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Supplementary Materials for

The activities of drug inactive ingredients on biological targets

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Materials and Methods

Similarity Ensemble Approach (SEA) Calculations

We assembled a set of 639 excipients with a defined molecular structure (http://excipients.ucsf.bkslab.org) (*15*). From this list were removed inorganic ions and duplicate salt forms. For target prediction, we used the ChEMBL 20/21/22 databases releases (*68*) (versions were released throughout the study). Ligands with potencies <10 μM were compiled into ligand-target lists; suspect aggregators (*22*) were removed. Using the "Library Search" function on the SEA webserver (http://sea16.ucsf.bkslab.org), each excipient was individually computationally screened against over 3000 such targets, using 1024-bit folded ECFP4 fingerprints (*69*). The Tanimoto coefficient (Tc) (*70*) was used as the similarity metric; the value, between 0 and 1, represents the overlap of the fingerprints between two molecules, with 1 signifying a perfect match. The sum of all pairwise Tc values greater than 0.28 is compared to what one expects at random to calculate a SEA score (*7*). The SEA scores, or E-values, are calculated using the BLAST formalism (*16-18*), and reflect the likelihood that an excipient-target prediction would occur at random—lower E-values are more significant.

Predictions with SEA score < 10^{-5} were prioritized, as in a previous large-scale study, (*12*) as were predictions against human target orthologs. Over 20,000 SEA predictions were ultimately inspected. 69 of these excipient-target predictions were selected for testing based on several criteria: (1) pharmacologically interesting excipients (e.g. amino acids were deprioritized), (2) orally dosed excipients were prioritized, (3) protein target availability for testing in vitro activity, (4) chemical similarity upon visual inspection (e.g. we insured that the molecular charge of the

excipient matched that of the target ligands, and that if there was a obvious key warhead among the target ligands, the excipient shared it).

OATP2B1 SEA predictions were made using internal ligand sets for the 1B1, 1B3, and 2B1 isoforms. These datasets were larger than those in the ChEMBL databases and are provided as additional material (see attached "OATP Inhibitors.xlsx"). Since the 2B1 dataset was small, we searched for consensus within any of the transporter sets. Ultimately, a SEA hit within any subset was used as justification to test the excipient at the 2B1 isoform. We also iterated one additional cycle of SEA predictions after a first round of experimental testing, feeding the newly discovered inhibitors back into the SEA engine. To use a custom reference set such as this, one can use the "Library Search" tab on the SEA landing page. In section 2, select "Custom Targets" and input group(s) of SMILES and ID as indicated. In section 3, enter your query molecules—in this instance, the list of excipients.

Pharmacological investigations

GPCR and transporter radioligand binding assays

Binding assays were performed according to standard procedures published in the literature (*71-77*). Both filtration and scintillation proximity assay (SPA) technologies were used.

Assay details

1. Typical filtration assay protocol

Incubations were performed in 96-well plates (Costar) in a total volume of 200 µL, consisting of 100 µL of radioligand prepared in assay buffer, 40 µL of various concentrations of test

compound or buffer, 60 µL of membrane preparation (cells or rat brain membranes). Non-specific binding (NSB) was determined in the presence of an appropriate ligand specific for the receptor under study. The plates were incubated to steady state for the time specified. Reactions were terminated by flash filtration and inverse transfer to 96-well filter plates (Packard 96-well cell harvester, filter plates GF/C). The plates were dried for 30 min at 50°C in an oven and sealed at the bottom using an adhesive sheet. Subsequently, 40-50 µL of scintillation cocktail (Microscint-20, PerkinElmer) were added, sealed on top with an adhesive sheet (TopSeal, PerkinElmer) and the radioactivity was counted in a 96-well plate counter (TopCount, PerkinElmer).

2. Typical SPA assay protocol

See Cook, 1996 (*78*) for a general introduction to Scintillation Proximity Assay (SPA) technology. Wheatgerm agglutinin SPA beads, lyophilized (RPNQ0001) were purchased from PerkinElmer (Boston, MA, USA). One vial (500 mg) was reconstituted using 5 mL of distilled water to give a final concentration of 100 mg/mL. Reconstituted SPA beads were stored at 4°C and not frozen. 384-well white, clear bottom plates (CAT # 3706) were purchased from Corning (Lowell, MA, USA). The incubations were performed in a final volume of 50 µL per well in a 384-well white, clear bottom polystyrene plate. The components of the incubation were added to each well as follows:

0.25 µL test compound in 100 % DMSO

9.75 µL distilled water

20 µL radioligand

20 µL SPA bead / membrane mixture

The final concentration of beads ranged from 2 to 7 mg/mL and that of membranes from 0.25 - 800 µg/mL, depending on the assay. The plates were then sealed and allowed to sit at room temperature for the required incubation time. Plates were counted in a PerkinElmer Microbeta Trilux or TopCount reader for 90 seconds per well. Total binding was determined by adding 0.25 µL DMSO to 8 separate wells and non-specific binding was determined by the addition of 0.25 µL of the respective compound (see Table 7-2) to 8 separate wells, instead of the test compound.

3. GPCR functional assays

a. Typical cAMP quantification assay

The assay was performed in white 384-well flat bottom assay plate (Nunc #262360) using the Homogenous Time Resolved Fluorescence Technology (HTRF) following standard procedure as described elsewhere (*79*). HTRF cAMP immunoassay kits were purchased from Cisbio International (Cisbio #62AM4PEC - Bagnols-sur-Cèze, France). Aliquots of cryopreserved cells overexpressing the target of interest were used.

i. Agonist mode

On the day of the experiment, the cells were thawed rapidly, re-suspended in HBSS/20 mM HEPES with 1 mM IBMX buffer at a concentration of $2.5x10^5$/mL and plated at 20 µL/well into the assay plate containing 100 nL of test compounds serially diluted in 100 % DMSO. Low controls (unstimulated cells) and high controls (reference agonist at EC_{100} concentration) were also included on each plate. The final DMSO concentration in the assay plate was 0.5 %.

ii. Antagonist mode

On the day of the experiment, the frozen cells were re-suspended in HBSS/20 mM HEPES buffer at a concentration of $5x10^5$/mL and plated at 10 µL/well into the assay plate containing 100 nL of test compounds serially diluted in 100 % DMSO. Plates were incubated at RT for 5 min and 10 µL of reference compound at EC_{80} concentration diluted in assay buffer with 2 mM IBMX was added to the appropriate wells. Low controls were determined with a known antagonist at high concentration (IC_{100}); high controls (reference agonist EC_{80}) were included on each plate. The final DMSO concentration in the assay plate was 0.5 %.

HTRF reagents were prepared by diluting stock solutions of anti-cAMP cryptate and cAMP D2 1:20 in lysis buffer supplied with the kit. After 30 min of incubation at room temperature, 10 µL of cAMP-D2 and 10 µL of anti-cAMP cryptate were added to the assay plates. After one hour of incubation time at room temperature, the plates were read on an Envision reader (PerkinElmer; Excitation wavelength: 330 nm, Emission wavelengths: 620 and 665 nm). Results were calculated from the 665 nm/620 nm ratio following standard protocols (*79*).

b. Typical calcium flux assay

Experiments were performed using a Hamamatsu FDSS7000 instrument. The day before the experiment, batches of cryopreserved cells (10 million of cells per mL) were thawed in a water bath at 37°C, centrifuged for 5 minutes at 1000 rpm and the cell pellet was resuspended in cell culture medium. Cells were seeded into black 384-well plates with clear bottom at a density of 20,000 cells per well in 40 µL of cell culture medium. After overnight incubation at 37°C, 5% CO_2 growth media was replaced with 25 µL of loading buffer (1X HBSS with Ca^{2+}/Mg^{2+}, 20

mM HEPES, 2.5 mM Probenecid, 2 µM calcium indicator Fluo4-AM or Calcium6 (Molecular Devices) and plates were incubated for 1h at 37°C/5% CO_2 followed by loading buffer replacement with 25 µL assay buffer at pH 7.4 (1X HBSS without Ca^{2+}/Mg^{2+}, 20 mM HEPES). Plates were incubated for at least 15 minutes at room temperature before performing the experiment.

For G_i- or G_s-coupled GPCRs, cells were first sensitized by a prestimulation with a G_q agonist, and later exposed to various ligands. Using this experimental paradigm, activation of most G_s- and G_i-coupled receptors becomes possible using the calcium-flux technology. 20 µL ATP at 20 µM diluted in assay buffer was added using the 384-head for cell priming. Cells were incubated at room temperature for 30 minutes. Compounds were then characterized in the agonist and/or antagonist mode. For G_q-coupled GPCRs, this step is not necessary and cells can be directly stimulated by compounds.

i. Agonist mode

The basal fluorescent signal was recorded for 9 seconds followed by the addition of the compounds and 3 minutes continuous recording intervals of 1 second. Calcium kinetic measurements were performed at 488 nm using an Ex 488 nm/Em 540 nm filter. Fluorescence was recorded by a CCD camera with exposure time of 0.2 sec. Low controls (unstimulated cells) were determined in the wells to be used later for antagonist controls and high controls with the reference agonist at high concentration (EC_{100}).

ii. Antagonist mode

After performing the agonist experiment, plates were incubated at room temperature during 15 minutes and a known agonist, at EC_{80} concentration, was added in each well. Low controls were determined with a known antagonist at high concentration (IC_{100}), high controls with the reference agonist at EC_{80} concentration.

Intracellular calcium kinetic measurements were expressed as the ratio of maximal fluorescent signal over background.

c. GTPγS

Cell membranes prepared from recombinant cell lines expressing human receptors (10 µg protein) are mixed with GDP (equal volumes) in assay buffer and incubated for at least 15 minutes on ice. In parallel, GTPγS[^{35}S] is mixed with PVT-WGA scintillation proximity assay beads (GE Healthcare #RPNQ0001; equal volumes) just before starting the reaction. The following reagents are successively added in the wells of an Optiplate (PerkinElmer #6007290): 10 µL of test compound or reference ligand, 10 µL of reference agonist at historical EC_{80} for antagonist testing or 10 µL buffer for agonist testing, 20 µL membrane/GDP mix, and 20 µL GTPγS[^{35}S]/SPA beads mix. The plates were sealed and allowed to stand at room temperature for 6 hours. The plates were then counted using a PerkinElmer TopCount reader, each well being counted for 2 minutes.

d. GABA A receptor functional assay

A CHO-K1 cell line expressing recombinant human GABA A $\alpha_2\beta_2\gamma_2$ receptor was transduced with a lentivirus containing a mutant form of yellow fluorescent protein (YFP). This YFP mutant

(H148Q, I152L) exhibits a bright fluorescence and shows sensitivity to various anions at cytoplasmic pH (I->SCN->NO_3->Br->Cl->acetate>sulfate>F-). Cytoplasmic YFP(H148Q) expression is quenched in the presence of iodine (used as a more sensitive surrogate for chlorine). This property is used to follow the activity of the chloride channel of the GABA A receptor. Upon activation by GABA or any other GABA A agonist or positive modulator, an iodine influx occurs and results in a quench of the YFP fluorescence.

One day before the experiment, cells were seeded in black 384-well plates with clear bottom at a density of 20,000 cells per well in 50 µl of cell culture medium (MEM alpha without nucleotide, supplemented with 10% (v/v) FBS, 700 µg/mL geneticin G418, 5 µg/mL of blasticidin and 250 µg/mL hygromycin B, 100 units of penicillin and 100 µg of streptomycin). Plates were incubated overnight at 37°C in 5% CO_2. On the day of the experiment, growth medium was removed by washing once with 100 µL of assay buffer (NaCl 140 mM, KCl 5 mM, HEPES 10 mM, $CaCl_2$ 2 mM, $MgCl_2$ 1 mM, D-Glucose 10 mM, pH 7.4). After the wash, 20 µL of fresh assay buffer were added to each well for both agonist plate and antagonist plate. Compounds were then characterized in agonist and antagonist mode for GABA A activity.

i. Agonist mode

20 µL of test compound diluted in NaI buffer1 (i.e. assay buffer as defined above in which NaCl 140 mM replaced by NaI 67 mM) were added to agonist assay plate and the quench of fluorescence was measured with a Hamamatsu FDSS7000. The active control was 50 µM GABA. A concentration response curve for a reference agonist (GABA) was run on each plate.

ii. Antagonist mode

20 µL of test compound dissolved in NaCl buffer were added to antagonist assay plate and incubated at room temperature for 15 minutes before the addition of 20 ul of GABA diluted in NaI buffer 2 (i.e. assay buffer as defined above in which NaCl 140 mM replaced by NaI 100 mM) to a final concentration of 1 µM (EC_{80}) in all wells. The changes of the fluorescence quenching were measured with a Hamamatsu FDSS7000. A concentration response curve for a reference antagonist (bicuculline) was run on each plate.

4. Nuclear receptor binding assays using a fluorescence polarization method

Assays were performed in 384-well black flat-bottom plates (Corning 3654) with a total volume of 50 µL: 10 µL of sample (test compound, buffer or reference compound), 20 µL of fluorescent ligand and 20 µL of receptor. Ligand, receptor and their corresponding buffers were purchased from Life Technologies (Carlsbad, CA, USA) as complete kits (ER alpha #P3029, ER beta #P3032, PR #P2962, GR #P2893 and AR #PV4293). The kits were run according to the instructions provided. 20 µL of ligand and 20 µL of receptor were added to plates already containing 10 µL of test compound using a Tecan EVO. The plates were then incubated for set times before reading on an Amersham LeadSeeker in fluorescence polarization mode with CY3 filters and epi-mirror. Total binding was determined by adding 10 µL 2.5 % DMSO in water to 8 separate wells and non-specific binding was determined by the addition of 10 µL of the respective compound to 8 separate wells, instead of the test compound.

5. Acetylcholinesterase assay method

The assay was performed in 384-well black, clear-bottom plates (Greiner 781091). Reaction and reagents were contained in 1X phosphate buffered saline pH 7.4 (Ambion AM9625). Total reaction volume was 50 µL. All steps were performed at room temperature: 20 µL, 35 ng/mL human recombinant acetyl cholinesterase enzyme purified from HEK-293 cells (Sigma C-1682) was added to 10 µL test compound (0.5% DMSO final). Reaction was initiated by the addition of 20 µL substrate/detection mix: 0.2 mM acetylthiocholine iodide (Sigma A5751) and 0.2 mM 5.5'-dithiobis-2-nitrobenzoic acid (Sigma D218200). The thiocholine generated reacts continuously with the dithiobisnitrobenzoic acid to produce 5-thio-2-nitrobenzoic acid, and its spectrophotometric 405 nm absorbance was read at 10 minutes on a PerkinElmer Envision. The reference physostigmine was tested up to 10 µM in an 8-point dose response, in duplicate, on each plate. The maximum inhibitory effect was determined by 10 µM physostigmine.

6. Cyclooxygenase assays using an amplex red method

Assays were performed in 384-well black flat-bottom plates (Corning Cat# 3654) with a total volume of 50 µL consisting of: 10 µL of sample (test compound, buffer or the reference diclofenac), 20 µL of COX enzyme and 20 µL arachidonic acid (AA) substrate with amplex red (AR). COX enzymes were purchased from Cayman Chemicals (Ann Arbor, MI, USA; ovine COX-1 Cat# 60100, human COX-2 Cat# 60122). Arachidonic acid (COX substrate) was purchased from Biomol (Plymouth Meeting, PA, USA; Cat# FA-003). Amplex red was purchased from Anaspec (San Jose, CA, USA; Cat # 85500). Diclofenac was purchased from Sigma (Cat# D6899).

Assay plates were prepipetted with 10 µL test compound. 20 µL of enzyme (COX) was added to all wells, except the minus COX control wells, using a Multidrop (Thermo Scientific, Waltham, MA, USA). The plates were covered and incubated for 30 minutes at room temperature. Arachidonic acid and amplex red were prepared from frozen stocks immediately before addition to plates to minimize unwanted reduction of amplex red to resorufin. 20 µL of the AA/AR solution was added to all wells using a Multidrop and plates were covered and incubated for another 5 minutes at room temperature. The final concentrations of the various reagents were: ovCOX-1 0.6 units/well, hCOX-2 6 units/well, AA 6 µM, AR 25 µM. Plates were read in an Envision Plate Reader (PerkinElmer) at the following wavelengths: excitation 530 nm, emission 590 nm, top read only.

The maximum signal was determined by adding 10 µL 2.5 % DMSO in water to 8 separate wells and the minimum signal was determined by the addition of 10 µL of diclofenac to 8 separate wells, instead of the test compound.

7. Monoamine Oxidase (MAO) Assay

The MAO luminescence assay was performed in a final volume of 40 µL per well in a 384 well plate using a kit sold by Promega (Cat No. V1402) using a luminogenic substrate (a derivative of beetle luciferin ((4S)-4.5-dihydro-2(6-hydroxybenzothiazolyl)-4-thiazole-carboxylic acid)). The components of the wells were added in two steps:

Step 1: Enzymatic step

0.1 µL test compound in DMSO

10 µL MAO substrate. Final concentration: 40 µM

10 µL MAO-A enzyme from Sigma (ref. M7316). Final concentration: 0.65 µg/well.

The plates were incubated at room temperature for 2 hours (volume: 20 µL). The maximum signal was determined by adding 0.1 µL DMSO instead of test compound and the minimum signal was determined by the addition of 0.1 µL clorgyline (10 µM final concentration).

Step 2: Detection step

30 µL Luciferin detection reagent was added

The plates were then incubated at room temperature for 20 minutes and were counted using an Envision reader (measurement interval time = 1s).

8. Phosphodiesterase (PDE) assays

The assay was performed in a final volume of 50 µL per well in a 384-well Optiplate. The reagents were added as follows: 10 µL of test compound in 2.5 % DMSO, 10 µL of a 2.5 % DMSO in water solution or an appropriate PDE inhibitor in the control wells (for the determination of the total and non-specific signals), 20 µL of the [^{3}H]cAMP tracer, 20 µL of the PDE enzyme solution. The plate was then incubated at room temperature for the time specified and the reaction was ended by the addition of 30 µL of a solution containing Yttrium silicate beads (PerkinElmer - RPNQ0150) in 30 % glycerol. The plates were incubated at room temperature for at least 30 minutes, sealed and counted in a Microbeta Trilux or TopCount (PerkinElmer), each well counted for 1 min.

9. Bile salt export pump (BSEP) vesicular [^{3}H]taurocholate uptake assay

This assay measures the uptake of [^{3}H]taurocholate by everted vesicles from insect cell membranes overexpressing human BSEP (ATP-dependent) (*80*). Human BSEP vesicles were

purchased from Solvo (Budapest, Hungary) and the Solvo protocol was used with some modifications.

Test compounds (1 µL) in 100% DMSO were added to 16 µL/well of a mixture of assay buffer (2 mM HEPES/Tris, pH 7.4, 100 mM KNO_3, 10 mM $Mg(NO_3)_2$, and 50 mM sucrose) containing 25 µg vesicle-protein and [^{3}H]taurocholate (0.5 µM final concentration) in 384-well polypropylene plates (Greiner), followed by 16 µL of 10 mM ATP (in assay buffer). The highest compound concentration tested was 300 µM and the final DMSO concentration was 3% (v/v). The plate was covered and incubated for 30 minutes at room temperature. The reaction was stopped by addition of 60 µL cold wash buffer (50 mM sucrose, 100 mM KNO_3, 100 mM HEPES/Tris, pH 7.4, 100 µM taurocholate). The contents of each well were transferred to a 384-well GF/B filter plate (Millipore). Under aspiration each well was washed four times with ice-cold stop buffer using a Millipore vacuum filtration system (Millipore Corporation, Billerica, MA). The plate was then dried overnight at room temperature before 20 µL of MicroScint-20 was added. The plate was counted on a Microbeta Trilux (PerkinElmer).

10. Vesicular monoamine transporter (VMAT2) uptake assay (96-well)

This assay measures the uptake of [^{3}H]dopamine by synaptic vesicles from rat brain cortex. The vesicle preparation was custom prepared by Analytical Biological Services Inc. (Wilmington, Delaware) using the protocol described by Nickell et al (*81*) with some modifications. Synaptic vesicles (100 µL, ~38 µg of protein) were incubated at room temperature for 40 mins in assay buffer (25 mM HEPES, 100 mM potassium tartrate, 1.7 mM ascorbic acid, 0.05 mM EGTA, 0.1 mM EDTA and 2 mM ATP-Mg^{2+}, pH 7.4) in the presence of [^{3}H]dopamine (100 nM

final concentration). Incubations were performed in a 96-well plate. The reaction was terminated by addition of 50 μL of cold wash buffer (25 mM HEPES, 100 mM potassium tartrate, 5 mM $MgSO_4$, 10 mM NaCl, pH 7.4) and rapid filtration through a Millipore MultiScreen HTS GF/B 96-well filter plate. The filters were soaked previously in 0.5 % polyethylenimine. Filters were washed three times with cold wash buffer using a Tecan filtering manifold. Radioactivity trapped in filters was counted using a liquid scintillation counter. Non-specific values were determined by measuring [^{3}H]dopamine uptake in the presence of 1 μM reserpine.

11. Protein arginine methyltransferase HotSpot assay

The assay was performed by Reaction Biology Corporation, a contract research organization. PRMT1 (RBC Cat# HMT-11-119): Human recombinant PRMT1 (GenBank Accession No. NM_001536) (amino-acids 2-371(end)), with N-terminal GST-tag, MW = 68.3 kDa, expressed in *E. coli*.

The substrate (5 μM Histone H4) was prepared in freshly prepared Reaction Buffer (50 mM Tris-HCl (pH 8.5), 50 mM NaCl, 5 mM $MgCl_2$, 0.01% Brij35, 1 mM DTT, 1% DMSO). The enzyme was delivered into the substrate solution and gently mixed. The testing compounds in DMSO were delivered into the enzyme/substrate reaction mixture by using Acoustic Technology (Echo 550, LabCyte Inc. Sunnyvale, CA) in nanoliter range, and incubated for 20 min at room temperature. 1 μM [^{3}H]-SAM was delivered into the reaction mixture to initiate the reaction and incubated for 1 hour at 30 °C. The reaction mixture was delivered to filter-paper for detection. The data was analyzed with Excel and GraphPad Prism software for IC_{50} curve fits.

12. Tyrosine hydroxylase inhibition assay

The assay was performed by Eurofins Cerep, a contract research organization. The catalog number was 214. The enzyme is sourced from rat striatum, stimulated with 10 μM [^{3}H] tyrosine and incubated for 40 minutes at 37 °C. Scintillation counting is the measured response. 3-iodo L-tyrosine was the reference inhibitor (460 nM measured IC_{50}). The methodology is adapted from (*82*).

13. Fat mass and obesity associated gene (FTO) and lysine demethylase (KDM) enzymatic assays

The assays were performed by BPS Bioscience, a contract research organization. All of the enzymatic reactions were conducted in duplicate at room temperature for 60 min (KDM4C) or 960 minutes (FTO) in a 50 μL mixture containing enzyme assay buffer, enzyme, and the test compound. These 50 μl reactions were carried out in wells of JMJD2 or FTO substrate pre-coated plate.

After enzymatic reactions, the reaction mixtures were discarded and each of the wells was washed three times with TBST buffer, and slowly shaken with Blocking Buffer for 10 minutes. Wells were emptied, and 100 μL of diluted 1° antibody was added. The plate was then slowly shaken for 60 minutes at room temperature. As before, the plate was emptied and washed three times, and shaken with Blocking Buffer for 10 minutes at room temperature. After discarding the Blocking Buffer, 100 μL of diluted 2° antibody was added. The plate was then slowly shaken for 30 minutes at room temperature. As before, the plate was emptied and washed three times, and shaken with Blocking Buffer for 10 minutes at room temperature. Blocking Buffer was

discarded and a mixture of the HRP chemiluminescent substrates was freshly prepared. 100 μL of this mixture was added to each empty well. Immediately, the luminescence of the samples was measured in a BioTek Synergy™ 2 microplate reader.

Enzyme activity assays were performed in duplicates at each concentration. The chemiluminescence intensity data were analyzed and compared. In the absence of the compound, the intensity (C_e) in each data set was defined as 100% activity. For FTO assay, since signal was inversely proportional to the activity, raw luminescence values were inversed. In the absence of enzyme, the chemiluminescence intensity (C_0) in each data set was defined as 0% activity. The IC_{50} value was determined by the concentration causing a half-maximal percent activity.

14. Bromodomain (BRD4) binding assay

The assay was performed by BPS Bioscience, a contract research organization, using AlphaScreening technology using a recombinant bromodomains and their corresponding ligands or nonacetylated ligands. The AlphaScreening signal from the assay is correlated with the amount of ligand binding to the bromodomain. The compounds were diluted in 4% DMSO and 2.5 μL of the dilution was added to a 10 μL reaction so that the final concentration of DMSO is 1 % in all of reactions.

All of the binding reactions were conducted at room temperature. The 10 μl reaction mixture in Assay Buffer contains either bromodomains and the indicated amount of inhibitor. For the negative control (blank), 2.5 μL of the assay buffer was added instead of the bromodomain. The reaction mixture incubated for 30 min followed by additional 30 min incubation after the addition of 1 μL of ligand (BET ligand, 30 nM). After the 30 min incubation with the ligand, 10

µl of detection buffer containing 20 µg/mL glutathione acceptor beads were added to the reaction mix and incubated for 30 min in a dark room. 10 µL of 40 µg/mL streptavidin donor beads were then added and the final 30 µL mixture was incubated for 15 min in a dark room.

15. AlphaScreening

Binding experiments were performed in duplicate at each concentration using an EnSpire Alpha 2390 Multilabel reader (Perkin Elmer*)*. The AlphaScreening data were analyzed using Graphpad Prism. In the absence of the compound, the AlphaScreening signal (At) in each data set was defined as 100% activity. In the absence of the BET Ligand, the AlphaScreening signal (Ab) in each data set was defined as 0% activity. The IC_{50} value is the concentration causing a half-maximal percent activity from a fit to this curve.

16. Testing for colloidal aggregation

Excipients were tested for colloidal aggregation by Dynamic Light Scattering (DLS) and detergent-sensitive inhibition of common counter-screening enzymes, AmpC β-lactamase (expressed and purified as previously described (*83*)) or Malate dehydrogenase (Millipore Sigma, 442610); the excipients were tested at up to 200 µM in these assays, as previously described (*84*).

BioMAP® Diversity PLUS Profiling (Eurofins) materials and methods

List of BioMAP Systems

System	Icon	Cell Type	Stimuli	Disease Relevance	Biomarker Readouts	Description
3C		Venular endothelial cells	IL-1β + TNFα + IFNγ	Cardiovascular Disease, Chronic Inflammation	MCP-1, VCAM-1, TM, TF, ICAM-1, E-selectin, uPAR, IL-8, MIG, HLA-DR, Proliferation, SRB	The 3C system models vascular inflammation of the Th1 type, an environment that promotes monocyte and T cell adhesion and recruitment and is anti-angiogenic. This system is relevant for chronic inflammatory diseases, vascular inflammation and restenosis.
4H		Venular endothelial cells	IL-4 + histamine	Asthma, Allergy, Autoimmunity	MCP-1, Eotaxin-3, VCAM-1, P-selectin, uPAR, SRB, VEGFRII	The 4H system models vascular inflammation of the Th2 type, an environment that promotes mast cell, basophil, eosinophil, T and B cell recruitment and is pro-angiogenic. This system is relevant for diseases where Th2-type inflammatory conditions play a role such as allergy, asthma, and ulcerative colitis.
LPS		Peripheral blood mononuclear cells + Venular endothelial cells	TLR4	Cardiovascular Disease, Chronic Inflammation	MCP-1, VCAM-1, TM, TF, CD40, E-selectin, CD69, IL-8, IL-1α, M-CSF, $sPGE_2$, SRB, sTNFα	The LPS system models chronic inflammation of the Th1 type and monocyte activation responses. This system is relevant to inflammatory conditions where monocytes play a key role including atherosclerosis, restenosis, rheumatoid arthritis, metabolic disease and other chronic inflammatory conditions.
SAg		Peripheral blood mononuclear cells + Venular endothelial cells	Superantigens	Autoimmune Disease, Chronic Inflammation	MCP-1, CD38, CD40, E-selectin, CD69, IL-8, MIG, PBMC Cytotoxicity, Proliferation, SRB	The SAg system models chronic inflammation of the Th1 type and T cell effector responses to TCR signaling with co-stimulation. This system is relevant to inflammatory conditions where T cells play a key role including organ transplantation, rheumatoid arthritis, psoriasis, Crohn's disease and multiple sclerosis.
BT		Peripheral blood mononuclear cells + B cells	Anti-IgM + Superantigens	Asthma, Allergy, Oncology, Autoimmunity	B cell Proliferation, PBMC Cytotoxicity, Secreted IgG, sIL-17A, sIL-17F, sIL-2, sIL-6, sTNFα	The BT system models T cell dependent B cell activation and class switching as would occur in a germinal center. This system is relevant for diseases and conditions where B cell activation and antibody production are relevant. These include autoimmune disease, oncology, asthma and allergy.
BF4T		Bronchial epithelial cells + Dermal fibroblasts	IL-4 + TNFα	Asthma, Allergy, Fibrosis, Lung Inflammation	MCP-1, Eotaxin-3, VCAM-1, ICAM-1, CD90, IL-8, IL-1α, Keratin 8/18, MMP-1, MMP-3, MMP-9, PAI-1, SRB, tPA, uPA	The BF4T system models lung inflammation of the Th2 type, an environment that promotes the recruitment of eosinophils, mast cells and basophils as well as effector memory T cells. This system is relevant for allergy and asthma, pulmonary fibrosis, as well as COPD exacerbations.
BE3C		Bronchial epithelial cells	IL-1β + TNFα + IFNγ	Lung Inflammation, COPD	ICAM-1, uPAR, IP-10, I-TAC, IL-8, MIG, EGFR, HLA-DR, IL1α, Keratin 8/18, MMP-1, MMP-9, PAI-1, SRB, tPA, uPA	The BE3C system models lung inflammation of the Th1 type, an environment that promotes monocyte and T cell adhesion and recruitment. This system is relevant for sarcoidosis and pulmonary responses to respiratory infections.
CASM3C		Coronary artery smooth muscle cells	IL-1β + TNFα + IFNγ	Cardiovascular Inflammation, Restenosis	MCP-1, VCAM-1, TM, TF, uPAR, IL-8, MIG, HLA-DR, IL-6, LDLR, M-CSF, PAI-1, Proliferation, SAA, SRB	The CASM3C system models vascular inflammation of the Th1 type, an environment that promotes monocyte and T cell recruitment. This system is relevant for chronic inflammatory diseases, vascular inflammation and restenosis.
HDF3CGF		Dermal fibroblasts	EGF + basic-FGF + PDGF-BB + IL-1β + TNFα + IFNγ	Fibrosis, Chronic Inflammation	MCP-1, VCAM-1, ICAM-1, Collagen I, Collagen III, IP-10, I-TAC, IL-8, MIG, EGFR, M-CSF, MMP-1, PAI-1, Proliferation_72hr, SRB, TIMP-1, TIMP-2	The HDF3CGF system models wound healing and matrix/tissue remodeling in the context of Th1-type inflammation This system is relevant for various diseases including Fibrosis, Rheumatoid Arthritis, Psoriasis and stromal biology in tumors.
KF3CT		Keratinocytes + Dermal fibroblasts	IL-1β + TNFα + IFNγ + TGFβ	Psoriasis, Dermatitis, Skin Biology	MCP-1, ICAM-1, IP-10, IL-8, MIG, IL-1α, MMP-9, PAI-1, SRB, TIMP-2, uPA	The KF3CT system models model cutaneous inflammation of the Th1 type, an environment that promotes monocyte and T cell adhesion and recruitment. This system is relevant for cutaneous responses to tissue damage caused by mechanical, chemical, or infectious agents as well as certain states of psoriasis and dermatitis.
MyoF		Lung fibroblasts	TGFβ + TNFα	Fibrosis, Chronic Inflammation, Wound Healing, Matrix Remodeling	a-SM Actin, bFGF, VCAM-1, Collagen-I, Collagen-III, Collagen-IV, IL-8, Decorin, MMP-1, PAI-1, TIMP-1, SRB	The MyoF system models the development of myofibroblasts relevant to fibrotic diseases as well as other chronic inflammatory settings where tissue remodeling, and fibrosis is involved including scleroderma, SLE, psoriasis and arthritis.
/Mphg		Venular endothelial cells + Macrophages	TLR2	Cardiovascular Inflammation, Restenosis, Chronic Inflammation	MCP-1, MIP-1α, VCAM-1, CD40, E-selectin, CD69, IL-8, IL-1α, M-CSF, sIL-10, SRB, SRB-Mphg	The /Mphg System models chronic inflammation of the Th1 type and macrophage activation responses. This system is relevant to inflammatory conditions where monocytes play a key role including atherosclerosis, restenosis, rheumatoid arthritis, and other chronic inflammatory conditions.

Human Primary Cell Culture

All studies follow the guidelines for human subjects research under United States HHS human subjects regulations (45 CFR Part 46). Preparation and culture of primary human cell types and methods for the systems were as previously described (*28, 85*). Human umbilical vein endothelial cells (HUVEC) and human neonatal foreskin fibroblasts (HDFn) are cultured according to the supplier's (Lonza, Inc., Allendale, NJ) recommendation. Primary human bronchial epithelial cells (Cell Applications, Inc., San Diego, CA), arterial smooth muscle cells, adult lung fibroblasts (Lonza, Inc., Allendale, NJ), and keratinocytes (Cambrex, Inc., East Rutherford, NJ) were cultured according to methods recommended by the commercial suppliers. Peripheral blood mononuclear cells (PBMC) were prepared from buffy coats from normal human donors (*86*). CD20+ B cells and CD14+ monocytes were obtained from All Cells, Inc., Emeryville, CA. Macrophages were prepared by culturing CD14+ monocytes in M-CSF (50 ng/ml) for 7 days. All primary human cells utilized in this work were obtained via commercially available sources are used at early passage (≤ P4) to minimize adaptation to cell culture and preserve physiological signaling responses.

BioMAP Systems

Primary human cell types used in BioMAP systems and their stimuli include the following: 3C System (HUVEC/IL-1β, TNFα and IFNγ), 4H System (HUVEC/IL-4 and histamine), LPS System (PBMC and HUVEC/LPS), SAg System (PBMC and HUVEC/TCR ligands), BT System ($CD19^{+}$B cells and PBMC/anti-IgM + TCR ligands), BE3C System (bronchial epithelial cells/ IL-1β, TNFα and

IFNγ), BF4T System (bronchial epithelial cells and human dermal fibroblasts/TNFα and IL-4), HDF3CGF System (human dermal fibroblasts/ IL-1β, TNFα, IFNγ, EGF, basic-FGF and PDGF-BB), KF3CT System (keratinocytes and dermal fibroblasts/ IL-1β, TNFα and IFNγ), CASM3C System (coronary artery smooth muscle cells/ IL-1β, TNFα and IFNγ), MyoF System (differentiated lung myofibroblasts/TNFα and TGFβ), /Mphg System (HUVEC and macrophages/TLR2 ligands). Concentrations of stimuli are as follows: cytokines (IL-1β, 1 ng/ml; TNF-α, 5 ng/ml; IFN-γ, 20 ng/ml; IL-4, 5 ng/ml), activators (histamine, 10 μM; SAg, 20 ng/ml or LPS, 2 ng/ml), growth factors (TGF-β, 5 ng/ml; EGF, basic-FGF, and PDGF-BB, 10 ng/ml; Zymosan 10 μg/ml; Anti-IgM, 500 ng/ml). Superantigens (SAg), Staphylococcal enterotoxin B and toxic shock syndrome toxin-1 (Staphylococcal enterotoxin F) from Staphylococcus aureus, and lipopolysaccharide (LPS) from Salmonella enteritidis were obtained from Sigma. Adherent cells are cultured to confluence prior to assay initiation. The number of lymphocytes are as follows for 96-well format: B cells (2.5×10^4), PBMC (7.5×10^4 cells/well for LPS and SAg systems or 2.5×10^4 cells/well for BT system) or macrophages (7500 cells/well). Assays are initiated by addition of compounds for 1 hr followed by addition of appropriate stimuli. Assay plates are then incubated for 24hr or as otherwise indicated. MyoF system is stimulated for 48hr and BT is stimulated for either 72hr (soluble readouts) or 6d (secreted IgG). Cell proliferation is determined using sulforhodamine B (SRB) assay for adherent cell types or Alamar Blue for PBMC cells. For proliferation assays, individual cell types are cultured at sub-confluence are read at specific times for different primary cell types (48, 72 or 96hr). After stimulation, plates and supernatants are harvested and biomarkers quantitated by ELISA and other methods (see below).

Test agents. Compounds are tested at indicated concentrations, in a single well per biomarker endpoint. Compounds are added 1 hr before stimulation of the cells, and are present during the subsequent 24hr-6d stimulation period. Final DMSO concentration is <0.1%. Colchicine, 3.3 μM, and non-stimulated samples are included as controls on every plate. DMSO 0.1% is tested at 8 replicates per plate.

Biomarker endpoint measurements. The levels of biomarker endpoints (cell surface levels unless indicated as soluble by the prefix "s"), are measured by ELISA as described (*28, 85*). Proliferation of PBMC (T cells) is quantified by Alamar blue reduction (*87*) and proliferation of adherent cell types was quantified by SRB staining (*88*). For PBMC proliferation, cells are plated (75,000/well in a 96-well plate) and then test agents added for 1 hr before addition of activators, SEB and TSST (20ng/ml final concentration each). Cells are then incubated for 90 hr and then Alamar Blue, 20 ul/well, (Invitrogen, Cat #DAL1100) is added for 6 hrs and then plates are read with a fluorescence microplate reader at 546 (excitation)/580 (emission). For PBMC viability (PBMC Cytotoxicity), cells are plated and activated as above, but incubated for only 16 hr, prior to addition of Alamar blue. After 6 hr, plates are read as described above. SRB is performed by staining cells with 0.1% sulforhodamine B after fixation with 10% TCA and reading wells at 560 nm.

Data analysis. Measurement values for each endpoint measurement in a treated sample is divided by the mean value from eight DMSO control samples (from the same plate) to generate a ratio. All ratios were then log10 transformed. Significance prediction envelopes were

calculated for historical controls (99% and 95%). Overtly cytotoxic compounds are identified as generating profiles with one or more of the following readouts below the indicated thresholds: SRB < -0.3, PI or PBMC cytotoxicity <-0.3 in one or more systems. For analysis of profile similarities, overtly cytotoxic compound profiles are removed. The correlation metric is a combination of similarity metrics in addition to Pearson's correlation (*89*). Similar profiles are identified as those having Pearson correlations above a selected threshold > 0.7 (or as otherwise indicated).

Mechanism classification was performed (*89*). For this, BioMAP profile data for the tested agents are assessed using predictive models for 28 mechanism classes developed using support vector machines. Mechanism classes assessed encompass safety and efficacy-related mechanisms, include both target-specific and pathway-based classes, and cover the most common mechanisms identified in phenotypic screens, such as inhibitors of mitochondrial and microtubule function, histone deacetylase, and cAMP elevators.

Toxicity Signature Analysis. Evaluation of the presence or absence of Toxicity Signatures within the BioMAP profile of the tested agent was performed at each concentration. Toxicity Signatures are made up of 2 – 5 biomarker activities that have been correlated to an increased risk of certain toxicity effects *in vivo*. Signature development is based on drugs with known clinical adverse effects and refined and validated by data mining the BioMAP Reference Database (*90*). Concentrations are listed if the signature for the indicated alert was detected at two or more concentrations (indicated as ≥ the lowest concentration), or at the top concentration (concentration is listed without a symbol). Not detected (nd) indicates that the

alert signature was not detected at any of the concentrations tested. Not assessed (NA) indicates that the alert signature cannot be assessed at the concentrations tested (for example, if the agent is overtly cytotoxic at all concentrations tested). Biomarker activity patterns for 9 BioMAP Toxicity Signatures (Acute Toxicity, Immunosuppression, Skin Irritation, Liver Tox, Organ Tox, Skin Rash, Skin Sensitization, Thrombosis and Vascular Toxicity) were developed by data mining the BioMAP Reference Database to identify common activities between the profiles of drugs with the same reported clinical adverse effects or in vivo effects (e.g., acute toxicity). Knowledge of key activities identified in BioMAP profiles was combined with clinical data to determine which of the biomarker activities is associated with a positive and negative impact on the particular biology involved. The strength of clinical associations was tested by comparing this biomarker pattern against the BioMAP Reference Database to determine consistency in the presence or absence of the signature across other drugs with reported adverse effects. While these alerts may not represent all of the possible mechanisms by which these outcomes occur (showing greater accuracy than sensitivity), the compounds used to define Toxicity Signatures allow mechanistic insight into underlying events regulating these clinically reported side effects. Details of each Toxicity Signature, including the drugs or chemicals used to identify the signature, the key biomarker readouts, and mechanisms associated with each signature, are described in detail elsewhere (*90*).

Assay acceptance criteria. The BioMAP platform generates multi-parameter data sets for each compound tested. Assays are plate-based and performance is assessed by positive and negative controls for each assay. Negative controls include buffer (e.g. DMSO). For stimulated

systems, positive controls include the non-stimulated condition (non-stim) and a positive control test agent (colchicine). Data acceptance criteria are based on plate performance (%CV of negative control wells), and the performance of positive controls across assays with a comparison to historical controls. The performance of each BioMAP system in a given assay is evaluated using the Pearson statistic for the positive control, calculated individually for each assay compared to the positive control reference dataset. This test, the QA/QC Pearson Test, is performed by first establishing the 1% false negative Pearson cutoff from the positive reference dataset. The process is iterated through each profile in the positive control reference dataset, calculating Pearson values between this profile and the mean of the rest of the profiles in the dataset, so the number of Pearson values calculated is the number of profiles in the reference dataset. The Pearson at the one percentile of all Pearson values calculated is the 1% false negative Pearson cutoff. If the Pearson between a new positive control profile and the mean of positive control reference profiles exceeds this 1% false negative Pearson cutoff, then these plates pass the test. Assays are accepted when the positive control passes the Pearson test and 95% of plates have % CV <20%.

Pharmacokinetics experiments

All animal related procedures were conducted under a Novartis IACUC approved protocol in compliance with Animal Welfare Act regulations and the Guide for the Care and Use of Laboratory Animals.

Rat pharmacokinetics

For rat pharmacokinetics (PK), blood concentration versus time profiles were obtained from three male Sprague Dawley rats for each respective compound. For intravenous PK, the compound was administered intravenously by bolus injection (0.5 mL/kg) at a dose of 1 mg/kg, solubilized in N-Methyl-2-pyrrolidone (10%) and PEG200 (90%). For oral PK an oral gavage was administered to the same animals at a dose of 1, 3, or 10 mg/kg. The oral dose was dispersed in phosphate buffered solution (100%). Blood samples (10–50 μL) were collected at 0.08 (i.v. only), 0.25, 0.5, 1, 2, 4, 7, and 24 h after dosing.

Analyses of parent compound concentrations were carried out in blood using LC-MS/MS . An aliquot of 30 μL was taken and added to 200 μL acetonitrile (including Glyburide (50 ng/mL) as internal standard) and supernatants (2 μL) were injected directly onto an LC/MS/MS system for analysis. PK parameters were derived from concentration−time data by non-compartmental analyses. All PK parameters were calculated with the computer program WinNonlin (Version 6.4) purchased from Certara Company (St. Louis, MO). The area under the concentration−time curve (AUClast) was calculated using the linear trapezoidal rule. The bioavailability was estimated using following equation:

$$\%F = (AUCpo \times doseiv)/ (AUCiv \times dosepo)$$

Where %F is bioavailability, AUCpo is area under the curve from oral dose, doseiv is the intravenous dose, AUCiv is the area under the curve from the intra venous dose and dosepo is the oral dose.

OAT1, OATP1B1, OATP1B3, and OATP2B1 inhibition assays

Flp-In™ 293 cells (Thermo Fisher Scientific, Waltham, MA) overexpressing human OAT1, OATP1B1, OATP1B3, OATP2B1 or empty vector-transfected cells were seeded in poly-D-lysine-coated 96 well plates (8×10^4/well) overnight to reach confluence. Before the uptake experiment, cell culture medium was removed and the cells were washed with Hank's balanced salt solution (HBSS). For human OAT1, 0.1 μM of [^{3}H]-para-aminohippuric acid (PerkinElmer Health Sciences Inc. (Shelton, CT), specific activity of 5 Ci/mmol) was used as the substrate. For OATP1B1 and OATP1B3, 0.02 μM of [^{3}H]-estrone sulfate (Perkin Elmer (Waltham, MA), specific activity of 54 Ci/mmol) was used as the substrate. For OATP2B1, 2 μM of 4,5'-dibromofluorescein (DBF) was used as the substrate. The inhibition of the uptake was performed in three replicates at 37°C in the presence of excipients at designed concentrations or vehicle (dimethyl sulfoxide, DMSO). The uptake was terminated at 3 minutes. Cells were washed twice with ice-cold HBSS buffer. For OAT1, OATP1B1 and OATP1B3, the cells were lysed with lysis buffer containing 0.1 N NaOH and 0.1% SDS, and the radioactivity in the lysate was determined by liquid scintillation counting. For OATP2B1, fluorescence in cells was measured by a fluorescence microplate reader right after the uptake, with excitation and emission wavelengths at 485 nm and 560 nm, respectively. Transporter-mediated uptake was determined by subtracting from the values in empty vector-transfected cells at each substrate concentration. The IC_{50} values were obtained by fitting the data to the Hill equation by non-linear regression using GraphPad Prism; inhibition constants, Ki, were estimated using the Cheng-Prusoff equation (*91*). The Km value of DBF for OATP2B1 is 4.7 μM and S is 2 μM.

Experimental measurement of permeation with MDCK-LE assays

Cell culture

MDCK-LE cells are a low efflux (LE) cell line developed internally at Novartis using methodology similar to that previously reported (*92*). MDCK-LE cells were cultured at 37°C under a 5% CO_2 atmosphere, at 95% relative humidity in DMEM containing 10% FBS, penicillin-streptomycin (100 µg/mL), and 2 mM Ala-Gln. Cells were passaged weekly into an Omnitray (Nunc, Thermo Fisher Scientific, Rochester, NY) at a density of approximately 5750 cells/cm2 for continuous culture. For assay purposes cells were seeded at a density of approximately 265,000 cells/cm^2 of a 96-well Transwell plate (Corning Life Sciences, Acton, MA) and cultured in the same media noted above for a period of four days.

Assay

The determination of the apparent permeability (Papp) was performed in the A>B direction where each compound was assayed in triplicate in a pool of three compounds, similar to that previously reported (*92*). The zwitterion bestatin, a poorly permeably compound, was used as marker of monolayer integrity and was included as a fourth compound in the cassette of three compounds. To initiate the assay, media was aspirated and the cells and basal chambers were washed three times with Hank's Balanced Salt Solution (HBSS) containing 10 mM HEPES (pH 7.4). Compound test solutions were prepared in triplicate in HBSS containing 10 mM HEPES (pH 7.4) and 0.02% bovine serum albumin (BSA) to a final concentration of 10 µM and centrifuged for 2 minuntesminutes at 4000g, then applied to the apical compartment (donor compartment) at time zero. Additionally at time zero, a 37°C solution without test articles (HBSS+10 mM HEPES (pH 7.4) plus 0.02% BSA) was added to the receiver chamber (basal) of the Transwell plate. A time zero sample of the donor solution was sampled for further analysis. The assay was conducted for a period of 120 minutes at 37°C without shaking. At the time of

assay termination samples were taken from each donor compartment and each acceptor compartment of the Transwell plate. To each of the 0 and 120 minute sample was added an internal standard solution containing glyburide in water:acetonitrile, 50:50 (v:v). Concentration curves were prepared using a Labcyte Echo in the same matrix noted above. Sample and concentration curve sample were analyzed by mass spectroscopy.

Mass Spectroscopy

Samples were loaded onto a Phenomenex Polar-RP column (50×2 mm, 4 μm; Torrance, CA) by means of a Leap autosampler (Carrboro, NC). Chromatography was performed at a flow rate of 0.9 mL/min, using a biphasic gradient: 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Chromatography was executed using the following gradient profile: 0-0.25 min, 5% B; 0.25-1.30 min, linear gradient to 95% B; 1.30-1.50 min, 95% B; 1.50-1.60 min, linear gradient to 5% B; and 1.60-2.00 min 5% B. Mass spectroscopy was performed using a Thermo Quantum Ultra (Thermo Scientific, Waltham, MA) equipped with an electrospray ionization source. The probe substrate concentration was calculated from the chromatographic peak area ratio of analyte to internal standard (glyburide), using Gubb's software (Alpharetta, GA). Assay samples were loaded onto a RapidFire C4 cartridge by means of a RapidFire autosampler (Agilent, Santa Clara, CA). Chromatography was performed at a flow rate of 1.25 mL/min, loading with 0.1% formic acid in water and eluting in 0.1% formic acid in methanol. Mass spectroscopy was performed using an AB Sciex API5500 (Sciex, Framingham, MA) equipped with a turbo ion spray source. The analyte concentration was calculated from the chromatographic peak area ratio of analyte to internal standard glyburide, using Multiquant software V3 (Sciex, Framingham, MA).

Data analysis

Calculations:

Papp values were determined using:

$$Papp = (VA/A*[D0])*([A120]/t)$$

Percent recovery values were determined using:

$$\%recovery = 100*([A120]+[D120])/[D0]$$

Where VA is the volume of the acceptor (mL), A is the surface area of the membrane, D0 is the donor solution concentration at t=0, D120 is the donor solution concentration at t=120, A120 is the acceptor solution concentration at t=120, and t = time (seconds).

Allometric scaling

We extrapolated mercury clearance from monkey- to human-infants using the following equation:

$$Y_2 = Y_1 (M_1/M_2)^{0.3}$$

Where Y is the clearance rate and M is the body mass (*93*). **Table S7** shows the parameter estimates derived from one-compartment analysis of blood or brain total Hg concentration from Burbacher's paper (after four weekly intramuscular injections of vaccine thimerosal (20 μg/kg Hg) and scaled values from monkey infant to human infants. In addition, using similar allometric scaling approach as described above, we scale clearance rate of ethyl mercury in blood from human adult (*94*) or mice (*95*) to human infants to estimate ethyl mercury concentration in blood. **Table S8** shows the parameter estimates derived from one-compartment analysis in human adult and scaled values to human infants.

Supplementary Figures and Tables

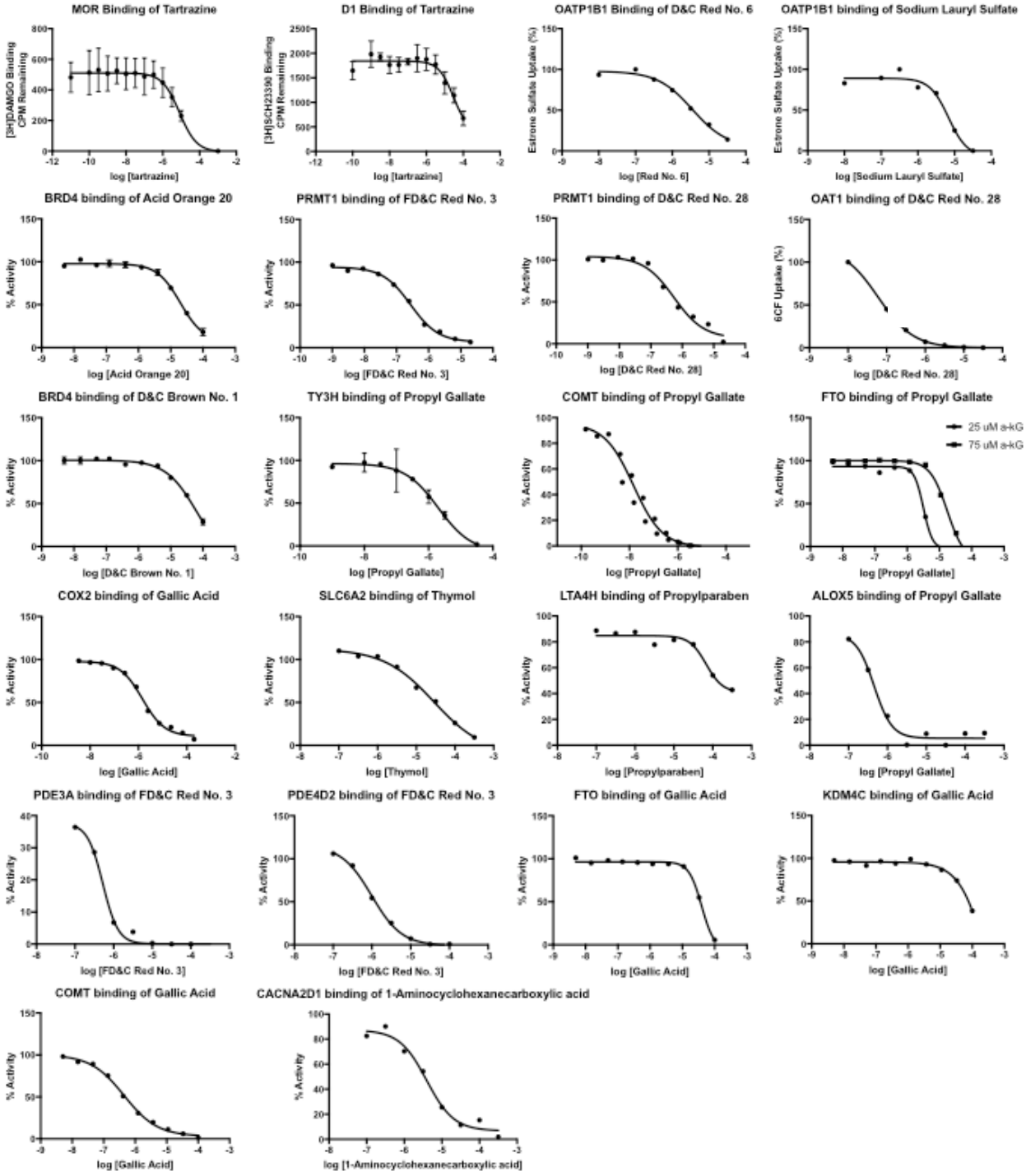


Fig. S1. Additional dose-response curves from in-vitro assays.

Twenty-two additional concentration-response binding curves for several excipients, including gallic acid, the primary metabolite of propyl gallate (Tables 1a-b and S1-S3).

General outline of Figures S2-S13:

BioMAP profiles: The X-axis lists the quantitative protein-based biomarker readouts measured in each system. The Y-axis represents a log-transformed ratio of the biomarker readouts for the drug-treated sample (n = 1) over vehicle controls (n ≥ 6). The grey region around the Y-axis represents the 95% significance envelope generated from historical vehicle controls. The profile is generated at different concentrations (unless otherwise specified); red: 30 μM, orange: 10 μM, yellow: 3.3 μM, and green: 1.1 μM. Biomarker activities are annotated when 2 or more consecutive concentrations change in the same direction relative to vehicle controls, are outside of the significance envelope, and have at least one concentration with an effect size > 20% ($|\log_{10}$ ratio$|$ > 0.1). Biomarker key activities are described as modulated if these activities increase in some systems, but decrease in others. Cytotoxicity is indicated on the profile plot by a thin black arrow above the X-axis, and antiproliferative effects are indicated by a thick grey arrow. Cytotoxicity and antiproliferative arrows only require one concentration to meet the indicated threshold for profile annotation (*26-28*).

Overlays of the top similarity match (in blue) from an unsupervised search of the BioMAP Reference Database of > 4,000 agents are provided for each excipient (in red). Common biomarker readouts are annotated when the readout for both profiles is outside of the significance envelope with an effect size > 20% (|log10 ratio| > 0.1) in the same direction. Similarity search results are filtered and ranked. Profiles are considered to have mechanistically relevant similarity if the Pearson's correlation coefficient ≥ 0.7. (*26-28*)

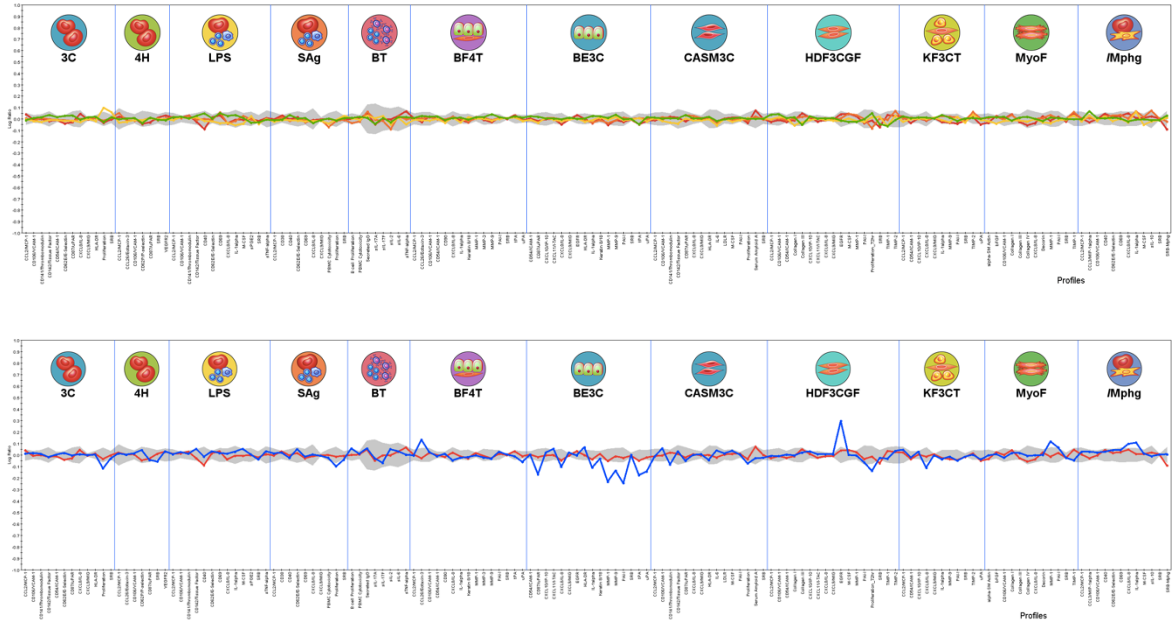


Fig. S2. BioMAP profile of aspartame and nearest drug match.

Top: No activities meet annotation criteria, nor are there cytotoxic or antiproliferative effects.

Bottom: Nearest drug match of aspartame (30 μM) was with an EGFR inhibitor (120 nM). The match is poor and insignificant; Pearson's correlation is 0.35.

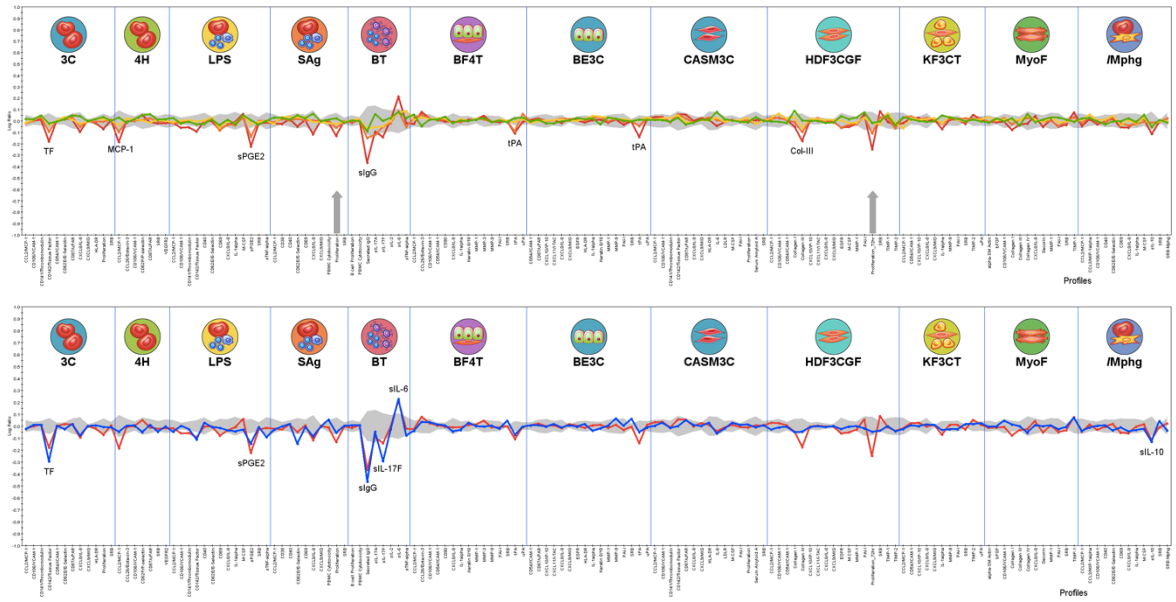


Fig. S3. BioMAP profile of butylparaben and nearest drug match.

Top: Butylparaben had seven annotated readouts, did not have cytotoxic effects, and was antiproliferative to fibroblasts and T-cells. Bottom: Nearest drug match of butylparaben (30 μM) was with Nabumetone (30 μM, COX inhibitor), sharing six annotated readouts. The match is mechanistically relevant; Pearson's correlation is 0.74.

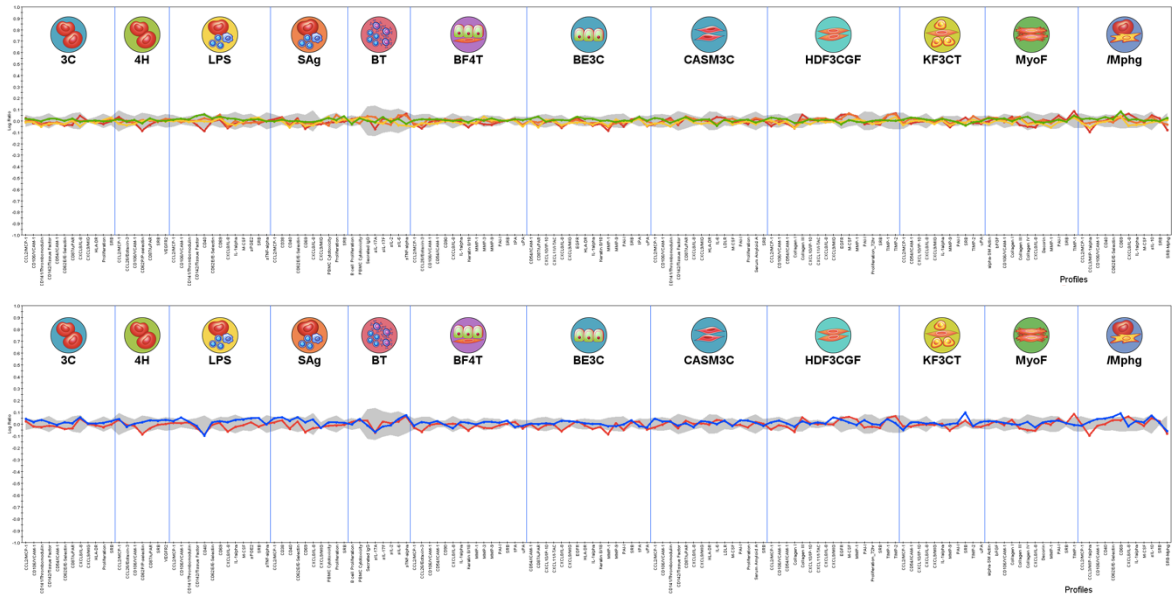


Fig. S4. BioMAP profile of propylene glycol and nearest drug match.

Top: No activities meet annotation criteria, nor are there cytotoxic or antiproliferative effects. Bottom: Nearest drug match of propylene glycol (30 μM) was with Nicardipine hydrochloride (1.1 μM, calcium channel blocker). The match is poor and insignificant; Pearson's correlation is 0.38.

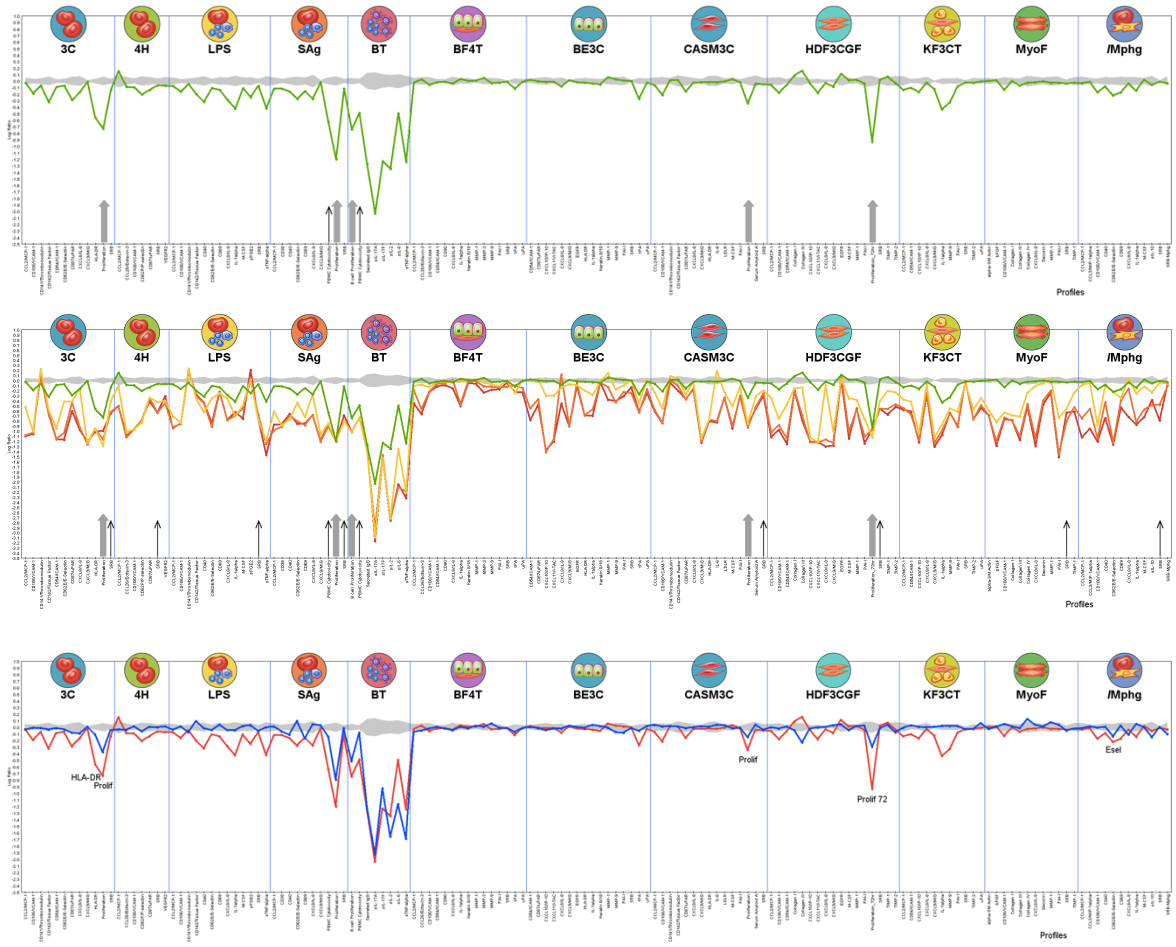


Fig. S5. BioMAP profile of thimerosal and nearest drug match.

Top: Thimerosal was cytotoxic to two systems at 1.1 µM and antiproliferative to coronary artery smooth muscle cells, endothelial cells, and fibroblasts. Middle: Above 1.1 µM, cytotoxicity was observed in three or more systems making it impossible to annotate readouts. Bottom: Nearest drug match of thimerosal (1.1 µM) was with cetylpyridinium chloride (1.1 µM, antiseptic)—another "inactive ingredient"—sharing five annotated readouts. The match is mechanistically relevant; Pearson's correlation is 0.86.

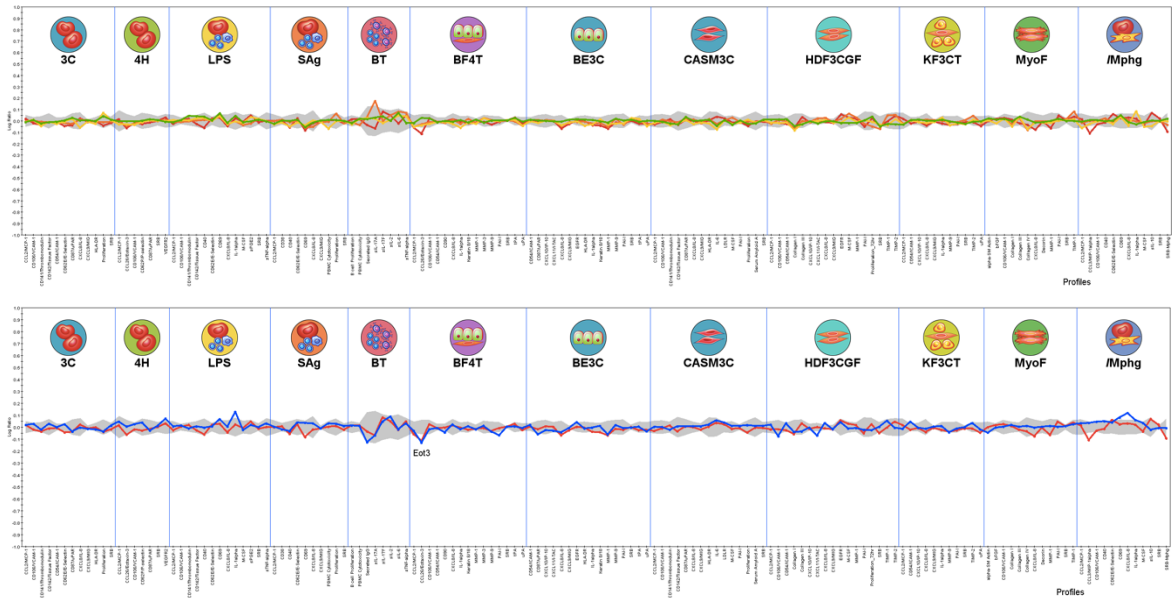


Fig. S6. BioMAP profile of tartrazine and nearest drug match.

Top: No activities meet annotation criteria, nor are there cytotoxic or antiproliferative effects. Bottom: Nearest drug match of tartrazine (30 μM) was with Crizotinib (4.6 nM, c-Met, ALK, and ROS1 inhibitor). The match is poor and insignificant; Pearson's correlation is 0.38.

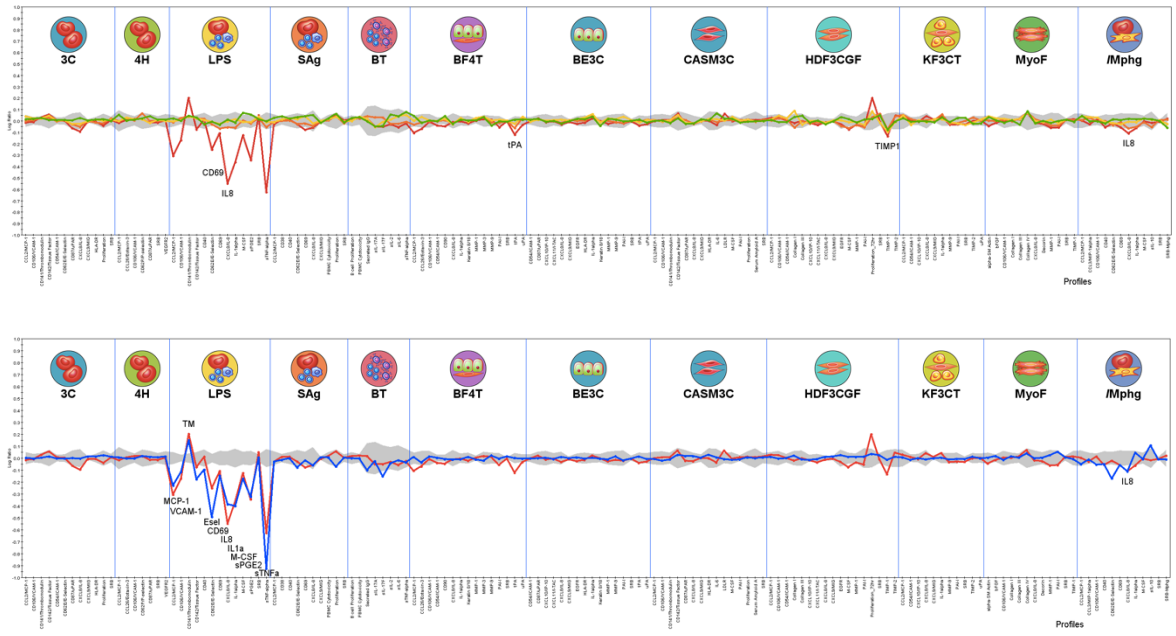


Fig. S7. BioMAP profile of polysorbate 80 and nearest drug match.

Top: Butylparaben had five annotated readouts and did not have cytotoxic effects or antiproliferative effects. Bottom: Nearest drug match of polysorbate 80 (30 µM) was with Interleukin 10 (30 µM, cytokine), sharing eleven annotated readouts. The match is mechanistically relevant; Pearson's correlation is 0.84.

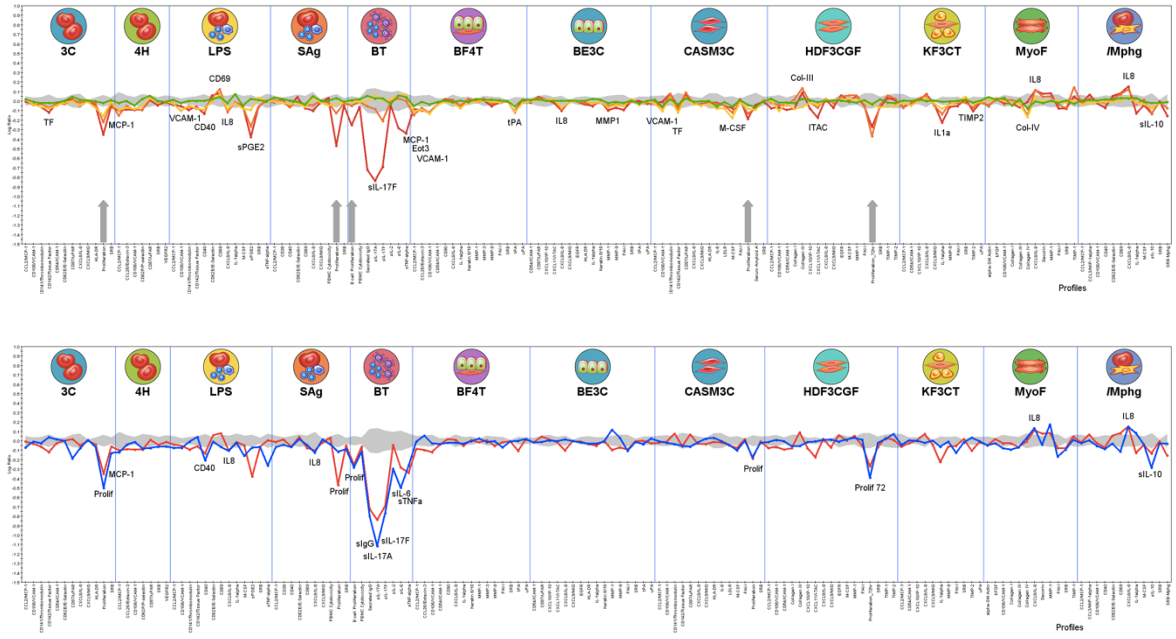


Fig. S8. BioMAP profile of propyl gallate and nearest drug match.

Top: Propyl gallate had twenty-five annotated readouts, did not have cytotoxic effects, however was antiproliferative to B cells, coronary artery smooth muscle cells, endothelial cells, fibroblasts and T cells. Bottom: Nearest drug match of propyl gallate (30 μM) was with phenazopyridine hydrochloride (30 μM, local anesthetic), sharing seventeen annotated readouts. The match is mechanistically relevant; Pearson's correlation is 0.81.

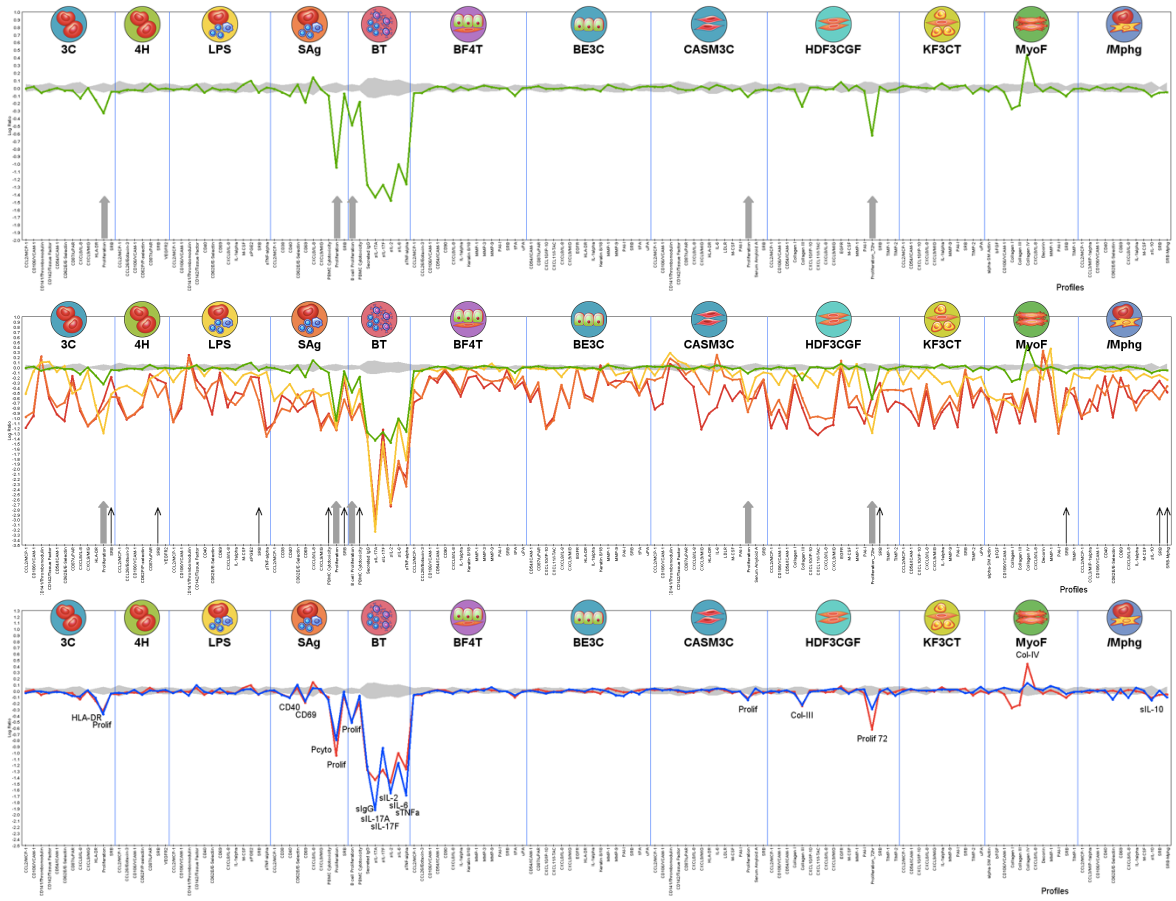


Fig. S9. BioMAP profile of cetylpyridinium chloride and nearest drug match.

Top: Cetypyridnium chloride was non-cytotoxic only at 1.1 μM and antiproliferative to B cells, T cells, coronary artery smooth muscle cells, endothelial cells, and fibroblasts. Middle: Above 1.1 μM, cytotoxicity was observed in three or more systems making it impossible to annotate readouts. Bottom: Nearest drug match of cetylpyridinium chloride (1.1 μM) was with cetylpyridinium chloride (1.1 μM, antiseptic)—a good sanity-check—sharing eighteen annotated readouts. The match is mechanistically relevant; Pearson's correlation is 0.95.

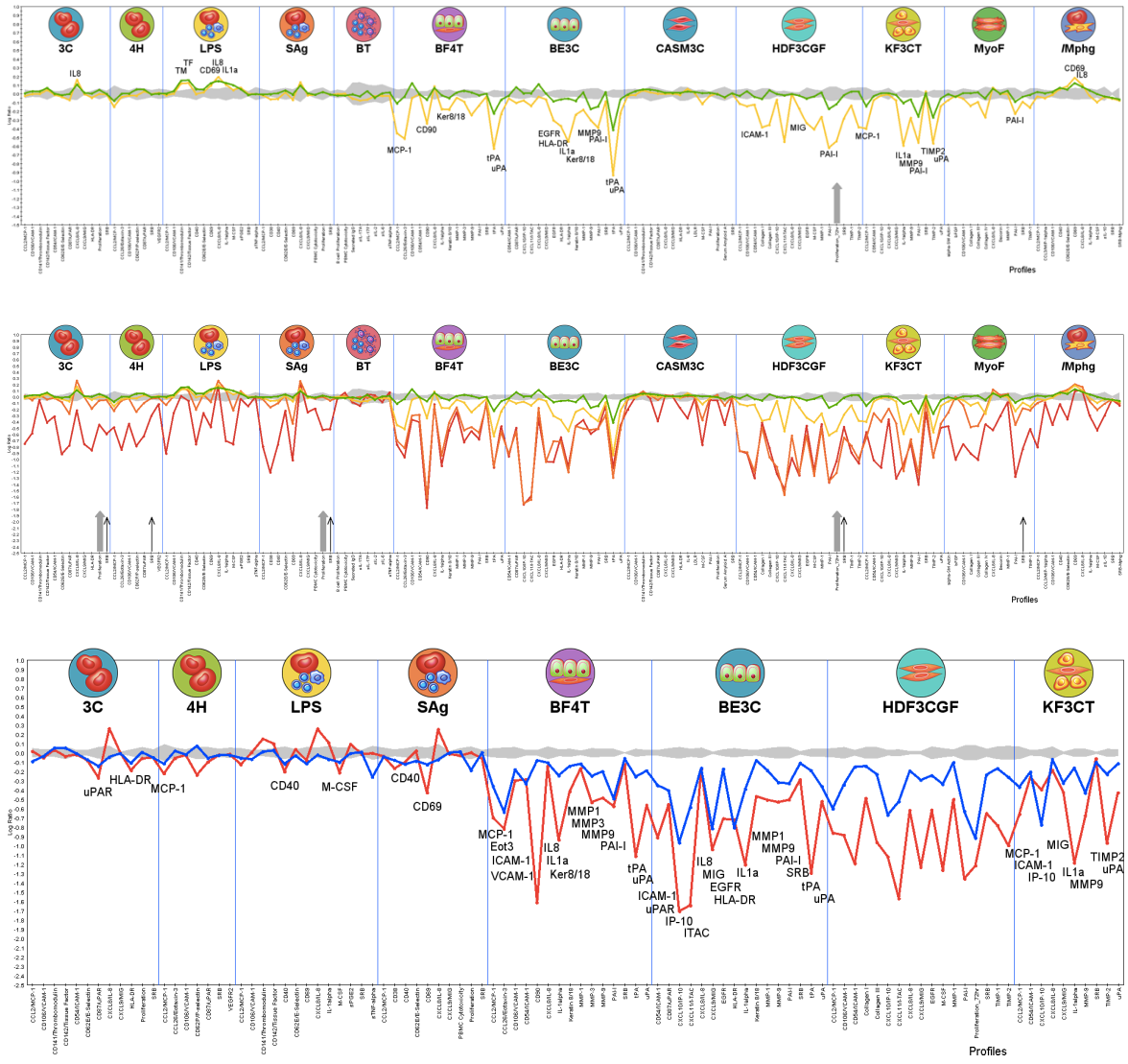


Fig. S10. BioMAP profile of D&C Red No. 28 (Phloxine B) and nearest drug match.

Top: Phloxine B had thirty-one annotated readouts, was cytotoxic in one system at 10 µM (thus not shown), and was antiproliferative to fibroblasts. Middle: At 30 µM, cytotoxicity was observed in three or more systems making it impossible to annotate readouts. Bottom: Nearest drug match of Phloxine B (10 µM) was with zafirlukast (10 µM, leukotriene receptor antagonist), sharing fourty-three annotated readouts in eight systems not impacted by cytotoxicity at this concentration. The relevance of the match is unclear; Pearson's correlation is 0.69.

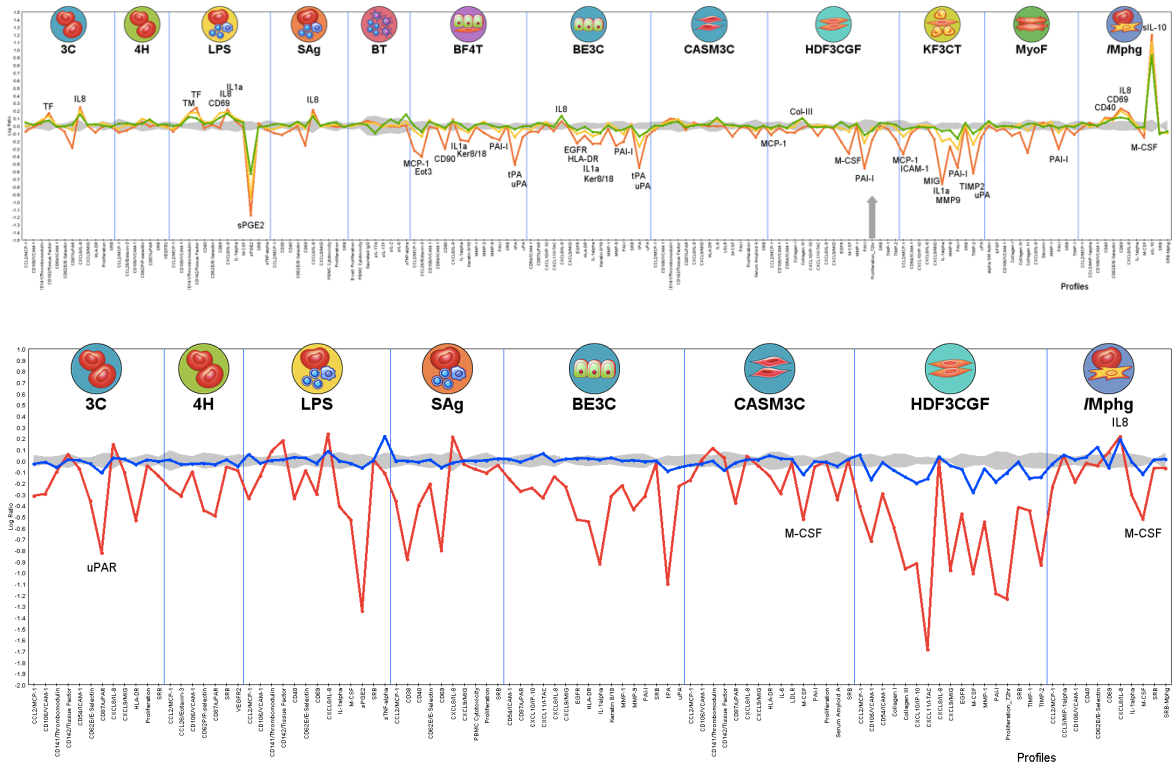


Fig. S11. BioMAP profile of FD&C Red No. 3 and nearest drug match.

Top: FD&C Red No. 3 had fourty-three annotated readouts, was cytotoxic in one system at 30 μM (thus not shown), and was antiproliferative to fibroblasts. Bottom: Nearest drug match of FD&C Red No. 3 (30 μM) was with dibenzazepine (370 nM, γ-secretase inhibitor), sharing four annotated readouts in eight systems not impacted by cytotoxicity at this concentration. The relevance of the match is unclear; Pearson's correlation is 0.64.

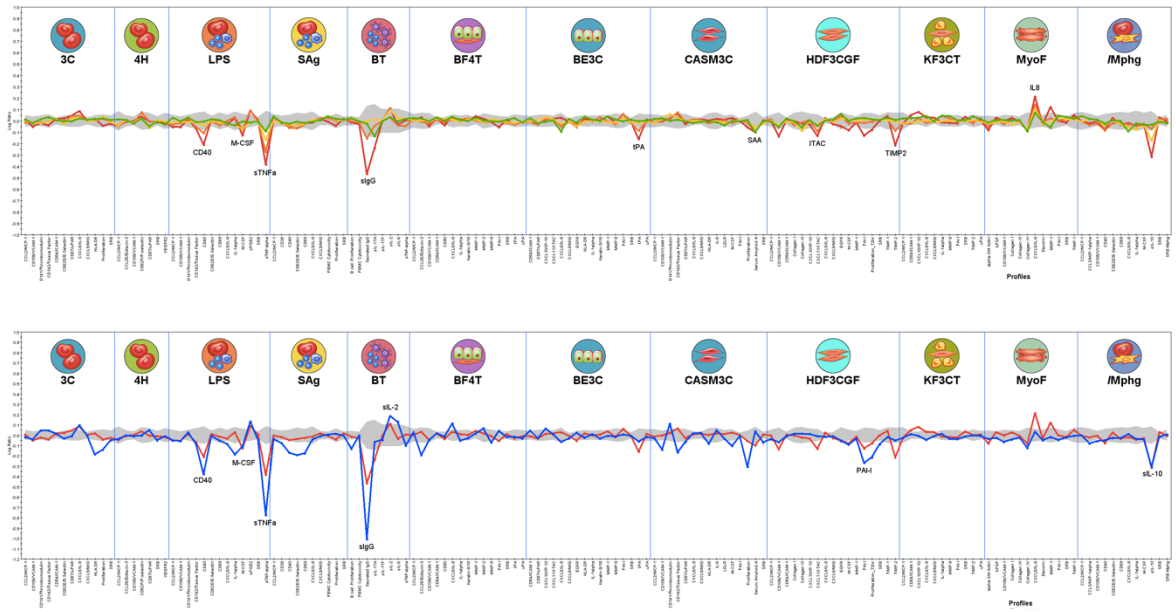


Fig. S12. BioMAP profile of diethyl phthalate and nearest drug match.

Top: Diethyl phthalate was tested at 400, 130, 44, and 15 µM. Diethyl phthalate had nine annotated readouts and did not have cytotoxic or antiproliferative effects. Bottom: Nearest drug match of diethyl phthalate (400 µM) was with roflumilast (90 µM, PDE4 inhibitor), sharing seven annotated readouts. The match is mechanistically relevant; Pearson's correlation is 0.72.

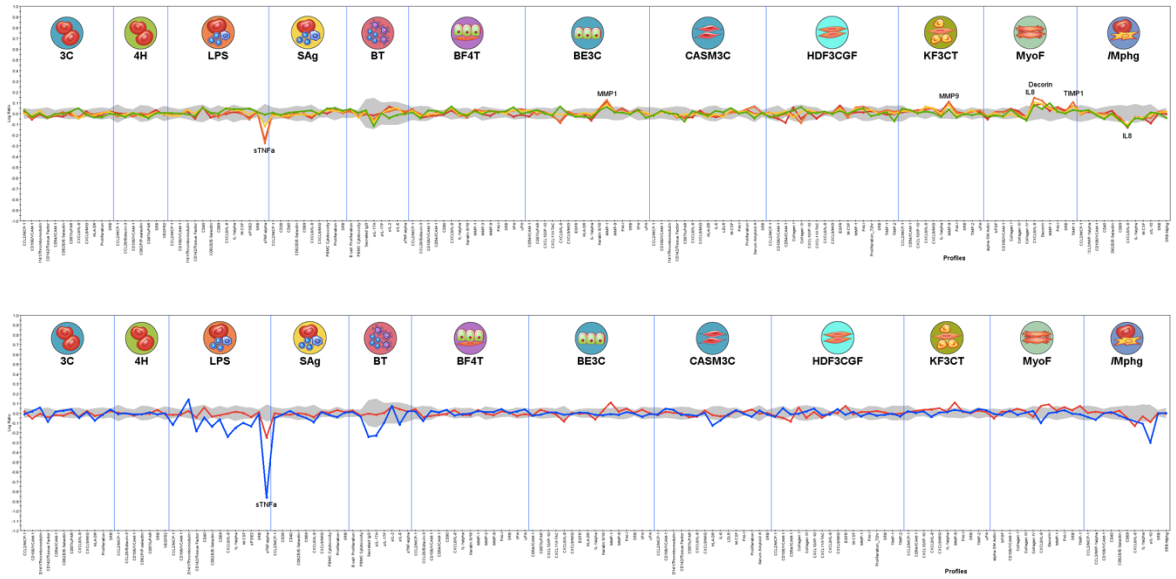


Fig. S13. BioMAP profile of hydroxypropyl methyl cellulose (HPMC) and nearest drug match.

Top: HPMC was tested at 360000, 120000, 40000, and 13000 ng/mL. HPMC had seven annotated readouts and did not have cytotoxic or antiproliferative effects. Bottom: Nearest drug match of HPMC (360000 ng/mL) was with Ro 320-1195 (3.3 μM, p38 MAPK inhibitor), sharing one annotated readout. The relevance of the match is unknown; Pearson's correlation is 0.54.

Excipient	Nearest drug match (Pearson's correlation: >0.7)	Conclusion	Toxicity signature/mechanism of action
Aspartam	not determined	No effect	none
Propylene glycol	not determined	Does not have any cytotoxic or antiproliferative impacts across the Diversity PLUS panel.	none
Propyl gallate	Mycophenolate Mofetil (CellCept) (0.81)	Active with 25 annotated readouts. There are no cytotoxic impacts across the Diversity PLUS panel; however, antiproliferative to B cells, coronary artery smooth muscle cells, endothelial cells, fibroblasts and T cells. It impacted inflammation-related activities (decreased Eotaxin 3, VCAM-1, MCP-1, I-TAC, IL-1α, sPGE2; modulated IL-8), immunomodulatory activities (decreased CD40, sIL-10, M-CSF, sIL-17F; increased CD69), tissue remodeling activities (decreased TIMP-2, Collagen IV, tPA, MMP-1; increased Collagen III), and hemostasis-related activities (decreased TF).	**Immunosupression**: ≥10 uM; Organ tox: ≥3.3 uM
Hydropropyl methyl cellulose	not determined	Hydroxypropyl methyl cellulose is active with 7 annotated readouts. It mediated changes in key biomarker activities are listed by biological and disease classifications. It impacted inflammation-related activities (decreased sTNFα; modulated IL-8) and tissue remodeling activities (increased Decorin, TIMP-1, MMP-1, MMP-9). It did not have any antiproliferative or cytotoxic impacts across the Diversity PLUS panel.	none
Diethyl phthalate	Roflumilast (0.72)	Diethyl phthalate is active with 9 annotated readouts. Diethyl phthalate mediated changes in key biomarker activities are listed by biological and disease classifications. It impacted inflammation-related activities (decreased SAA, sTNFα, I-TAC; increased IL-8), immunomodulatory activities (decreased CD40, sIgG, M-CSF), and tissue remodeling activities (decreased TIMP-2, tPA). It did not have any antiproliferative or changes in key biomarker activities are listed by biological and disease classifications. It did not have any antiproliferative or cytotoxic impacts across the Diversity PLUS panel.	none **EP agonist @ 44 uM**
Tartrazine	not determined	No effect	none
Butyl paraben	Nabumetone (0.74)	Did not have any cytotoxic impacts across Diversity PLUS systems. Antiproliferative to fibroblasts and T cells. Impacted in inflammation-related activities (decreased MCP-1, sPGE2); immunomodulatory activities (decreased sIgG); tissue remodeling activities (decreased tPA, Collagen III), and hemostasis-related activities (decreased TF).	**Immunesupression**: ≥1.1uM; Organ tox: ≥3.3 uM
Polysorbate 80	IL-10 (0.84)	There are no cytotoxic or antiproliferative impacts across the Diversity PLUS panel. It mediated changes in key biomarker activities are listed by biological and disease classifications. JA-13-RB49 impacted inflammation-related activities (decreased IL-8), immunomodulatory activities (decreased CD69), and tissue remodeling activities (decreased TIMP-1, tPA). **It was classified as a p38 MAPK inhibitor at 30uM only.**	none **p38 MAPK inhibition @ 30 uM**
Thimerosal	Ceriylpyridinium chloride (0.86)	Cytotoxic in >3 Diversity PLUS systems at 30 uM (3C, 4H, LPS, SAg, BT, CASM3C, HDF3CGF, MyoF and IMphg). 10 uM (3C, 4H, LPS, SAg, BT, HDF3CGF, MyoF and IMphg). 3.3 uM (3C, 4H, LPS, SAg, BT, HDF3CGF, MyoF), and also cytotoxic to PBMCs in the SAg and BT systems at 1.1 uM. These concentrations were excluded from annotation.	**Acute tox**: ≥3.3 uM; not assessed
FD&C Red #3 (Erythrosine extra bluish)	DBZ (7.5)	Active with 69 annotated readouts. Cytotoxic in the HDF3CGF system at 30 µM. Activities in this system are annotated for non-cytotoxic concentrations. It is antiproliferative to fibroblasts and T cells. Impacted inflammation-related activities (decreased Eotaxin 3, E-selectin, MCP-1, SAA, I-TAC, MIG, ICAM-1, sPGE2; increased IL-8; modulated IL-1α), immunomodulatory activities (decreased HLA-DR, M-CSF, CD38; increased sIL-10; modulated CD40, CD69), tissue remodeling activities (decreased Collagen I, TIMP-2, Collagen IV, tPA, uPA, CD90, EGFR, bFGF, MMP-3, PAI-1, uPAR, Ker8/18, MMP-9; modulated Collagen III) and hemostasis related activitiwes (increased FT, TM).	**Thrombosis**: ≥1.1 uM
D&C Red #28 (Phloxine)	Zafirlucast (0.69)	Active with 61 annotated readouts. Cytotoxic in the HDF3CGF system at 10 µM. This system was annotated at non-cytotoxic concentrations. Antiproliferative to fibroblasts. Impacted inflammation-related activities (decreased Eotaxin 3, MCP-1, I-TAC, MIG, ICAM-1, IP-10; increased E-selectin, IL-8; modulated IL-1α), immunomodulatory activities (decreased CD40, HLA-DR, M-CSF, CD38; modulated CD69), tissue remodeling activities (decreased Collagen I, TIMP-2, Collagen IV, TIMP-1, tPA, uPA, CD90, Collagen III, α-SMA, EGFR, MMP-1, MMP-3, PAI-1, uPAR, Ker8/18, MMP-9), and hemostasis-related activities (increased TF, TM). Cytotoxic in ≥ 3 Diversity PLUS system at 30 µM, and this concentration was excluded from annotation and Mechanism Classification Prediction analyses. Overtly cytotoxic in ≥ 3 Diversity PLUS systems at 30 µM (3C, 4H, SAg, HDF3CGF, and MyoF) and this concentration was excluded from annotation and Mechanism Classification Prediction analyses.	**Acute toxicity**: ≥30 uM
Cetylpyridinium Chloride (monohydrate)	Cetylpiridinium chloride (0.95) Bafilomycin (0.9)	Cytotoxic in ≥ 3 Diversity PLUS systems at 30 µM (SAg, BT, HDF3CGF, MyoF and IMphg). 10 µM (3C, 4H, LPS, SAg, BT, HDF3CGF, MyoF and IMphg), and 3.3 µM (3C, SAg, BT, HDF3CGF, MyoF). These concentrations were excluded form annotation and Mechanism Classification Prediction analyses. It was classified as a **Microtubule Disruptor at a single concentration of 1.1 µM** (DV = 0.49). Note, the other concentrationswere cytotoxic in 3 or more systems. There are 6 common activities that are annotated within the following systems: 3C (HLA-DR, Prolif), SAg (CD40, Prolif), CASM3C (Proif) and HDF3CGF (Proiif 72).	**Acute Toxicity**: ≥3.3 uM; **not assessed** **Microtubule disraptor @ 1.1 uM**

Fig. S14. Raw description of results provided from BioMAP profiling. More detailed results provided related to Figure 2 and Figs. S2-S13 above.

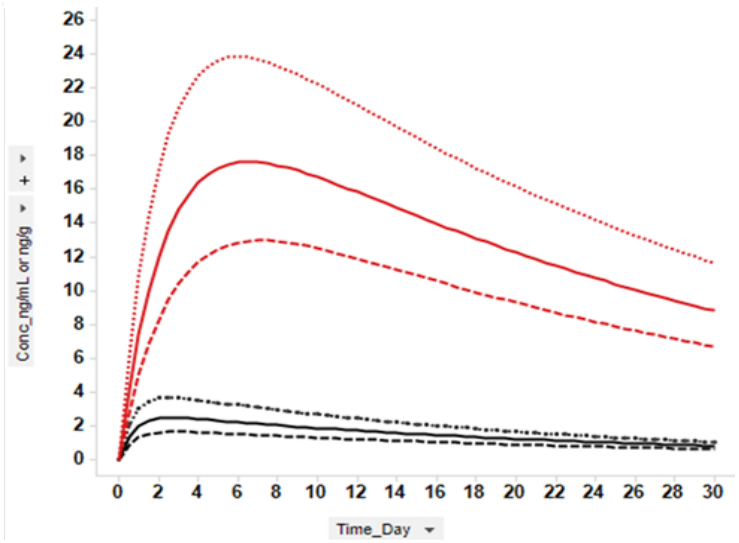
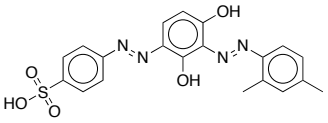
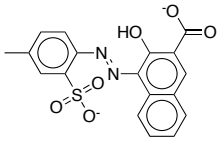
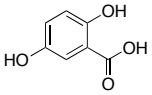
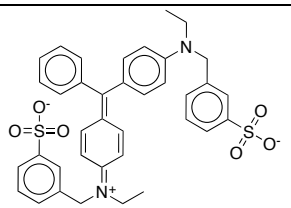
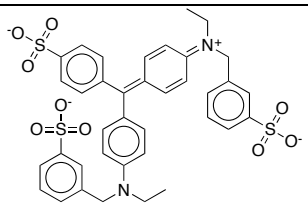
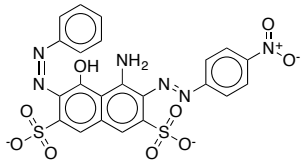
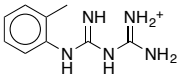
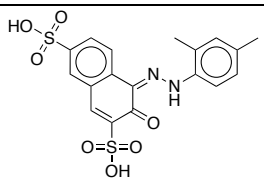


Fig. S15. Allometrical scaling of Hg or EtHg pharmacokinetics in blood or brain from preclinical species or human adult to human infants.

Predicted Hg blood or brain concentration – time profile in human infants allometrically scaled from monkey infants (*43*) after receiving vaccination including thimerosal with 25µg ethylmercury. ⋯.... Newborn; ― 2-mo old; ---6-mo old; Red (Hg in brain); Black (Hg in blood).

Excipient	Function	Structure	Highest excipient amount/dose	Target	SEA Score	K_i/AC_{50} (μM)
D&C Brown No. 1	Colorant		0.005 mg [a]	BRD4 SLCO2B1	$1.1x10^{-27}$ $5.2x10^{-27}$	46 2.8
D&C Red No. 6	Colorant		1.5 mg [a]	SLCO1B1 SLCO2B1	$1.7x10^{-30}$ $1.7x10^{-30}$	3.1 10
Gentisic Acid	Antioxidant		0.63 mg/mL [b]	PTGS1 PTGS2	NA	18 5.8
Guinea Green B	Colorant		0.12 mg [a]	SLCO2B1	NA	0.7
Light Green SF	Colorant		40 mg [a]	SLCO2B1	NA	0.7
Naphthol Blue Black	Colorant		0.08 mg [a]	SLCO2B1	NA	0.4
O-tolylbiguanide	Antioxidant		0.013% w/w [c]	5HT3 ADRA1A ADRA2A	$2.0x10^{-12}$ NA NA	88 5.0 7.1
Ponceau Xylidine	Colorant		0.18 mg [a]	SLCO2B1	NA	3.1

a. Oral, b. Intravenous, c. Topical
Activity values accurate to +/- 20%

Table S1.

New excipient-off targets determined by computational prediction and/or side-effect panel experiments that are listed in the IID, but for which a currently marketed drug was not found

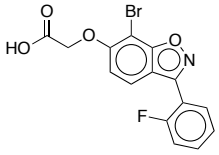
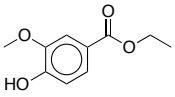
Excipient	Function	2D Structure	Target	SEA Score (if applicable)	K_i/AC_{50} (μM)
Brocrinat	Diuretic		PTPN6 PPARG	$5.0x10^{-06}$ NA	200 23
Ethyl Vanillate	Flavoring agent		ALOX5 PDE4D	$4.3x10^{-25}$ $4.3x10^{-39}$	270 67

Table S2.

New excipient-off targets determined by computational prediction and/or side-effect panel experiments are listed in the IID without a dosage and are assumed to not be in any currently marketed drugs.

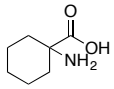
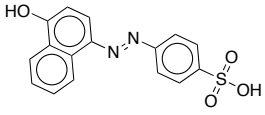
Excipient	Function	2D Structure	Target	SEA Score	K_i/AC_{50} (μM)
1-Aminocyclo-hexane-carboxylic acid, C-11	Probe of BBB amino acid transport		CACNA2D1	$6.4x10^{-31}$	4.3
Acid Orange 20	Colorant		BRD4 SLCO2B1	$1.4x10^{-25}$ $7.9x10^{-35}$	25 3.3

Table S3.

Molecular targets determined for excipients that were current in the IID at the outset of this study (2016), but have since been removed from that database.

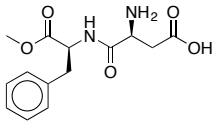
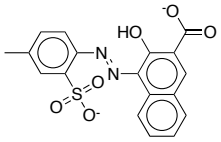
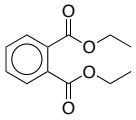
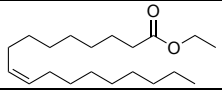
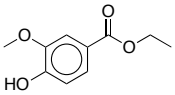
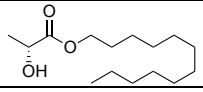
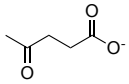
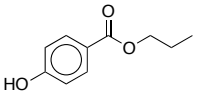
Excipient	Function	2D Structure	Target	SEA Score	Activity
Aspartame	Sweetener		CAPN1 TACR1 GPR142 OPRK1 SLC6A3 HTR2B	$2.6x10^{-194}$ $9.3x10^{-11}$ $1.2x10^{-37}$ $9.0x10^{-08}$ $3.5x10^{-07}$ $2.0x10^{-06}$	0% @ 40 µM 0% @ 10 µM >30 µM >30 µM >30 µM >30 µM
D&C Red No. 6	Colorant		P2RX5	$1.6x10^{-14}$	>30 µM
Diethyl Phthalate	Film-forming agent; plasticizer; solvent		PDE4B CACNA1C CACNA1D ADRB1 CHRM5 CHRM1 CHRM4 CHRM2 ADORA2A	$1.0x10^{-09}$ $1.1x10^{-43}$ $2.6x10^{-43}$ $1.5x10^{-23}$ $5.9x10^{-11}$ $9.6x10^{-11}$ $2.2x10^{-09}$ $1.1x10^{-06}$ $2.3x10^{-06}$	>100 µM >30 µM >30 µM >30 µM >30 µM >30 µM >30 µM >30 µM >30 µM
Ethyl Oleate			CNR1	$5.2x10^{-15}$	0% @ 10 µM
Ethyl Vanillate	Flavoring agent		AKR1B1 DUSP3 CAH14 HTR2B ADRB1	$1.0x10^{-11}$ $1.3x10^{-14}$ $1.4x10^{-23}$ $1.5x10^{-14}$ $3.4x10^{-13}$	2% @ 10 µM 14% @ 10 µM No inhibition >30 µM >30 µM
Lauryl Lactate			FAAH	$3.3x10^{-21}$	9% @ 10 µM
Levulinic Acid			KDM5C	$2.5x10^{-11}$	0% @ 100 µM
Propylparaben	Antimicrobial preservative		CAH14	$1.2x10^{-19}$	No inhibition

Table S4.

Predictions made by SEA that were ultimately tested experimentally and proved to be false. Does not include cases where non-specific inhibition as a result of aggregation was deemed to be the culprit. Testing concentrations provided where available. No follow-up experiments were conducted with these excipient-target pairs.

Excipient
Acetyltryptophan
Aminobenzoate sodium
Ascorbyl palmitate
D&C Red No. 36
Erythritol
Eugenol
FD&C Yellow No. 5
Glycerin
Lactic Acid
Mannitol
Ponceau 3R
Propyl gallate
Propylparaben
Sodium propionate
Sorbitol
Stannous 2-ethylhexanoate
Tagatose
Vanillin
Xylitol

Table S5.

List of excipients predicted by SEA to target OATP2B1/1B1 that were selected as described above in Materials and Methods, and ultimately showed minimal/no activity at 200 μM.

	5-HT1A	5-HT2B_ag	A3	a1A_ag	a1A_antag	alpha2A	D1	D3	H3	M1_ag	M1_antag	M2	MOP	TP_ag	TP_antag	GABAA	AR	ERa	PR-B	DAT	NET	SERT	ACES	COX1	COX2	MAO-A	PDE3	PDE4	Thrombin	KDR	hERG bind	hERG QP
Excipient	**HTR1A**	**HTR2B**	**ADORA3**	**ADRA1A**	**ADRA1A**	**ADRA2A**	**DRD1**	**DRD3**	**HRH3**	**CHRM1**	**HRM1**	**CHRM2**	**OPRM1**	**TBXA2R**	**TBXA2R**	**GABRA1**	**AR**	**ESR1**	**PGR**	**SLC6A3**	**SLC6A2**	**SLC6A4**	**ACHE**	**PTGS1**	**PTGS2**	**MAOA**	**PDE3A**	**PDE4D**	**F2**	**KDR**	**KCNH2**	**KCNH2**
1-(o-Tolyl)biguanide	>30	>30	>30	5.0		7.1	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
1-Aminocyclohexanecarboxylic acid	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
2,5-Dihydroxybenzoic acid (Gentisic acid)	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
2-Isopropyl-5-methylphenol (Thymol)	>30	>30	>30	>30	26	>30	>30	>30	>30	>30	14	>30	>30	>30	>30	>30	>30	>30	>30	>30	23	>30	>30	>30	>3	>30	>30	>30	>30	>30	>30	
4-chloro-3,5-dimethylphenol (Chloroxylenol)	>30	>30	>30	>30	14.2	>30	>30	>30	>30	>30	9.9	>30	>30	>30	28	>30	>30	>30	>30	>30	>30	>30	>30	>30	>3	>30	>30	>30	>30	>30	>30	>30
Ammonio methacrylate copolymer Type B	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>1		
Aspartame	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
Benzethonium chloride	>30	>30	5.3	>10	4.7	11	3.8	1.6	27	>10	1.8	1.1	6.0	>10	>3	>30	>30	11	6.2	0.9	1.18	2.3	2.3	>30	24	>30	>30	14	>30	>30	0.49	1.9
Benzoic acid	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30
Benzyl Alcohol	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
Brocrinat	>30	>30	24	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	15	>30	>30	>30	
Butyl paraben	>30	>30	>30	>30	16.4	>30	>30	>30	>30	>30	12	>30	>30	>30	19	>30	>30	3.5	>30	19	20	>30	>30	>30	>3	>30	>30	>30	>30	>30	>30	>30
Butylated hydroxyanisole	>30	>30	>30	>30	19	>30	>30	>30	>30	>30	19	>30	>30	>30	>30	>30	>30	29	>30	>30	>30	>30	>30	>30	2.6	>30	>30	>30	>30			
Calcium carbonate	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Cellulose, microcrystalline	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
Cetylpyridinium Chloride (monohydrate)	9.9	>30	3.3	>10	6.4	6.1	4.8	0.55	13	18		0.8	5.1	>10	3.4	>30	>30	18	4.5	2.4	2.3	3.0	4.7	7.5	2.5	>30	>30	13	>30	>30	1.3	21.5
Citric Acid	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Coco monoethanolamide (Cocamide)	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	18	>30	>30	>30	23		>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
Croscarmellose sodium	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30			
D&C Red #28 (Phloxine)	2.4	>30	0.48	>3	0.48	14	2.1	1.5	21	>1		0.68	0.8	>1		>30	28	18	30	>30	>30	>30	2.0	2.6	3.0	>30	0.1	0.9	25	12	17	>30
D&C Red #6 (Lithol Rubine Na)	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>10	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30			
D&C Yellow #10 (Quinoline Yellow WS)	>30	>30	>30	>30	>30	28	>30	26	>30	>30	>30	>30	>30	>30	>30		>30	19	>30	29	>30	>30	>10	>30	>30	>30	>30	>30	>30			
D-Arginine	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Diethyl phthalate	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	16	>30	>30	>30	>30
D-Mannitol	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30
Ethyl Cellulose	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Ethyl vanillate	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30	>30	>30	>30	>30	>30	>30	>30	>3	>30	>30	>30	>30	>30	>30	
Ethylene diamine	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
Ethylparaben	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>1	>30	>30	>30	>30	>30	>30	>30
FD&C Blue #1 (Brilliant Blue FCF)	>30	>10	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>10	>30	22	>30	>30	28	>30			
FD&C Blue #2 (Indigo carmine)	>30	>30	1.0	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30	>10	>30	>30	>30	>30	>30	>30	24	>3	>30	>30	>30	3.1	>30	
FD&C Red #3 (Erythrosine extra bluish)	1.9	>30	3.3	>0.3	>0.1	>30	3.9	0.8	>30	>0.3	>0.1	0.8	0.6	>0.3		25	>30	>30	16	>30	>30	>30	0.42	5.7	>30	>30	0.092	0.32	16	>3	2.8	>30
FD&C Red #40 (Allura Red AC)	19	>30	>30	>30	>30	>30	29	>30	>30	>30	>30	>30	>30	>30	>30	22	28	15	30	>30	>30	>30	>10	>30	26	>30	17	>30	>30			
FD&C Yellow # 5 (Tartrazine)	>30	>30	>30	>30	>30	>30	23	>30	>30	>30	>30	>30	16	>30	>30	13	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
FD&C Yellow # 6 (Sunset yellow)	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>10	>30	>30	>30	>30	>30	>30			
Ferric oxide	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Glycerine	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
Glyceryl laurate	>30	>30	23	>30	6.8	>30	29	4.8	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	13	>30	>30	>30	>30	>30	>10	>30	>30	>30	>30	
Hydroxy propyl cellulose	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>3	>3	>30	>30		>30
Hydroxyl propyl methyl cellulose	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>10	>3	>30	>30	>30	
Hypromellose, unspecified	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>10	>3	>30	>30	>30	
Lactose	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
L-Arginine	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
L-cysteine	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	4.7	>30	>30	>30	>30	>30		
Methyl 4-hydroxybenzoate (methylparaben)	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>1	>30	>30	>30	>30	>30	>30	
Methyl cellulose	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
Methylene Blue hydrate	>30	>30	>30	>30	>30	5.5	>30	>30	0.88	>30	6.3	0.19	6.2	>30	>30	>30	>30	>30	>30	4.6	2.9	8.3	0.09	24	1.8	0.0	>30	>30	24	>3	2.5	27.5
m-Iodobenzylguanidine hemisulfate	2.5	>30	>30	>30	11	0.49	13	8.2	>30	>30	>30	>30	22	>30	>30		>30	>30	>30	6.4	8.8	>30	>30	>30	>30	26	>30	>30	>30	>30	>30		
Oleic acid	>30	>30	12	>30	>30	>30	>30	>30	>30	>30	13	>30	>30	>30	25	>30	>30	>30	12	>30	27	>30	>30	6.7	3.5	>30	>30	>30	>30	>30	>30	>30
Palmitic acid	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>3	>30	4.1	6.6	>30	>30	>30	>30
PEG 400	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
Phenol	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>3	>30	>30	>30	>30	>30	>30	
Phenylmercuric acetate	1.9	>30	1.4	8.7		2.0	2.2	0.024	5.2	8.1		>30	3.8	6.2		>30	>30	>30	>30	1.8	1.7	5.1	5.8	>30	>30	6.6	>30	7.0	>30	22	3.2	6.1999998
Polysorbate 80	>30	>30	9.3	>30	>30	>30	>30	22	5.3	>30	2.6	1.3	>30	>30	12	>30	>30	>10	21	>30	>30	>30	>30	1.0	2.9	>30	>3	>10	>30	>30	>30	>30
Potassium metabisulfite	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Povidone or PVP	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
Pramoxine hydrochloride	>30	>30	>30	>30	>30	>30	>30	>30	2.5	>30	17	4.0	>30	>30	>30		>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	7.5	20.5
Propyl gallate	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	7.2	>30	>30	26	>30	>30	>30	
Propylene glycol	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30
Propylparaben	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	23	>30	>30	>30	>30	>30	>30	11	>30	>30	30	>30	>30	>30	>3	>30	>30	>30	>30	24		>30
Saccharin	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30
Silicon dioxide (Colloidal silicon dioxide, silica)		>30		>30	>30	>30	>30			>30	>30	>30		>30	>30							>30					>30					
Sodium benzoate	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		>30
Sodium bicarbonate	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Sodium citrate tribasic dihydrate	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Sodium dodecyl sulfate	15	>30	15	>30	15	>30	>30	18	>30	>30	29	>30	>30	>30	>10	>30	>30	>30	15	>30	>30	>30	>30	>30	>30	>30	>30	23	>30	>30		
Sodium lauryl sulfate	15	>30	15	>30	15	>30	>30	18	>30	>30	29	>30	>30	>30	>10	>30	>30	>30	15	>30	>30	>30	>30	>30	>30	>30	>30	23	>30	>30		
Sodium L-glutamate (monohydrate)	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Sodium metabisulfite	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30		
Stearic acid	>30	>30	10	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	16	>30	>30	>30	>30	>30	>30	>30	>30	3.1	6.7	>30	>30	>30	
Succinic acid	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
Triethyl citrate	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	>30	
Thimerosal	30	15	1.6	>30	17	3.8	2.4	see table S5	>30	30		>30	27	>30	18	>30	>30	>30	>30	>30	>30	>30	>30	5.3	19	9.7	>30	>30	>30	16	>30	>30
Ethyl mercury (Thimerosal metabolite)	1.92	>30	1.28	>30	>3	2.11	4.68	0.207	9.95	25.09		>30	3.67	1.81		>30	>30	>30	>30	1.59	2.92	6.05	11.8	>30	>30	>30	>30	>30	>30		3.31	

Table S6. Excipients tested in the secondary pharmacology assay panel, including the hERG assay. Assays represent frequently encountered "off-targets" associated with observed clinical adverse drug reactions (ADRs). The panel is broadly used in drug discovery for off-target mitigation. All excipients were tested up to 30 μM concentration and IC_{50} or EC_{50} values were determined in case more than 50% target engagement was observed at 30 μM. Rows highlighted in yellow denote excipients having no on-target activity in this panel. For extended description of the assay protocols, see Methods.

Site	Ki/IC50 (µM)	Geo. mean IC50 (µM)	St. dev. (µM)
NIBR lab	0.052 0.085 0.09 0.059 0.05 0.077 0.029 0.072	0.061	0.020
Eurofins	0.059 0.31	0.135	0.177
UNC lab	0.352 0.946 1.205 1.72 1.49 1.55 1.25 1.22 0.508 0.595 0.478 0.567 0.721 0.62 0.745 0.568 0.384 0.348	0.745	0.445
All		**0.323**	**0.514**

Table S7.

Thimerosal was tested at D_3 dopamine receptor binding assays in three different laboratories: Department of Pharmacology, University of North Carolina, Chapel Hill (PDSP), Eurofins laboratories and Novartis Institutes for Biomedical Research (NIBR), Data are listed from the individual assays and the geometric mean from all K_i/IC_{50} values were calculated.

Parameter		Monkey	Newborns	2 mo old	6 mo old
Brain	Ka (1/Day)	0.0330			
	V/F (mL/kg)	15.3			
	Cl/F (mL/(kg*Day))	14.5	7.27	6.44	5.69
	Residual error	0.195 (Multiplicative)			
Blood	Ka (1/Day)	1.38			
	V/F (mL/kg)	1546			
	Cl/F (mL/(kg*Day))	146	73.2	64.8	57.3
	Residual error	0.228 (Multiplicative)			

Table S8.

Parameter estimates derived from one-compartment analysis of blood or brain total Hg in monkey infants and allometrically scaled values to human infants.