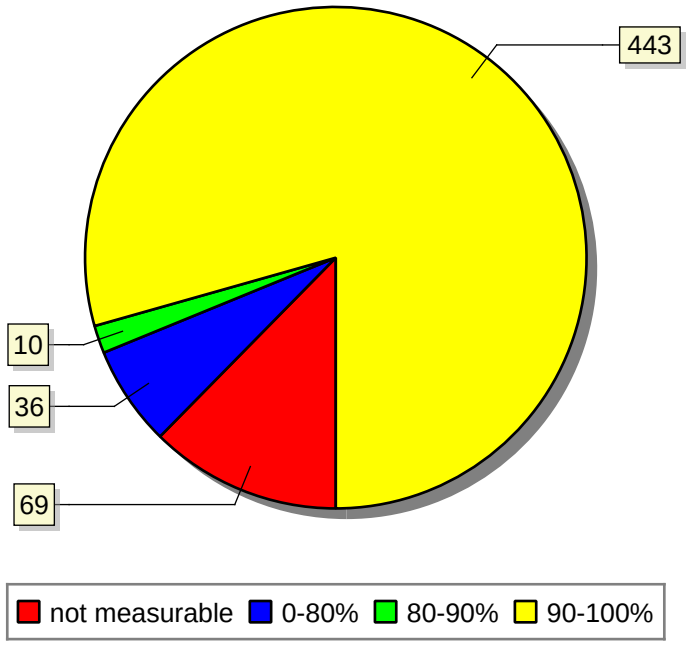
CBB3: Carboxylate/ketone and amine fragments. Library designed by Dr. Michael Lisurek, 4576 compounds.

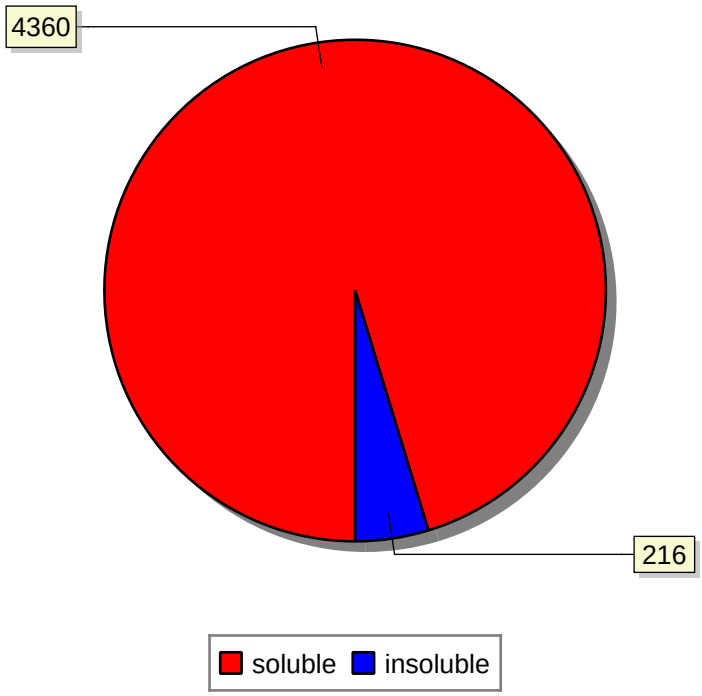
LC-MS purity distribution of compounds that were active in primary screening



Purity Range	Number of Samples	Percentage
not measurable	69	12
0-80%	36	6
80-90%	10	1
90-100%	443	79

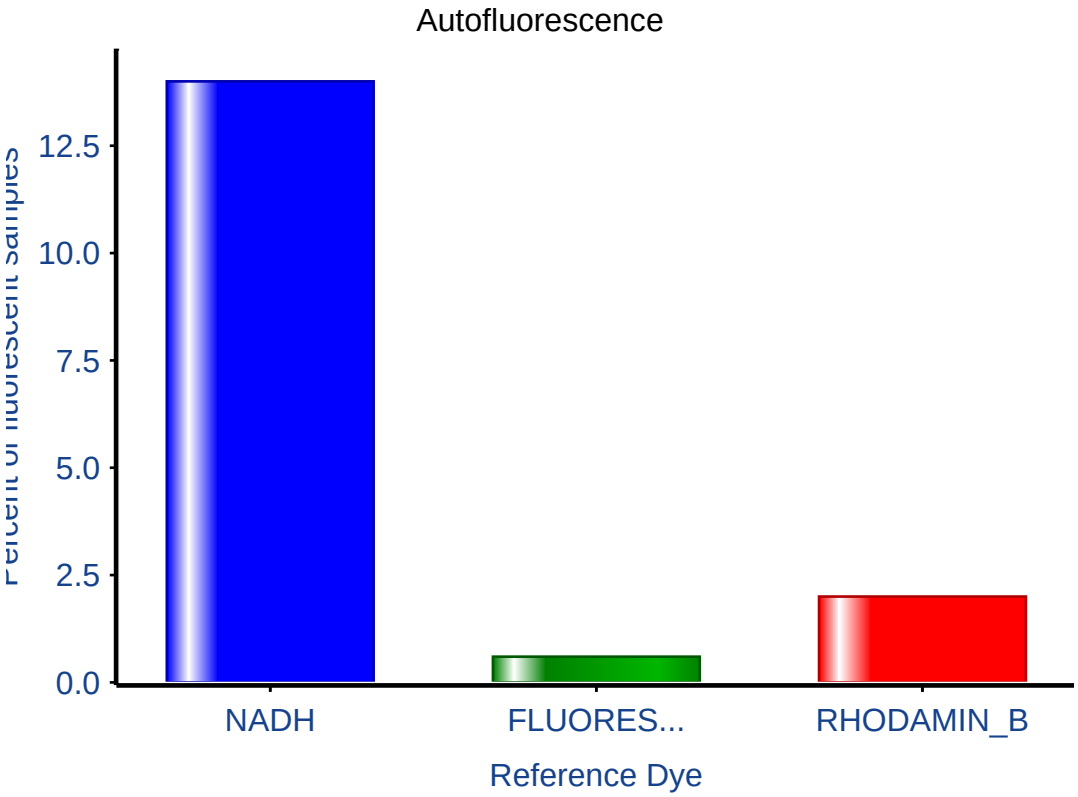
For LC-MS analysis compounds were dissolved in DMSO, diluted with acetonitril/water (1:1) to a concentration of 25 uM and filtered before measurement. Purity was determined according to the UV absorbance at 254 nm. Data generated by Katy Franke.

DMSO Solubility



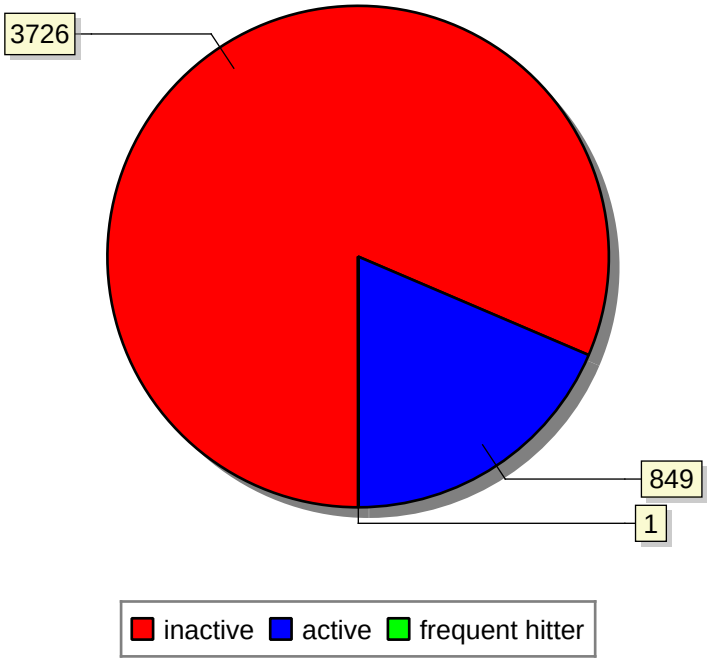
DMSO_Solubility	Number of Samples	Percentage
soluble	4360	95
insoluble	216	4

For DMSO solubility determination, the compound glass vials were visually inspected when the compounds were dissolved with DMSO to a concentration of 20 mM. If the solution appeared clear, the sample was judged as soluble. Data generated by Keven Mallow.



For autofluorescence determination, compounds where diluted in PBS to 100 uM and their fluorescence intensities compared to reference dyes (NADH at 100 uM;340/20 ex;465/20 em, Fluorescein at 0.1 uM; 485/20 ex; 535/20 em, Rhodamine at 0.1 uM; 560/20 ex; 610/20 em). If the fluorescence intensitiy exceeded 5% intensity of the reference dye, the sample was counted as autofluorescent. The concentrations of the reference dyes approximate concentrations that are typically used in biochemical assays. Data generated by Katy Franke.

Biological Activity



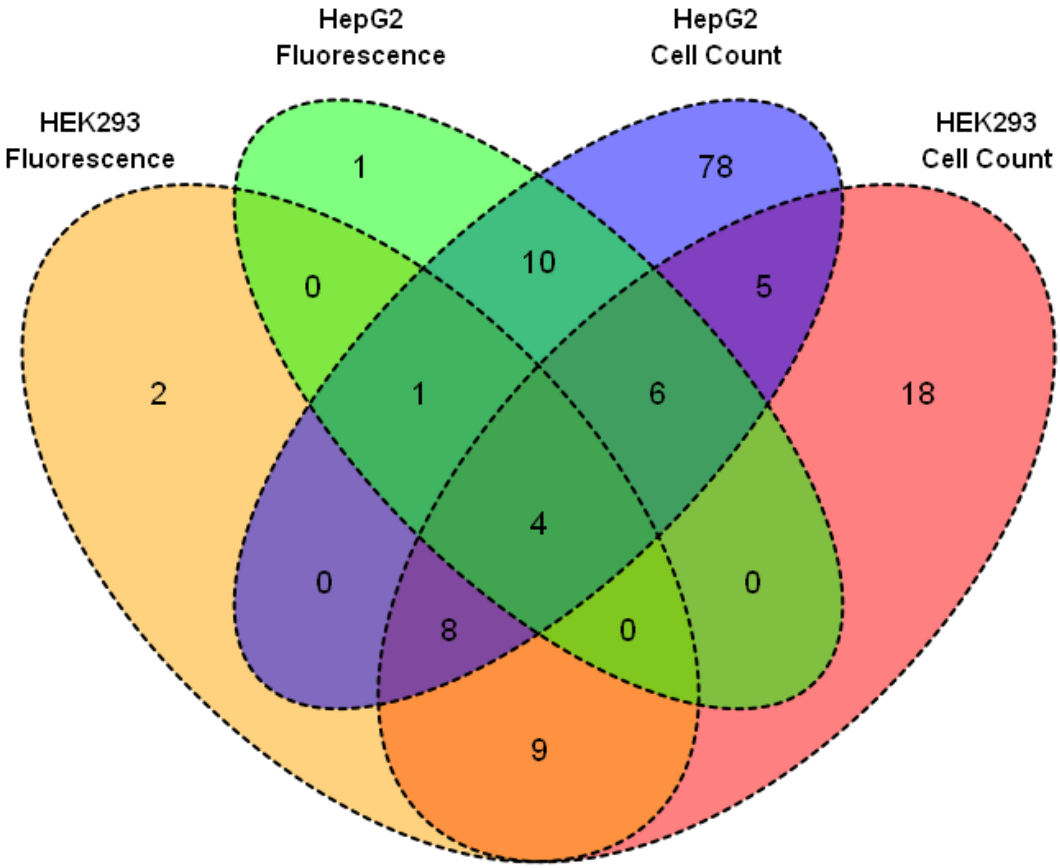
Category	Number of Samples	Percentage
inactive	3726	81
active	849	18
frequent hitter	1	0

A compound was judged as active if it showed an absolute z_score > 4 in at least one primary screen. If the occurence as active sample is significantly higher than the occurence of all compounds that were at least active once (with a p-value < 0.01), then the sample is marked as frequent hitter. Data generated by Screening Unit.

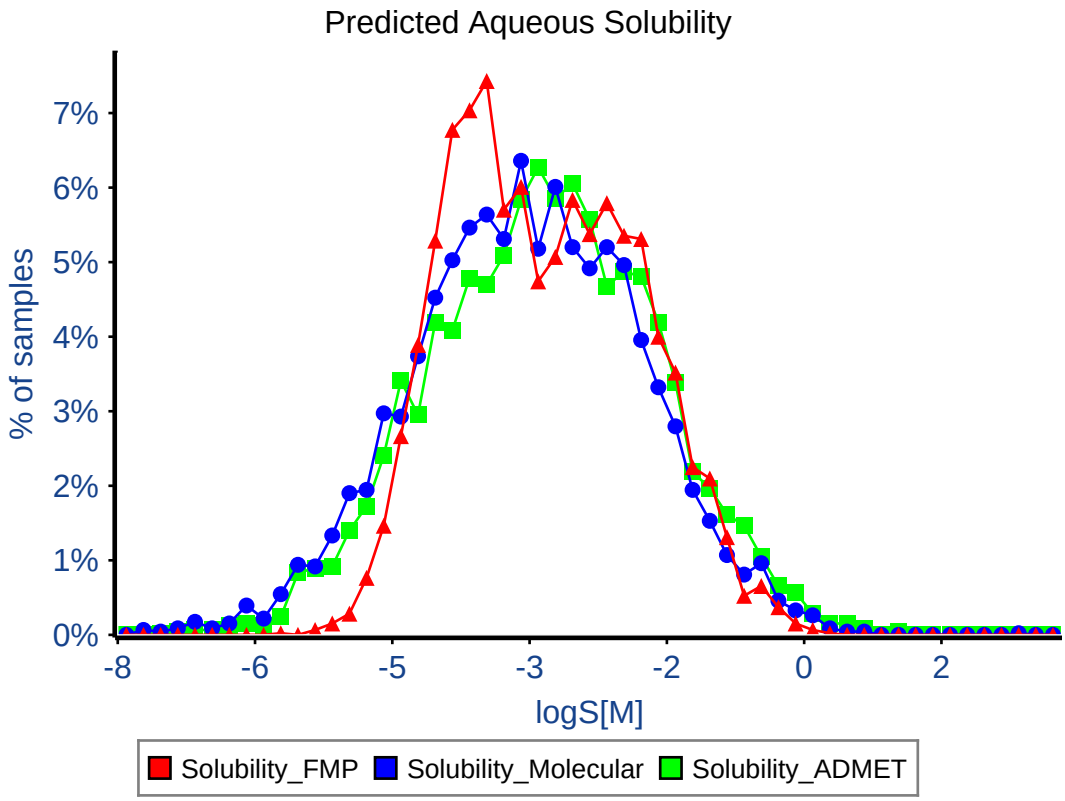
Cytotoxicity measured by resazurin assay and cell number at 10 uM compound concentration

Assay	Tested Compounds	Active Compounds	Percentage
HEK293 Cell Count	3872	50	1.3
HEK293 Fluorescence	4576	24	0.5
HepG2 Cell Count	4576	112	2.4
HepG2 Fluorescence	3872	22	0.6

Overlap between different assays:



HepG2 or HEK293 cells were seeded at a cell density of 2000 cells per well onto a 384-well plate and incubated for 24 hours. Compound was added to a final concentration of 10 uM (0.1% DMSO) and incubation was continued for another 72 hours. Resazurin was added and after 7 hours incubation, the extent of resazurin reduction was determined using a fluorescence plate reader ("Fluorescence"). After cell fixation using PFA and nuclei staining with Hoechst, cells were counted using a high content microscope ("Cell Count"). A compound was judged as active if it showed a z_score < -3 and a signal reduction of at least 50% compared to the assay controls (DMSO and Etoposide). The activity must have been observed in at least 2 independent measurements (out of 2 or 3 measurements performed). Data provided by Silke Radetzki, Sabrina Kleissle, and Marc Wippich.



Method	P25	P50	P75
Solubility_FMP	6611.2 uM	805.0 uM	128.0 uM
Solubility_Molecular	4983.1 uM	599.8 uM	75.5 uM
Solubility_ADMET	7906.8 uM	975.0 uM	116.1 uM

For aqueous solubility prediction, 3 different algorithms were used and the percentiles are given at which 25%, 50%, and 75% of the compounds should still be soluble.

Solubility_Molecular: Tetko et al. "Estimation of Aqueous Solubility of Chemical Compounds Using E-State Indices" J Chem Inf. Comput. Sci, 2001, 41, 1488-1493.

Solubility_ADMET: Cheng, A. and Merz, Jr., K. "Prediction of aqueous solubility of a diverse set of compounds using quantitative structure-property relationships," J. Med. Chem., 2003, 46, 3572-3580.

Solubility_FMP: Wichard DJ., Kuehne R. "Predicting aqueous solubility from structure" J. University of Applied Sciences Mittweida, Proceedings of the 20. IWKM, 28.-29. Oct 2009.