
$$J(A,B) = \frac{|A \cap B|}{|A \cup B|} \frac{\#\text{ shared structural features}}{\text{total number of measured features}}$$

Structural similarity



Results: Screen of 1199 chemicals

Identification of bioactive chemicals



➔ **49% of the tested chemicals were active**

Fig.1: Results of screening 1199 chemicals from the ToxCast library. Note that three chemicals had < 4 non-cytotoxic concentrations left and could not be analyzed for concentration-responsiveness.

Clustering of phenotypic profiles

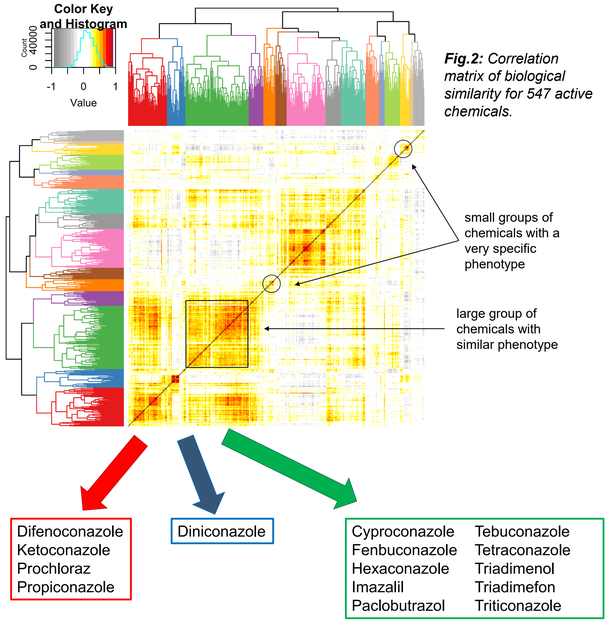


Fig.2: Correlation matrix of biological similarity for 547 active chemicals.

➔ **Diniconazole clusters away from all other conazoles.**

Aims

1. **Screen a set of 1199 environmental chemicals in the HTPP assay**
2. **Examine similarities in phenotypic profiles observed amongst active chemicals.**
3. **Case study of conazoles:**
   a) **Can conazoles be grouped based on their phenotype?**
   b) **Can similarities and differences in phenotypic profiles of conazoles be confirmed in a secondary screen?**

Results: Conazoles (I)

The main screen included 18 conazoles, of which 16 were bioactive:

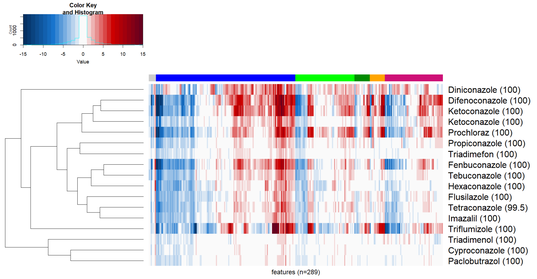


Fig.3: Heatmap of the phenotypic profile of 16 conazoles. The number in brackets indicates the test concentration in μM. Note that ketoconazole was tested twice.

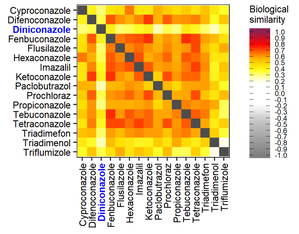


Fig.4: Correlation matrix of biological similarity for the 16 active conazoles.

➔ **The phenotypic profile of diniconazole is different from that of all other tested conazoles.**

Conclusions

1. **49% of the tested chemicals were active at ≤ 100 μM**
2. **Multiple phenotypic profiles were observed, some were shared among many chemicals while others were specific to a small group of chemicals.**
3. **Diniconazole induces a phenotypic profile different from structurally similar conazoles. Instead, diniconazole induces a profile similar to known disruptors of microtubules.**

➔ **HTPP can be used to group chemicals based on biological similarity.**

Results: Conazoles (II)

For the follow up screen, chemicals available in the ToxCast inventory were selected if they had structural similarity (Jaccard similarity > 0.5) with one of the initial conazoles. A total of 69 conazole-like chemicals were retested in HTPP, of which 42 were active.

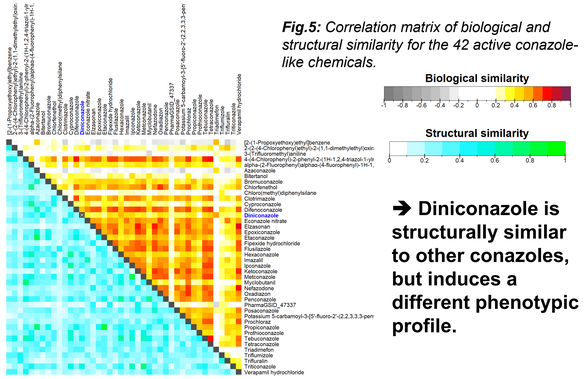


Fig.5: Correlation matrix of biological and structural similarity for the 42 active conazole-like chemicals.

➔ **Diniconazole is structurally similar to other conazoles, but induces a different phenotypic profile.**

Chemicals with the highest biological similarity to diniconazole:

DTXSID	Chemical Name	Conc [uM]	Biol Sim	
1 DTXSID0021125	Phenolphthalein	100	0.55	★
2 DTXSID2032398	Fludioxonil	30.1	0.547	
3 DTXSID2032500	Fenpyroximate (Z,E)	100	0.546	
4 DTXSID8046666	Methyl Violet	3	0.535	
5 DTXSID2037712	4,4',4''-Ethane-1,1,1-triyltriphenol	100	0.516	
6 DTXSID1024338	Thiophanate-methyl	100	0.511	★
7 DTXSID4022442	Bisphenol B	100	0.509	
8 DTXSID5020576	17alpha-Ethinylestradiol	100	0.503	★
9 DTXSID6032192	Tri-o-cresyl phosphate	100	0.491	
10 DTXSID9020114	Acarimine hydrochloride	10	0.482	★
11 DTXSID2023224	Loratadine	100	0.48	★
12 DTXSID6021248	Rotenone	1	0.478	
13 DTXSID3020465	Diethylstilbestrol	30	0.471	★
14 DTXSID1021243	Rhodamine 6G	10	0.458	
15 DTXSID8029868	Atorvastatin	94.5	0.456	
16 DTXSID9032379	Dithiopyr	99.6	0.455	

DTXSID	Chemical Name	Conc [uM]	Biol Sim	
16 DTXSID4020870	2,2'-Methylenebis(4-methyl-6-tert-butylphenol)	30	0.455	
16 DTXSID1034181	Raloxifene hydrochloride	30	0.455	
19 DTXSID8041248	2,5-Di-tert-butylbenzene-1,4-diol	100	0.451	
20 DTXSID0020573	17beta-Estradiol	100	0.449	★
21 DTXSID5032525	Bifenazate	100	0.448	
21 DTXSID7024081	Endothal	100	0.448	
23 DTXSID1026900	Dodecyltrimethylammonium chloride	30	0.443	
24 DTXSID1033664	17-Methyltestosterone	100	0.436	★
25 DTXSID8032113	Tebuconazole	100	0.435	
26 DTXSID6020226	Cadmium chloride	100	0.429	
27 DTXSID9032329	Bensulide	100	0.423	
28 DTXSID8032548	Fenbuconazole	100	0.422	
29 DTXSID5022871	Amitraz	100	0.42	
30 DTXSID7029879	Ketoconazole	100	0.417	

★: Literature evidence for modulators of microtubule assembly.

➔ **A subset of chemicals with biological similarity to diniconazole are known to affect microtubules.**

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