Analysis of Pfizer Compounds in EPA's ToxCast Chemicals-Assay Space

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Analysis of Pfizer Compounds in EPA's ToxCast Chemicals-Assay Space

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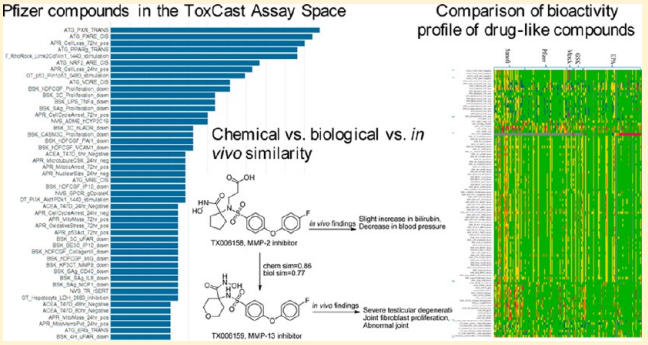
Supporting Information

ABSTRACT: The U.S. Environmental Protection Agency (EPA) launched the ToxCast program in 2007 with the goal of evaluating high-throughput *in vitro* assays to prioritize chemicals that need toxicity testing. Their goal was to develop predictive bioactivity signatures for toxic compounds using a set of *in vitro* assays and/or *in silico* properties. In 2009, Pfizer joined the ToxCast initiative by contributing 52 compounds with preclinical and clinical data for profiling across the multiple assay platforms available. Here, we describe the initial analysis of the Pfizer subset of compounds within the ToxCast chemical (n = 1814) and *in vitro* assay (n = 486) space. An analysis of the hit rate of Pfizer compounds in the ToxCast assay panel allowed us to focus our mining of assays potentially most relevant to the attrition of our compounds. We compared the bioactivity profile of Pfizer compounds to other compounds in the ToxCast chemical space to gain insights into common toxicity pathways. Additionally, we explored the similarity in the chemical and biological spaces between drug-like compounds and environmental chemicals in ToxCast and compared the *in vivo* profiles of a subset of failed pharmaceuticals having high similarity in both spaces. We found differences in the chemical and biological spaces of pharmaceuticals compared to environmental chemicals, which may question the applicability of bioactivity signatures developed exclusively based on the latter to drug-like compounds if used without prior validation with the ToxCast Phase-II chemicals. Finally, our analysis has allowed us to identify novel interactions for our compounds in particular with multiple nuclear receptors that were previously not known. This insight may help us to identify potential liabilities with future novel compounds.



1. INTRODUCTION

A challenge for the pharmaceutical industry is the identification of the potential toxicity of new chemical entities early in the drug discovery stages.[1] Despite the evolution of new safety assessment strategies in pharmaceutical organizations, safety issues remain a major cause of compound attrition in both preclinical and clinical stages, and, more importantly, have not prevented the withdrawal of drugs from the market.[2] The safety assessment of pharmaceuticals has long relied on *in vivo* studies in animals and extrapolation of observed findings to predict safe exposure levels and effective dosing in humans.[3] However, there are several limitations to this approach. *In vivo* animal toxicity data may not translate well enough to predict the safety of a xenobiotic agent in humans. In addition, these data provide little information about the actual mechanisms of toxicities. These studies are expensive, cumbersome, time-consuming, and require a large cohort of animals.[4] The paradigm of toxicity testing has been challenged recently to shift from more classical laboratory animal studies to *in vitro* assays that use primary human cells or human cell-lines and focus on biologically significant perturbations linked with potential toxicity pathways enabling insights into molecular mechanisms of observed toxicities.[5] The long-term goal for *in vitro* toxicity testing of compounds is to use *in vitro* assays as a tool for chemical prioritization that can limit the number of chemicals progressing to higher tier regulatory-required animal studies.[6]

Recently, there have been a number of predictive toxicology initiatives by regulatory agencies aimed at improving the ability to project *in vivo* toxicities using *in silico* methods[7] and/or *in vitro* assays.[8−11] One of these approaches is ToxCast, a project initiated by the US Environmental Protection Agency (EPA) in 2007 to predict the potential toxicity of the environmental chemicals using an array of *in vitro*, high-throughput screening assays, applied to a relatively large and diverse chemical space.[12] The goal of the ToxCast project is to develop predictive bioactivity signatures using *in vitro* assays and *in silico* data in order to predict *in vivo* toxicities or phenotypes observed in traditional animal toxicity testing such that the resulting signatures could then be applied to untested chemicals.[13,14] The first, proof-of-concept phase of ToxCast involved 309 unique chemicals, most of which were food-use pesticides, rich in

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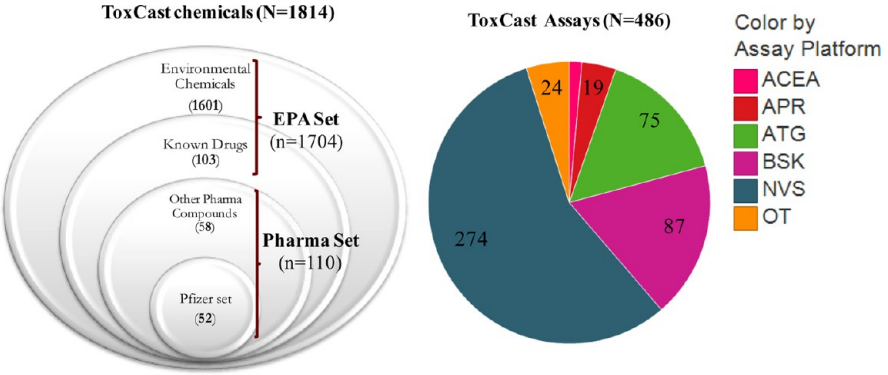

Figure 1. Summary of the ToxCast unique chemicals and assays used in this study.

animal toxicity data that were profiled in 467 biochemical or cell-based assays from 9 assay technologies.[13] The first phase of ToxCast was quite successful in confirming the expected interactions as well as developing *in vitro* assay signatures in a few instances that are predictive of *in vivo* phenotypes.[13−18]

To further validate their efforts, the EPA launched the second phase of ToxCast and expanded their chemistry space beyond food-use pesticides to include other environmental chemicals such as plasticizers, phthalates, and antimicrobials, as well as a small number of pharmaceutical compounds and marketed drugs.[19] In 2009, Pfizer joined the ToxCast initiative and contributed 52 discontinued pharmaceuticals with preclinical *in vivo* animal toxicity data. Subsequently, other pharma companies, including Merck, Sanofi, Roche, and GSK, joined the initiative which increased the number of pharma-donated compounds to a total of 110. It is worthwhile to mention that these pharma-donated compounds are development candidates that failed in preclinical and/or clinical studies primarily because of safety issues.

Herein, we describe our initial analysis of Pfizer compounds within the ToxCast chemical and assay spaces. The hit rates of compounds in individual assays and in each assay platform were analyzed. We also compared the bioactivity profiles of Pfizer compounds with failed pharmaceuticals from other companies and with marketed drugs. In addition, chemical and pharmacological similarities of the pharmaceuticals were compared to each other and to other nonpharmaceuticals to understand the overall applicability of ToxCast chemical assay space in the prediction of *in vivo* toxic effects. Our analyses identified novel interactions for our compounds that were previously not known and helped us to generate potential hypotheses to explain the observed *in vivo* toxicities of our compounds. They also highlighted the need for caution in the use and interpretation of these large scale assay data.

2. MATERIALS AND METHODS

2.1. Tools Used. Pipeline Pilot, version 8.5,[20] custom scripting was used for the ToxCast data manipulation and analyses. The results were visualized using the TIBCO Spotfire 3.3 DXP.[21]

2.2. Chemical Library. A chemical data set of 1831 compounds was provided to all companies by the EPA on March 30th, 2012 as part of the EPA-PHARMA collaboration, and this file was used for this analysis. Detailed information on individual ToxCast phases for these compounds can be obtained from the EPA Web site (http://www.epa.gov/ncct/dsstox/sdf_toxcst.html). These include a total of 1831 compounds with 1814 unique structures and 17 replicate samples. A subset (~800 of 1814 compounds) was referred to as the e1K subset by the EPA and was only profiled in a small set of ToxCast assays related to endocrine activities. The rationale for the selection of the ToxCast compounds and information on chemical quality control are described in other publications from the EPA.[19,22] The breakdown of the ToxCast chemicals by compound type and source is shown in Figure 1. The discontinued pharmaceuticals, labeled as the Pharma set, comprised unique compounds (n = 110) supplied by Pfizer, Merck, Sanofi, Roche, and GSK. The EPA set comprised marketed drugs (n = 103) with well-known therapeutic activities and other nonpharmaceuticals (n = 1601) such as plasticizers, pesticides, pthalates, food additives, and antimicrobials.

2.3. Pfizer Set. Pfizer contributed 52 compounds to the EPA's ToxCast initiative with their *in vivo* toxicology data. These compounds were selected based on their attrition in preclinical or clinical studies due to safety issues and the availability of *in vivo* animal toxicity data. The majority of these compounds were terminated due to hepatotoxicity either in preclinical or clinical studies. The primary mode of action of these compounds, along with their structures in SMILES representation, is shown in Table S1 (Supporting Information). These compounds spanned diverse mechanisms of action (MOA), interacting with G-protein coupled receptors, nuclear receptors, kinase, and ion channel targets and were designed to treat conditions such as inflammation, pulmonology, neurological diseases, etc. The distribution of physicochemical properties for the 52 compounds is given in the Supporting Information (Figure S1). The majority of the compounds fall in the drug-like space characterized by the Lipinski rule of five (41 out of 52 compounds satisfied the rule). In addition, the set had an equal distribution of acids and bases with relatively few neutral compounds as determined using acid−base dissociation constants (pK_a and pK_b) calculated by ACD, version 12, software (http://www.acdlabs.com).

2.4. *In Vitro* Assay Platforms. *In vitro* assay data were available for six assay platforms at the time of analysis: ACEA (http://www.aceabio.com, ACEA Biosciences, Inc., San Diego, CA), Apredica (http://www.apredica.com/, now a subsidiary of Cyprotex, Watertown, MA), Attagene (http://attagene.com/, Attagene Inc., Morrisville, NC), BioMap (http://bioseekinc.com, BioSeek Inc., now a subsidiary of Asterand, South San Francisco, CA), Novascreen (http://www.perkinelmer.com/, NovaScreen Biosciences, now a subsidiary of Caliper Life Sciences Inc., Hanover, MD), and Odyssey Thera (http://www.odysseythera.com/, Odyssey Thera, San Ramon, CA). These platforms offer a variety of assays that include cell-free biochemical assays covering molecular targets from protein super families to more complex cell-culture assays mostly in primary human cells or relevant cell-lines, covering key signaling pathways and phenotypic end points. A brief discussion of each of these six assay technologies is described previously.[22] These assays were run by the respective individual vendors, and data were processed further by EPA for normalization, curve-fitting, and for the calculation of the concentration causing half maximal response (AC_{50}) or the lowest effect concentration (LEC) for some platforms. The *in vitro* assay data for all the ToxCast compounds were retrieved from the EPA's SharePoint site accessible to the pharma partners in March of 2012. In total, at the time of this analysis, there were 973 assays, consisting of 486 unique end points (ignoring the directionality and different time points of individual assays) from six assay platforms available (Figure 1). Table S2 (Supporting Information)

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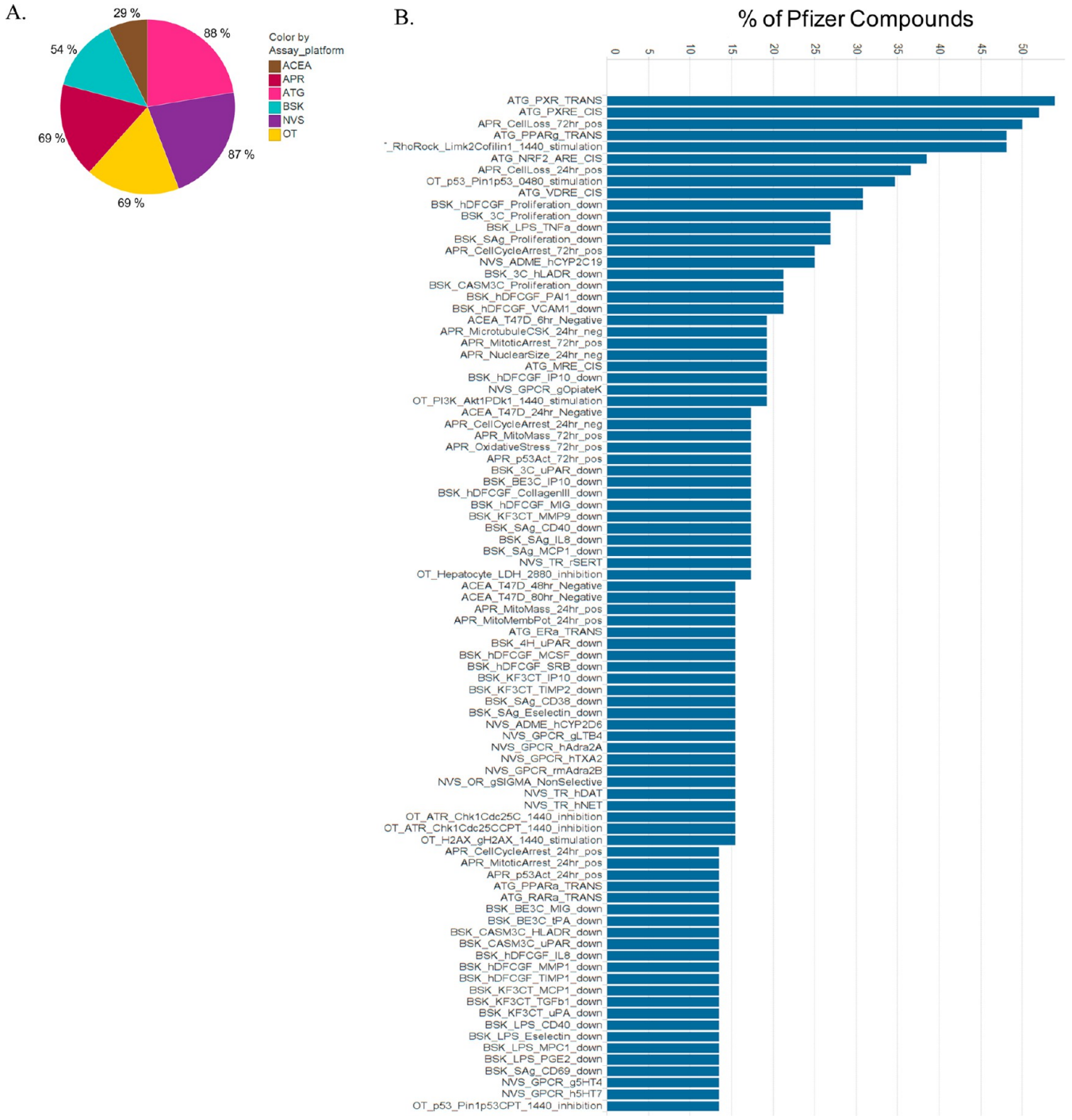

Figure 2. (A) Hit rates of Pfizer compounds across six assay platforms. The sector size in the pie chart is colored by individual assay platforms. (B) Hit rates of Pfizer compounds in individual ToxCast assays. An AC_{50} cutoff of <100 μM was used to define a hit in individual assays across each assay platform; the 88 ToxCast assays with a minimum of 7 hits are shown.

lists the available assay activities for all compounds used in these studies. The specific definitions of assay values to describe "inactives" and "not profiled" are also given in Table S2 (Supporting Information). Table S3 (Supporting Information) summarizes the descriptions of all assays, target genes (when available), their technical specifications, and the positive controls used in the assays as provided by the EPA. It is worth noting that the March 2012 version of the ToxCast assay data from the EPA provided to the Pharma partners may differ from the version that will be released to the public by the EPA in December 2013 due to additional retesting of activities and/or changes in the curve-fitting algorithms, etc.

2.5. Chemical Structure and Biological Profile Similarities of the Pharma vs EPA Set. Chemical structure and biological profile similarity analysis was carried out to compare the Pharma set (n = 110), comprising discontinued pharmaceuticals, with the EPA set (n = 1704) which included a majority of nonpharmaceuticals and a small set of marketed drugs (n = 103). Chemical similarity was calculated using the MDL public keys and the Tanimoto similarity coefficient calculated by the *Molecular Similarity* component of Pipeline Pilot, 8.5.[23,24] For the calculation of biological similarities, all compounds and assays having >50% missing values were removed. This resulted in a matrix of 982 compounds (799 environmental chemicals and 73 known drugs from the EPA set and 110 failed pharmaceuticals) and 802 ToxCast assays

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A.

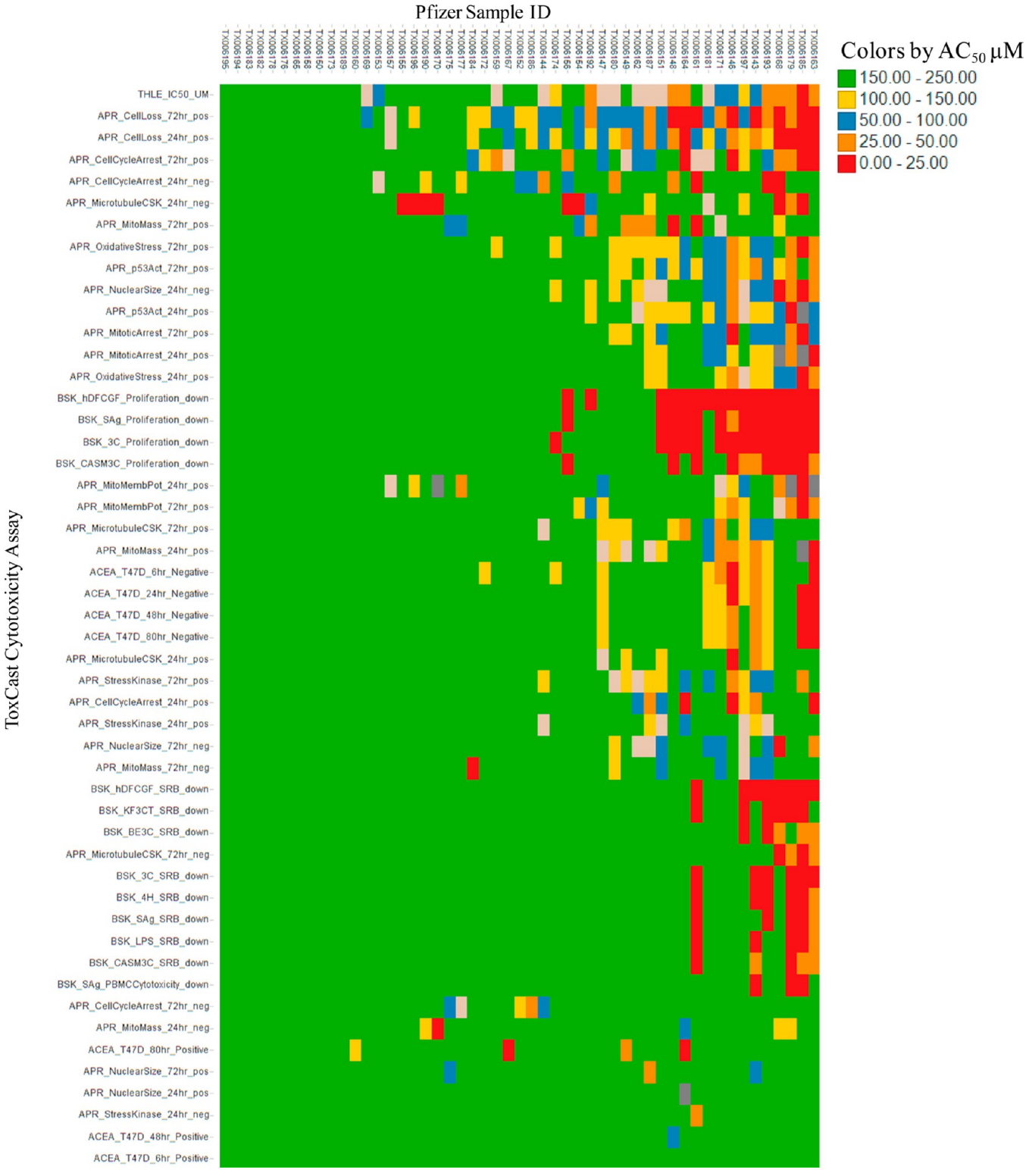

Figure 3. continued

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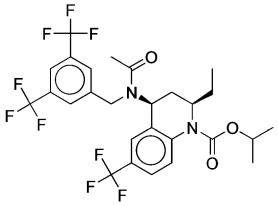
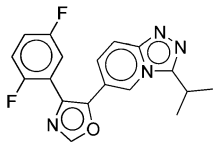
B.



TX006170, CETP inhibitor

THLE IC_{50}= >300 μM
APR_MicrotubuleCSK_24hr_neg= 0.39 μM
APR_MitoMass_24hr_neg=2.6 μM

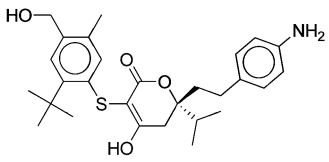
Organ affected:- Liver



TX006184, P38 MAPK Inhibitor

THLE IC_{50}= >300 μM
APR_MitoMass_72hr_neg =0.78 μM

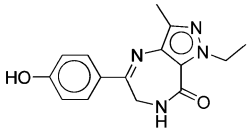
Organ affected:- Large intestine, Liver and Bone marrow



TX006155, HIV protease inhibitor

THLE IC_{50}= >300 μM
APR_MicrotubuleCSK_24hr_neg= 0.39 μM
ATG_NRF2_ARE_CIS= 9.5 μM

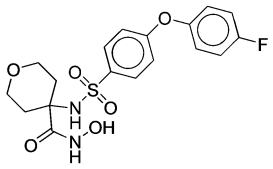
Organ affected:- Kidney, Gall bladder, Stomach



TX006156 , PDE4 inhibitor

THLE IC_{50}= >300 μM
APR_MicrotubuleCSK_24hr_neg= 2.5 μM
BSK_SAg_Proliferation_down = 4.4 μM
BSK_hDFCGF_Proliferation_down= 18 μM
BSK_CASM3C_Proliferation_down = 19 μM
APR_CellCycleArrest_72hr_pos= 43 μM

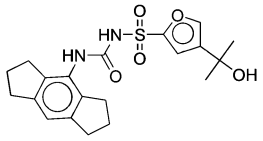
Organ affected:- Heart, Stomach, Small intestine , Pancreas



TX006159 , MMP-13 Inhibitor

THLE IC_{50}= >300 μM
APR_CellCycleArrest_72hr_pos= 41 μM
ATG_NRF2_ARE_CIS= 44 μM

Organ affected:- Testes, Liver



TX006190, IL-1 Releasing Inhibitor

THLE IC_{50}= >300 μM
APR_MicrotubuleCSK_24hr_neg =1.6 μM

Organ affected:- Lung, Kidney, Adrenal Gland

Figure 3. (A) Heat map of activity of Pfizer compounds in a Pfizer internal THLE cytotoxicity assay versus multiple ToxCast cytotoxicity assays from ACEA, APR, ATG, and BSK. An AC_{50} cutoff of <100 μM was used to define hits in the cytotoxicity assays. (B) Chemical structures and activities (AC_{50} < 50 μM) of selected Pfizer compounds active in cytotoxicity mechanistic assays from Apredica and BioSeek. The THLE IC_{50} of these compounds is also shown. Data from rodent and nonrodent preclinical studies of organ structural damage (necrosis, degeneration, inflammation, or hemorrhage) by these compounds are listed.

(with ~400 unique end points). As expected, 830 of 832 eliminated chemicals were from the e1K subset of the ToxCast library, as discussed above, that were profiled in only a select subset of the available assays. The AC_{50} values for all assays were transformed to molar −log10 values (pAC_{50}) in order to scale chemical−assay pairs between 0 (inactive) and 9 (AC_{50} = 1 nM). Subsequently, pairwise correlations were calculated to produce a correlation matrix containing Pearson correlation coefficient values for each pair of compounds using their normalized activities in each assay. A Pearson correlation coefficient cutoff of 0.7 and above was used to define two compounds as being biologically similar.

2.6. Chemical Structure and Biological Profile versus *In Vivo* Similarities between Pharmaceuticals. Biological and chemical similarity analysis of compounds from the Pharma set (n = 110) and marketed drugs (n = 103) from the EPA set (a total of 213 compounds) was carried out, as described above, to identify similar pairs within the drug-like compounds in the ToxCast set. Pharmaceuticals with high similarity in both chemical and biological spaces were then used to query ToxRefDB, a reference database that stores the *in vivo* animal toxicity data for the ToxCast compounds.[25−27] The *in vivo* profile similarities of the compounds pairs were then compared manually. The bloodstream exposure (C_{max}) data for Pfizer compounds, obtained from our internal database, was also used in the analysis.

3. RESULTS

3.1. Hit Rate Analysis of Pfizer Compounds. A hit was defined as a compound that exhibited activity at or below an AC_{50} value of 100 μM for this analysis. The hit rate of Pfizer compounds across the assay platforms and in the individual assays are shown in the pie chart in Figure 2A and as a histogram in Figure 2B. As can be seen in Figure 2A, 88% of the compounds showed activity in one or more of the Attagene (ATG) assays, which use a cellular biosensor system (Factorial) to assess a compound's impact on gene regulatory pathways (CIS-factorial), and across the panel of nuclear receptors (TRANS-factorial), which modulate a variety of cellular processes.[28] Generally, compounds showed activities in both the CIS and TRANS assay formats. The next highest hit rates were found in assays from Novascreen (NVS), Apredica (APR), and Odyssey Thera (OT) platforms, showing hit rates of 87%, 69%, and 69%, respectively. These platforms are of significant interest to us in correlating *in vitro* assay responses with the adverse events observed from the compounds' preclinical or clinical study data.

Figure 2B depicts the 88 individual ToxCast assays having a minimum of 7 hits and shows the percentage of hits per assay across the compounds. The pregnane X receptor (PXR) TRANS- and PXR response element (PXRE) CIS were among the top two assays in which more than half of the Pfizer compounds showed an activity below an AC_{50} of 100 μM. This finding is consistent with the promiscuous nature of PXR, which is known to be activated by a variety of xenobiotics, and natural steroids.[29] However, the correlation of both CIS-and TRANS-PXR assays for Pfizer-discontinued compounds was modest (R^2 of 0.45) as compared to the strong correlation (R^2 of 0.67) reported by Martin et al. for phase-I environmental chemicals

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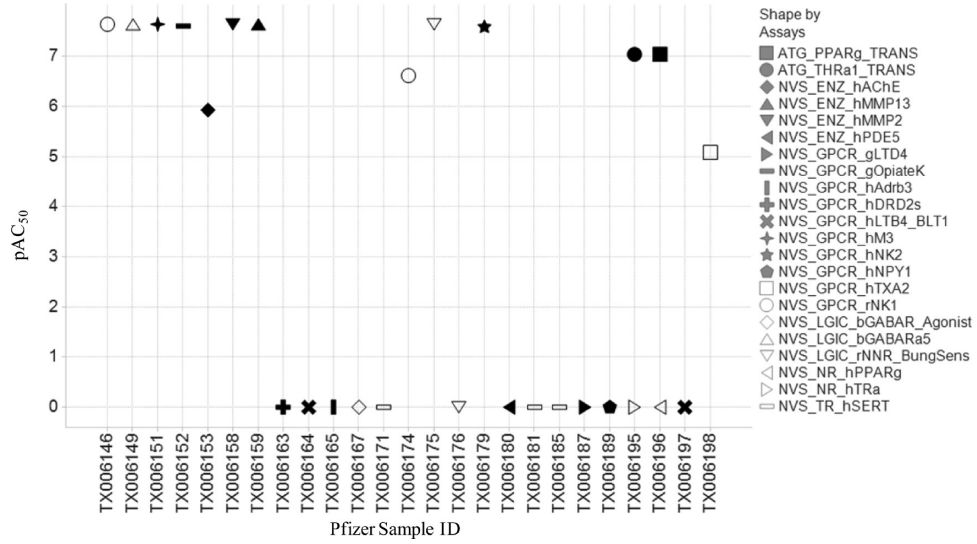

Figure 4. Plot of 25 Pfizer compounds and their pAC_{50} values in NVS and ATG assays.

showing activities in PXR assays.[16] The other nuclear receptor (NR) assays with high hit rates (30−48%) include the nuclear factor erythroid 2-related factor 2 (NRF2), the peroxisome proliferator-activated receptor (PPARg), and the vitamin D receptor from Attagene. NRF2 coordinates the up-regulation of cytoprotective genes in response to oxidative and electrophonic stress through binding to the antioxidant response element (ARE).[30,31] Thirty-eight precent of compounds were found to be inducers of the NRF2-ARE pathway. Twenty-five out of 52 (48%) compounds showed activity in the PPARg assay in the TRANS format, an up-regulation of which plays an important role in regulating genes related to lipid uptake and adipocyte differentiation.[32] Recently, several publications have implicated a role for PPAR gamma activation in hepatotoxicity,[33−35] which may be relevant to the observed hepatotoxicity of some Pfizer compounds.

Pfizer compounds also showed activity in multiple cytotoxicity assays from APR, BioSeek (BSK), and ACEA. APR offers high-content screening (HCS) multiparametric cytotoxicity assays to measure the effects of chemicals on toxicity biomarkers in a cell culture of human hepatocellular carcinoma cell-lines (HEPG2) and primary rat hepatocytes. Fifty percent and 34% of our compounds were active in the cell loss assay from APR at 72 and 24 h time points, respectively. Other sensitive cell health indicators in the HCS assay panel were cell-cycle arrest, mitotic arrest, a decrease in nuclear size, and disruption of cytoskeletal (CSK) integrity. In addition, a number of compounds showed activity in the antiproliferation assays from BSK, which utilize multiple primary human cells, stimulated with a variety of biological effectors, and measure the level of downstream response proteins in an enzyme-linked immunosorbent assay (ELISA).[36,37] Of the compounds that showed activity in the antiproliferation assay, about 30% of them were active in dermal fibroblasts (hDFCGF), followed by 27% and 21% active in endothelial cells (3C, SAg) and smooth muscle cells (CASM3C), respectively. BSK assays also measure other cytotoxicity end points in different cell types, such as sulforhodamine B (SRB) staining for total protein, an alamar blue assessment of peripheral blood mononuclear cell metabolic activity (PBMC Cytotox), and a morphologic score (Vis), for which a few compounds showed activity. Finally, some compounds from the Pfizer set also showed activities in cell-growth kinetics assays from ACEA, which utilize an impedance-based technique to measure changes in cell state (cell-morphology, cell-number, and cell−cell adhesions) upon compound exposure in a human mammary ductal carcinoma cell line (T-47D).[22] Twenty-one percent of compounds from this set showed inhibition of cell-growth at least at one time point (6, 24, 48, or 80 h). A few compounds (~6%) increased cell growth at 80 h post-treatment.

Figure 3A shows a heat map of ToxCast cytotoxicity assays and mechanistic end points from four platforms (ACEA, APR, ATG, and BSK) compared to a Pfizer internal cytotoxicity assay in a T-antigen immortalized human liver epithelial (THLE) cell line which is routinely used at Pfizer for an early assessment of *in vivo* toxicity.[38] As shown, 39 out of 52 compounds have THLE assay values above 100 μM and are considered noncytotoxic in this assay. Analysis of these same compounds in one of the cytotoxicity assays or mechanistic readouts (as discussed above) from the ToxCast data suggested that 24 of 39 (62%) compounds were active at an AC_{50} of $\leq$100 μM. Figure 3B shows the activities of some representative compounds from the Pfizer set in selected ToxCast cytotoxicity assays. As shown, TX006170, TX006184, TX006155, TX006156, TX006159, and TX006190 were inactive in the THLE cytotoxicity assay in concentrations up to 300 μM but were active via other cytotoxicity mechanisms available in ToxCast. Also, compounds such as TX006185 and TX006163 showed activity in 29 unique end points from APR and BSK at an AC_{50} below 50 μM but have only moderate activity in the THLE assay. A number of these compounds were found to cause structural damage to multiple organs in the available preclinical study data. Figure 3B lists some representative chemical structures, their activities in the ToxCast and Pfizer cytotoxicity assays and the organs affected by these compounds in preclinical studies. For example, TX006170, a cholesteryl ester transfer protein (CETP) inhibitor, showed liver necrosis in rats at a low C_{max} exposure of 1.9 μM. Likewise, TX006159, an MMP-13 inhibitor, caused degeneration of seminiferous tubules in the testes of dogs at a C_{max} exposure of 19.8 μM. TX006156, a phosphodiesterase (PDE-4) inhibitor, caused degeneration of the heart in monkeys and rats, as well as inflammation and degeneration of the stomach, pancreas, and small intestine in rats, at various exposures. It is worth mentioning that 13 of the Pfizer-donated compounds were not active in any of the ToxCast cytotoxicity mechanism assays or in our internal THLE assay at

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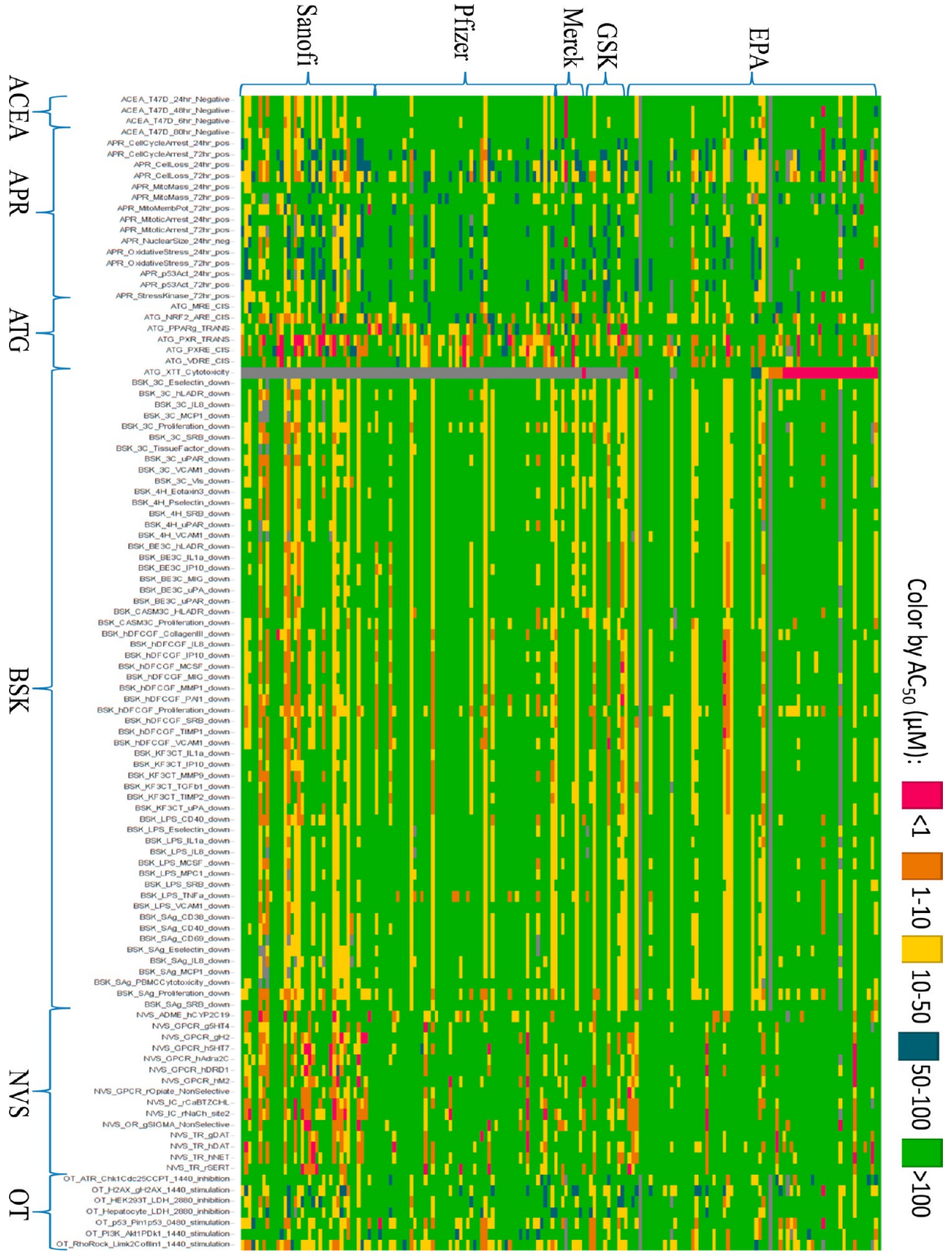

Figure 5. Heat map of bioactivity profiles of Pfizer compounds compared to compounds from other pharmaceutical companies (Merck, Sanofi, GSK) and known drugs from the EPA set. Assays with a minimum of 30 hits (AC_{50} <100 μM) are shown. Missing values are shown in gray. The *in vitro* assays from the respective vendors are shown on the *X*-axis, and the ToxCast source sets are displayed on the *Y*-axis.

concentrations up to 100 μM, although they did show multiple organ toxicities in preclinical studies.

3.2. Reproducibility of Primary Mechanism of Action of Pfizer Compounds. Next, we investigated the ability of the ToxCast assays to confirm the known primary mode of action (MOA) of the Pfizer compounds. Twenty NVS assays matched with the primary pharmacology of 25 Pfizer compounds. Figure 4A shows these compounds and their pAC_{50} ($-\log AC_{50}$) values in the corresponding NVS assays. As shown, NVS correctly identified the known MOAs for 11 out of 25 compounds. The other 14 compounds were inactive (pAC_{50} = 0) in the NVS assays corresponding to their known MOAs. The primary MOA

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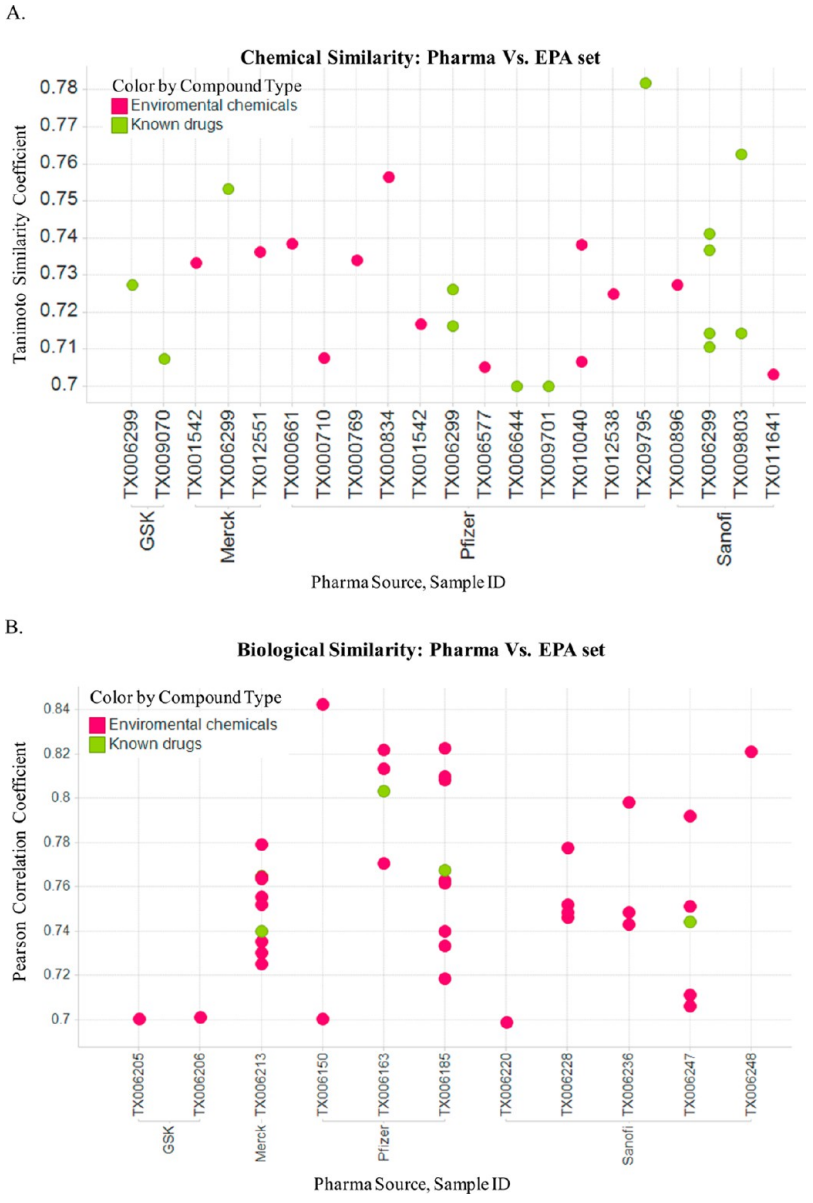

Figure 6. (A) Chemical similarity of Pharma compounds with the EPA set using MDL keys as a fingerprint and Tanimoto similarity coefficient. Only those compounds with a chemical similarity of 0.7 and above are shown. The values are colored by compound type in the EPA set. Red dots are environmental chemicals, and green dots are marketed drugs. (B) Biological similarity of the Pharma set with the EPA set as measured by the Pearson correlation coefficient. Only those compounds from the EPA set with a biological similarity of 0.7 and above to the Pharma set are shown. The values are colored by compound type in the EPA set. Red dots are environmental chemicals, and green dots are marketed drugs.

of two compounds, TX006195 (PPARg agonist) and TX006196 (THRa1 inhibitor), were found to be inactive in the NVS biochemical assays but were active in ATG PPARg and THRa1 TRANS-factorial reporter assays at pAC_{50} values of 7.

3.3. Comparison of the Bioactivity Profiles of Pfizer Compounds with Other Pharmaceutical Compounds. We investigated the similarity of activities of Pfizer compounds to other pharmaceutical compounds in the ToxCast assay space. Figure 5 compares the ToxCast assay bioactivity profiles of Pfizer compounds with compounds from the other pharmaceutical companies (Pharma set) as well as with the marketed drugs from the EPA set. As can be seen, a majority of the Pharma set, along with the marketed drugs, showed higher hit rates for Attagene PXR (pregnane X receptors) and NRF2 (nuclear receptor factor-II) assays than the other assay platforms. The Attagene PXR assay is indicative of metabolic stress, whereas the NRF2 (nuclear receptor factor-II) assay indicates oxidative stress. Moreover, compounds from the Pharma set, in particular, the Pfizer and Sanofi compounds had more activity in the PPARg TRANS-activation assay from ATG. In addition to activity at nuclear receptors, compounds from both sets were active in cell cycle arrest at 72 h, cell loss at 24 and 72 h in assays from APR as well as in diverse cytotoxicity readouts, such as antiproliferation assays, PBMCs, the SRB cytotoxicity assay from BSK, and the cell-growth assays from ACEA. Compounds from other pharma companies, such as Sanofi, GSK, and Merck, were also active in a diverse set of cytotoxicity assays at AC_{50} values below 100 μM. In summary, the trends observed for Pfizer compounds were similar to the trends observed for other pharma compounds. These findings emphasize the importance and applicability of mechanistic cytotoxicity assays in early detection of the toxicity of compounds.

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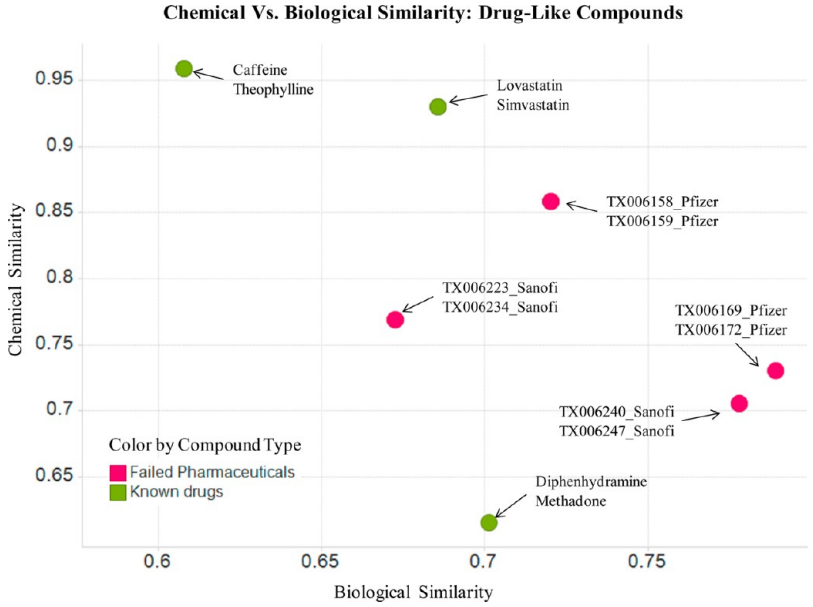

Figure 7. Plotting of compound similarities in the chemical and biological spaces calculated using the Tanimoto coefficient and Pearson correlation coefficient for chemical and biological spaces, respectively. Only those hits with chemical and biological similarities ≥0.6 (60%) are shown.

A number of known drugs from the EPA set were also found to be active in the mechanistic assays, some of them showing low micromolar activities. In particular, the XTT 2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2*H*-tetrazolium-5-carboxanilide) cell viability assays from ATG measure the mitochondrial dehydrogenase activity of the living cells.[39] A closer look at drugs with activity (AC_{50} <10 μM) in the XTT-cytotoxicity assay suggests that a majority of these compounds have drug-induced liver injury (DILI) liabilities, according to the DILI classification published by scientists at the Food and Drug Administration (FDA).[40] This classification is based on the FDA-approved drug label, and it classifies the DILI potential of 287 known drugs into three categories: Most-DILI, Less-DILI, and No-DILI.[40] For the subset of compounds showing activity in the XTT assay that had DILI annotations available, we found Isoniazid (an antituberculosis drug, TX005751) and methotrexate (an antineoplastic agent, TX006303) with XTT AC_{50} values <1 μM that were classified as compounds with Most-DILI concerns. In contrast, compounds such as busulfan (TX009225), diclofenec (TX006293), hydroxyurea (TX009231), methimazole (TX008381), methyldopa (TX006855), and Tamoxifen (TX008618), with XTT AC_{50} values <1 μM, were in the Less-DILI category. We examined the ability of this cytotoxicity assay to separate 25 compounds from Most-DILI (n = 11), Less-DILI (n = 13), and No-DILI (n = 1). However, the AC_{50} value in the XTT-cytotoxicity assay alone was not able to discriminate compounds with different DILI liabilities (data not shown).

3.4. Chemical Structure and Biological Profile Similarities: Pharma vs the EPA Set. The phase-I of ToxCast resulted in assay signatures for specific toxicities based on the response of the environmental chemicals set. We sought to investigate the applicability and relevance of these signatures in predicting the *in vivo* toxicity of pharmaceuticals by an analysis of how similar pharmaceuticals are to the environment chemicals space. The chemical structure similarities between the EPA set (n = 1704) and the Pharma set (n = 110) were calculated using the Tanimoto similarity[24] as a metric and are shown in Figure 6A. At a Tanimoto similarity threshold of ≥0.7 (or 70%), there are only 27 compounds, out of ∼1700 from the EPA set, that are chemically similar to any of the compounds from the Pharma set. Fourteen of the 27 compounds were known drugs. On the basis of the low numbers of chemically similar compounds, it can be concluded that the environmental chemicals within ToxCast are structurally very different from the pharmaceuticals when whole molecule similarities are considered.

Comparison of the biological similarity of the pharma compounds with those from the EPA set, using the assay responses as the descriptor fingerprint (Figure 6B), resulted in 41 compounds from the EPA set with a Pearson correlation coefficient at or above the cutoff of 0.7. Among these, four compounds were known drugs. However, only 11 out of 110 (10%) compounds from the pharma set had any biologically similar compounds in the EPA set (see Figure 6B). Our analysis suggests that Pharma compounds are different in both chemical and biological spaces than the environmental chemicals. Thus, no further attempts were made to compare the *in vivo* profile of pharma compounds with those of environmental chemicals.

3.5. Chemical and Biologcal vs *in Vivo* Similarity of Pharmaceuticals. In the analysis shown above, we discovered that compounds from the Pharma set differ in both chemical and biological spaces to the environmental chemicals from the EPA set. Here, we investigated whether similarities exist among pharmaceuticals or drug-like compounds (i.e., compounds from the Pharma set and the marketed drugs from the EPA set) in ToxCast. The chemical and biological similarities of compounds between failed pharmaceuticals (n = 110) and known drugs (n = 103) were calculated in a similar manner as described above (not shown) and plotted against each other to find compounds that are similar in both spaces at a similarity cutoff of 0.6 and above (Figure 7). Relatively few pharmaceuticals exhibited both pharmacological and chemical similarities. Unsurprisingly, a majority of these compound pairs share the same mode of action (see Figure 8A).

We investigated the *in vivo* profile of four pairs of discontinued compounds from the Pharma set that showed similarity in both chemical and pharmacological spaces: TX006159 and TX06158 (matrix metalloprotease (MMP) inhibitors), TX006169, and TX006172 (phosphodiesterase-4 (PDE-4) inhibitors),

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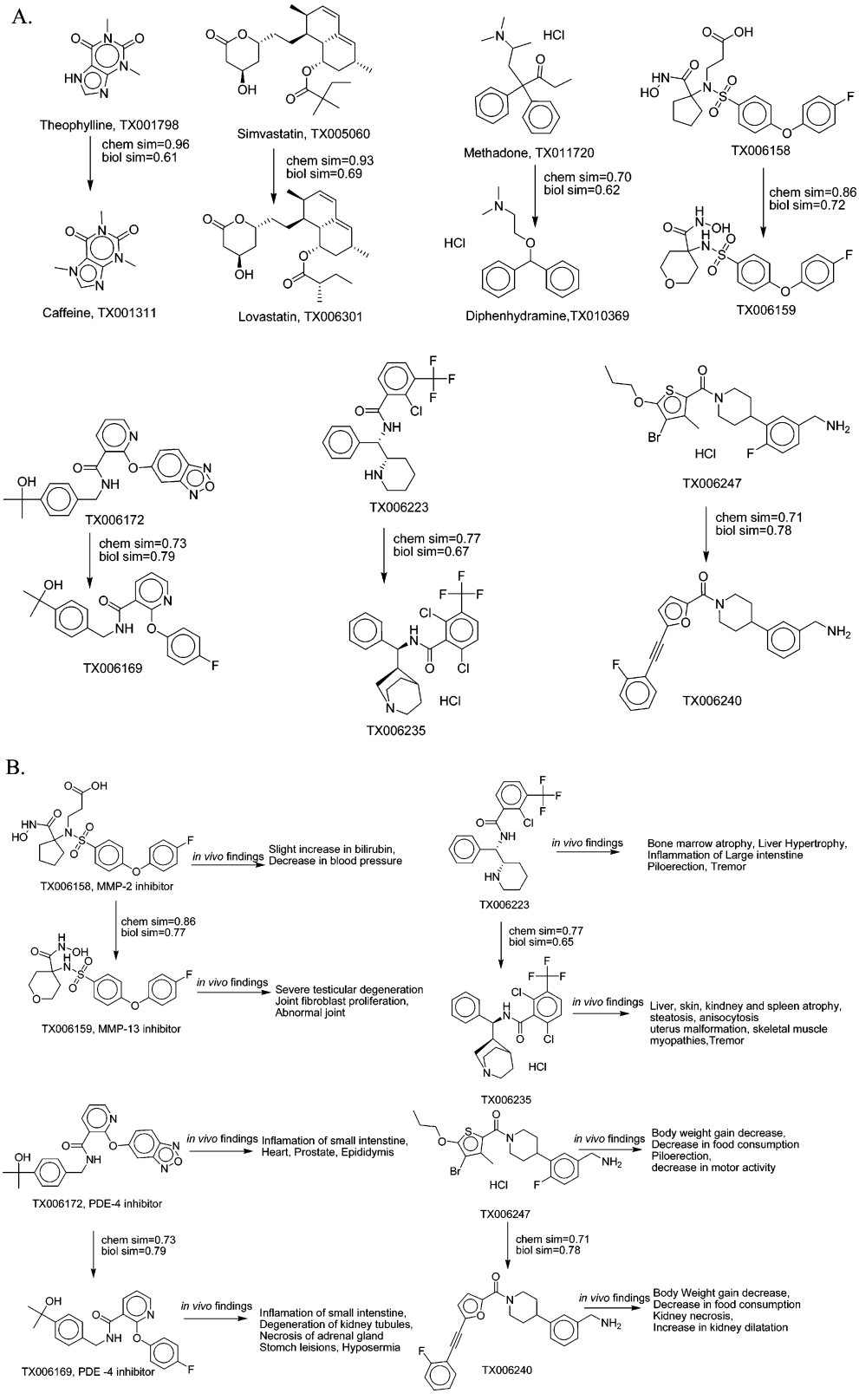

Figure 8. (A) Chemical structures of compounds from the Pharma set and marketed drugs from the EPA set with chemical and biological similarities above 0.6 are shown. (B) Chemical structures and the *in vivo* findings of four pairs of compounds from ToxCast that are highly similar in both chemical and biological spaces.

TX006223 and TX006235 (glycine transporter-1 inhibitors),[41] and TX006240 and TX006247 (Sanofi compounds, mechanisms unknown). The chemical structures of these pairs of compounds are shown in Figure 8A. These compounds showed similarity in both chemical and pharmacological spaces at a cutoff of >0.6 (Figure 7). The preclinical profiles of these compounds revealed distinct *in vivo* toxicity pathologies. For example, TX006159, an MMP-13 inhibitor, causes severe testicular degeneration in dogs

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at 19.8 μM bloodstream exposure, whereas TX006158, an MMP-2 inhibitor, did not show any evidence of testicular damage at any doses examined in dogs to a maximum exposure of 143 μM. Similarly, TX006223 and TX006235, despite sharing the same primary MOA (glycine transporter-1 inhibitors) and high chemical and pharmacological similarities, affected different target organs. TX006235 affected multiple organs and was found to be predominantly toxic, whereas TX006223 had a significantly cleaner toxicity profile (Figure 8B). Among the pairs of PDE-4 inhibitors, TX006169 causes inflammation of the small intestine as well as degeneration of kidney tubules at the lowest C_{max} of 0.29 μM in rats. It also causes necrosis of the adrenal gland (C_{max} = 2.5 μM), hypospermia (C_{max} = 5 μM), and stomach lesions (C_{max} = 45.2 μM) in rats, yet TX006169 did not exhibit findings in the heart or prostate in any of the species at the maximum plasma exposure of 13 μM unlike its nearest neighbor. TX006172 showed inflammation of the small intestine at a C_{max} of 4 μM and inflammation of the heart, prostate, and epididymes at a C_{max} of 11.4 μM. No findings were observed in the adrenal gland, stomach, or kidney for this compound up to a maximum exposure of 29 μM in rats in contrast to TX006169. TX006240 and TX006247 had similar *in vivo* profiles except that TX006240 additionally showed kidney necrosis. As our primary goal with this analysis was to investigate if higher chemical and biological similarities translate into similar *in vivo* findings, no attempts were made to understand the discordant structural features or the discordant assay results for these pairs of compounds, which may lead to observed differences in the *in vivo* findings due to either metabolism or other pharmacokinetic factors.

4. DISCUSSION

In vitro pharmacology assays have been increasingly used to address the potential for pharmacological promiscuity of candidate drugs early in the drug discovery process.[42] These assays can provide mechanistic insights into toxicities and enable opportunities to derisk them at the lead−development stages by means of structure−activity relationships. However, there are only a small number of pharmacology targets that have been linked to known toxicity phenotypes, and these are routinely profiled within the pharmaceutical industry.[43] In this context, the ToxCast approach, with ∼500 *in vitro* assays in human cell lines, could more comprehensively explore the mechanisms of toxicity observed in preclinical or clinical studies.

The ToxCast data analysis of Pfizer compounds provided us interesting hypotheses for the mechanisms of toxicity of some of our compounds. In particular, a majority of our compounds, most of which failed in preclinical or clinical trials due to hepatotoxicity, showed activity in three nuclear receptor assays: PXR, NRF2 and PPARg. These interactions were previously unknown for these compounds. A number of recent studies attempt to correlate NR activation with acute and chronic forms of hepatotoxicity,[44−46] although, clear association of activity in these NRs with hepatotoxicity requires a more comprehensive investigation. Nonetheless, the analysis showed NR activity as a hypothesis to investigate further. Other notable aspects of the ToxCast data was the activity of Pharma compounds in multiple cytotoxicity assays from APR and BSK. In particular, the majority of Pfizer compounds, including those not active in our internal THLE cytotoxicity assay, showed activity in a number of mechanistic assays from these platforms. However, we found limited utility of these assays in predicting organ-specific adverse events. This is evident from Pfizer compounds that are active in cytotoxicity assays but are found to affect multiple organs rather than a specific one.

While *in vitro* pharmacology assays in ToxCast have helped us to generate potential hypotheses for our compounds, the approach warrants caution in using this data without extensive validation of each platform. Our arguments are summarized here.

(1) The NVS platform offers biochemical assays for pharmacological targets of interest to pharmaceutical drug discovery, as well as assays that are standard in *in vitro* safety assessment panels used by the pharmaceutical industry.[43] However, these assays failed to reproduce the known MOA for ∼50% of the Pfizer compounds, some of which did show the expected activity in other platforms (ATG and OT). These compounds were active in our in-house biochemical or functional screens which eliminates the possibility of metabolism driven activity and, hence, cannot explain the inactivity in the NVS assays. These findings, rather disappointing, suggest that care should be taken in using or interpreting results of these biochemical assays.

(2) The vast majority of ToxCast compounds are environmental chemicals with a relatively small number of drug-like compounds (<10%). Our comparison between the pharmaceuticals and environmental chemicals revealed substantial differences in both chemical and biological spaces between these compounds. This dissimilarity calls into question the applicability of the *in vitro* assay signatures developed largely based on nonpharmaceuticals to predict the *in vivo* toxicity of drug-like compounds as such. The signatures developed using phase-I environmental chemicals should be validated with ToxCast phase-II chemicals to demonstrate their utility for drug-like compounds. In addition, the small size and diversity of the Pharma set in ToxCast limits our abilities to compare and contrast the *in vivo* toxicities of drug-like compounds in the ToxCast chemical and assay space.

(3) The *in vivo* data in ToxRefDb, which lacks the serum exposure information at which findings were observed in the preclinical studies, makes it difficult to draw meaningful comparisons between the *in vivo* findings of compounds that are similar in either the chemical or biological space. For example, compounds that exhibit liver hypertrophy at lower serum exposures (e.g., at a C_{max} of 1 μM) might have different biological profiles to those that exhibit such toxicity at higher exposures (e.g., at a C_{max} of 100 μM). One of the reasons for the relatively clean *in vivo* profile for TX006223 as compared to TX006235 (Figure 8B) might be lack of adequate preclinical exposures of the two compounds. This data gap limits the ability to build statistically significant quantitative structure−activity relationships models to develop *in vitro* assay signatures predictive of *in vivo* toxicities. A recent study, published by Thomas et al. supports these observations as they found limited utilities for ToxCast phase-1 assays and chemicals in predicting *in vivo* end points using different statistical classification methods.[47]

(4) The majority of the ToxCast assays utilize human-based cell lines and proteins. This may pose challenges in the correlatation of the *in vivo* effects of these compounds observed in rodent or nonrodent species during preclinical studies with *in vitro* data. This could be one of the possible reasons for the poor predictivity of *in vivo* toxicity using the ToxCast phase-I *in vitro* assays as reported by others.[47] There may be utility for the ToxCast assays in predicting clinical adverse events; however, they are out of the scope of the present work.

Overall, the present study provided possible insights into the mechanisms of toxicity for a few compounds and generated new

hypotheses for a few others. It highlighted the utility of cytotoxicity assays for early identification of a compound's potential to cause toxicity prior to more expensive preclinical or clinical studies. It has suggested novel assay platforms and/or individual mechanistic assays that warrant further investigation to help understand the specific toxicity of some compounds. However, profiling of additional compounds in these assays, to understand their utility as broad screens for toxicity, is warranted.

■ ASSOCIATED CONTENT

Supporting Information

The MOA and physicochemical properties of the Pfizer set, the AC_{50} values for the chemical and assay matrix, all assays, and a brief assay description. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

EPA, Environmental Protection Agency; MOA, mechanism of action; APR, Apredica; ATG, Attagene; BSK, BioSeek; NVS, NovaScreen; OT, Odyseey Thera; AC_{50}, half-maximal activity concentration; PXR, pregnane X receptor; NRF2, nuclear factor erythroid 2-related factor 2; PPARg, peroxisome proliferator-activated receptor; ARE, antioxidant response element; HEPG2, human hepatocellular carcinoma cell-lines; THLE, T-antigen-immortalized human liver epithelial cell; DILI, drug-nduced liver injury

■ REFERENCES

(1) Wilson, A. G. E. (2012) *New Horizons in Predictive Toxicology:Current Status and Application*, Vol. *12*, Royal Society of Chemistry, Cambridge, U.K.

(2) Zhang, W., Roederer, M. W., Chen, W. Q., Fan, L., and Zhou, H. H. (2012) Pharmacogenetics of drugs withdrawn from the market. *Pharmacogenomics 13*, 223−231.

(3) Olson, H., Betton, G., Robinson, D., Thomas, K., Monro, A., Kolaja, G., Lilly, P., Sanders, J., Sipes, G., Bracken, W., Dorato, M., Van Deun, K., Smith, P., Berger, B., and Heller, A. (2000) Concordance of the toxicity of pharmaceuticals in humans and in animals. *Regul. Toxicol. Pharmacol. 32*, 56−67.

(4) Hartung, T. (2009) Toxicology for the twenty-first century. *Nature 460*, 208−212.

(5) Krewski, D., Acosta, D., Jr., Andersen, M., Anderson, H., Bailar, J. C., III, Boekelheide, K., Brent, R., Charnley, G., Cheung, V. G., Green, S., Jr., Kelsey, K. T., Kerkvliet, N. I., Li, A. A., McCray, L., Meyer, O., Patterson, R. D., Pennie, W., Scala, R. A., Solomon, G. M., Stephens, M., Yager, J., and Zeise, L. (2010) Toxicity testing in the 21st century: a vision and a strategy. *J. Toxicol. Environ. Health, Part B 13*, 51−138.

(6) Judson, R., Kavlock, R., Martin, M., Reif, D., Houck, K., Knudsen, T., Richard, A., Tice, R. R., Whelan, M., Xia, M., Huang, R., Austin, C., Daston, G., Hartung, T., Fowle, J. R., III, Wooge, W., Tong, W., and Dix, D. (2013) Perspectives on validation of high-throughput assays supporting 21st century toxicity testing. *ALTEX 30*, 51−56.

(7) Worth, A. P., Bassan, A., De Bruijn, J., Gallegos Saliner, A., Netzeva, T., Patlewicz, G., Pavan, M., Tsakovska, I., and Eisenreich, S. (2007) The role of the European Chemicals Bureau in promoting the regulatory use of (Q)SAR methods. *SAR QSAR Environ. Res. 18*, 111−125.

(8) Collins, F. S., Gray, G. M., and Bucher, J. R. (2008) Toxicology. Transforming environmental health protection. *Science 319*, 906−907.

(9) Schmidt, C. W. (2009) TOX 21: new dimensions of toxicity testing. *Environ. Health Perspect. 117*, A348−353.

(10) Huang, R., Xia, M., Cho, M. H., Sakamuru, S., Shinn, P., Houck, K. A., Dix, D. J., Judson, R. S., Witt, K. L., Kavlock, R. J., Tice, R. R., and Austin, C. P. (2011) Chemical genomics profiling of environmental chemical modulation of human nuclear receptors. *Environ. Health Perspect. 119*, 1142−1148.

(11) Haynes, R. C. (2010) ToxCast on target: in vitro assays and computer modeling show promise for screening chemicals. *Environ. Health Perspect. 118*, A172.

(12) Dix, D. J., Houck, K. A., Martin, M. T., Richard, A. M., Setzer, R. W., and Kavlock, R. J. (2007) The ToxCast program for prioritizing toxicity testing of environmental chemicals. *Toxicol. Sci. 95*, 5−12.

(13) Judson, R. S., Houck, K. A., Kavlock, R. J., Knudsen, T. B., Martin, M. T., Mortensen, H. M., Reif, D. M., Rotroff, D. M., Shah, I., Richard, A. M., and Dix, D. J. (2010) In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. *Environ. Health Perspect. 118*, 485−492.

(14) Knudsen, T. B., Houck, K. A., Sipes, N. S., Singh, A. V., Judson, R. S., Martin, M. T., Weissman, A., Kleinstreuer, N. C., Mortensen, H. M., Reif, D. M., Rabinowitz, J. R., Setzer, R. W., Richard, A. M., Dix, D. J., and Kavlock, R. J. (2011) Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets. *Toxicology 282*, 1−15.

(15) Rotroff, D. M., Beam, A. L., Dix, D. J., Farmer, A., Freeman, K. M., Houck, K. A., Judson, R. S., LeCluyse, E. L., Martin, M. T., Reif, D. M., and Ferguson, S. S. (2010) Xenobiotic-metabolizing enzyme and transporter gene expression in primary cultures of human hepatocytes modulated by ToxCast chemicals. *J. Toxicol. Environ. Health, Part B 13*, 329−346.

(16) Martin, M. T., Dix, D. J., Judson, R. S., Kavlock, R. J., Reif, D. M., Richard, A. M., Rotroff, D. M., Romanov, S., Medvedev, A., Poltoratskaya, N., Gambarian, M., Moeser, M., Makarov, S. S., and Houck, K. A. (2010) Impact of environmental chemicals on key transcription regulators and correlation to toxicity end points within EPA's ToxCast program. *Chem. Res. Toxicol. 23*, 578−590.

(17) Kavlock, R., Chandler, K., Houck, K., Hunter, S., Judson, R., Kleinstreuer, N., Knudsen, T., Martin, M., Padilla, S., Reif, D., Richard, A., Rotroff, D., Sipes, N., and Dix, D. (2012) Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem. Res. Toxicol. 25*, 1287−1302.

(18) Houck, K. A., Dix, D. J., Judson, R. S., Kavlock, R. J., Yang, J., and Berg, E. L. (2009) Profiling bioactivity of the ToxCast chemical library using BioMAP primary human cell systems. *J. Biomol. Screening 14*, 1054−1066.

(19) Sipes, N. S., Martin, M. T., Kothiya, P., Reif, D. M., Judson, R. S., Richard, A. M., Houck, K. A., Dix, D. J., Kavlock, R. J., and Knudsen, T. B. (2013) Profiling 976 ToxCast Chemicals across 331 Enzymatic and Receptor Signaling Assays. *Chem. Res. Toxicol. 26*, 878−895.

(20) (2011) *Pipeline Pilot*, version 8.5, Accelrys, Inc., San Diego, CA.

(21) TIBCO Spotfire 3.3.1, http://spotfire.tibco.com/.

(22) Kavlock, R., Chandler, K., Houck, K., Hunter, S., Judson, R., Kleinstreuer, N., Knudsen, T., Martin, M., Padilla, S., and Reif, D. (2012) Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem. Res. Toxicol.*, 1287−1302.

(23) Hassan, M., Brown, R. D., Varma-O'brien, S., and Rogers, D. (2006) Cheminformatics analysis and learning in a data pipelining environment. *Mol. Diversity 10*, 283−299.

(24) Willett, P., Barnard, J. M., and Downs, G. M. (1998) Chemical similarity searching. *J. Chem. Inf. Comput. Sci. 38*, 983−996.

(25) Knudsen, T. B., Martin, M. T., Kavlock, R. J., Judson, R. S., Dix, D. J., and Singh, A. V. (2009) Profiling the activity of environmental chemicals in prenatal developmental toxicity studies using the U.S. EPA's ToxRefDB. *Reprod. Toxicol. 28*, 209−219.
(26) Martin, M. T., Mendez, E., Corum, D. G., Judson, R. S., Kavlock, R. J., Rotroff, D. M., and Dix, D. J. (2009) Profiling the reproductive toxicity of chemicals from multigeneration studies in the toxicity reference database. *Toxicol. Sci. 110*, 181−190.
(27) Judson, R. S., Martin, M. T., Egeghy, P., Gangwal, S., Reif, D. M., Kothiya, P., Wolf, M., Cathey, T., Transue, T., Smith, D., Vail, J., Frame, A., Mosher, S., Cohen Hubal, E. A., and Richard, A. M. (2012) Aggregating data for computational toxicology applications: The U.S. Environmental Protection Agency (EPA) aggregated computational toxicology resource (ACToR) system. *Int. J. Mol. Sci. 13*, 1805−1831.
(28) Romanov, S., Medvedev, A., Gambarian, M., Poltoratskaya, N., Moeser, M., Medvedeva, L., Gambarian, M., Diatchenko, L., and Makarov, S. (2008) Homogeneous reporter system enables quantitative functional assessment of multiple transcription factors. *Nat. Methods 5*, 253−260.
(29) Jones, S. A., Moore, L. B., Shenk, J. L., Wisely, G. B., Hamilton, G. A., McKee, D. D., Tomkinson, N. C., LeCluyse, E. L., Lambert, M. H., Willson, T. M., Kliewer, S. A., and Moore, J. T. (2000) The pregnane X receptor: a promiscuous xenobiotic receptor that has diverged during evolution. *Mol. Endocrinol. 14*, 27−39.
(30) Nguyen, T., Nioi, P., and Pickett, C. B. (2009) The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. *J. Biol. Chem. 284*, 13291−13295.
(31) Lee, J. M., and Johnson, J. A. (2004) An important role of Nrf2-ARE pathway in the cellular defense mechanism. *J. Biochem. Mol. Biol. 37*, 139−143.
(32) Spiegelman, B. M. (1998) PPAR-gamma: adipogenic regulator and thiazolidinedione receptor. *Diabetes 47*, 507−514.
(33) Guo, L., Zhang, L., Sun, Y., Muskhelishvili, L., Blann, E., Dial, S., Shi, L., Schroth, G., and Dragan, Y. P. (2006) Differences in hepatotoxicity and gene expression profiles by anti-diabetic PPAR gamma agonists on rat primary hepatocytes and human HepG2 cells. *Mol. Diversity 10*, 349−360.
(34) Tolman, K. G. (2000) Thiazolidinedione hepatotoxicity: a class effect? *Int. J. Clin. Pract., Suppl.*, 29−34.
(35) Panasyuk, G., Espeillac, C., Chauvin, C., Pradelli, L. A., Horie, Y., Suzuki, A., Annicotte, J. S., Fajas, L., Foretz, M., Verdeguer, F., Pontoglio, M., Ferre, P., Scoazec, J. Y., Birnbaum, M. J., Ricci, J. E., and Pende, M. (2012) PPARgamma contributes to PKM2 and HK2 expression in fatty liver. *Nat. Commun. 3*, 672.
(36) Kunkel, E. J., Plavec, I., Nguyen, D., Melrose, J., Rosler, E. S., Kao, L. T., Wang, Y., Hytopoulos, E., Bishop, A. C., Bateman, R., Shokat, K. M., Butcher, E. C., and Berg, E. L. (2004) Rapid structure-activity and selectivity analysis of kinase inhibitors by BioMAP analysis in complex human primary cell-based models. *Assay Drug Dev. Technol. 2*, 431−441.
(37) Kunkel, E. J., Dea, M., Ebens, A., Hytopoulos, E., Melrose, J., Nguyen, D., Ota, K. S., Plavec, I., Wang, Y., Watson, S. R., Butcher, E. C., and Berg, E. L. (2004) An integrative biology approach for analysis of drug action in models of human vascular inflammation. *FASEB J. 18*, 1279−1281.
(38) Greene, N., Aleo, M. D., Louise-May, S., Price, D. A., and Will, Y. (2010) Using an in vitro cytotoxicity assay to aid in compound selection for in vivo safety studies. *Bioorg. Med. Chem. Lett. 20*, 5308−5312.
(39) Mosmann, T. (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods 65*, 55−63.
(40) Chen, M., Vijay, V., Shi, Q., Liu, Z., Fang, H., and Tong, W. (2011) FDA-approved drug labeling for the study of drug-induced liver injury. *Drug Discovery Today 16*, 697−703.
(41) Boulay, D., Pichat, P., Dargazanli, G., Estenne-Bouhtou, G., Terranova, J. P., Rogacki, N., Stemmelin, J., Coste, A., Lanneau, C., Desvignes, C., Cohen, C., Alonso, R., Vige, X., Biton, B., Steinberg, R., Sevrin, M., Oury-Donat, F., George, P., Bergis, O., Griebel, G., Avenet, P., and Scatton, B. (2008) Characterization of SSR103800, a selective inhibitor of the glycine transporter-1 in models predictive of therapeutic activity in schizophrenia. *Pharmacol., Biochem. Behav. 91*, 47−58.
(42) Whitebread, S., Hamon, J., Bojanic, D., and Urban, L. (2005) Keynote review: in vitro safety pharmacology profiling: an essential tool for successful drug development. *Drug Discovery Today 10*, 1421−1433.
(43) Bowes, J., Brown, A. J., Hamon, J., Jarolimek, W., Sridhar, A., Waldron, G., and Whitebread, S. (2012) Reducing safety-related drug attrition: the use of in vitro pharmacological profiling. *Nat. Rev. Drug Discovery 11*, 909−922.
(44) Shah, I., Houck, K., Judson, R. S., Kavlock, R. J., Martin, M. T., Reif, D. M., Wambaugh, J., and Dix, D. J. (2011) Using nuclear receptor activity to stratify hepatocarcinogens. *PLoS One 6*, e14584.
(45) Wolf, K. K., Wood, S. G., Hunt, J. A., Walton-Strong, B. W., Yasuda, K., Lan, L., Duan, S. X., Hao, Q., Wrighton, S. A., Jeffery, E. H., Evans, R. M., Szakacs, J. G., von Moltke, L. L., Greenblatt, D. J., Court, M. H., Schuetz, E. G., Sinclair, P. R., and Sinclair, J. F. (2005) Role of the nuclear receptor pregnane X receptor in acetaminophen hepatotoxicity. *Drug Metab. Dispos. 33*, 1827−1836.
(46) Yamazaki, Y., Kakizaki, S., Horiguchi, N., Takagi, H., Mori, M., and Negishi, M. (2005) Role of nuclear receptor CAR in carbon tetrachloride-induced hepatotoxicity. *World J. Gastroenterol. 11*, 5966−5972.
(47) Thomas, R. S., Black, M. B., Li, L., Healy, E., Chu, T. M., Bao, W., Andersen, M. E., and Wolfinger, R. D. (2012) A comprehensive statistical analysis of predicting in vivo hazard using high-throughput in vitro screening. *Toxicol. Sci. 128*, 398−417.