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Prediction of drug-induced liver injury and cardiotoxicity using chemical structure and *in vitro* assay data

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Abstract

Drug-induced liver injury (DILI) and cardiotoxicity (DICT) are major adverse effects triggered by many clinically important drugs. To provide an alternative to *in vivo* toxicity testing, the U.S. Tox21 consortium has screened a collection of ~10K compounds, including drugs in clinical use, against >70 cell-based assays in a quantitative high-throughput screening (qHTS) format. In this study, we compiled reference compound lists for DILI and DICT and compared the potential of Tox21 assay data with chemical structure information in building prediction models for human *in vivo* hepatotoxicity and cardiotoxicity. Models were built with four different machine learning algorithms (e.g., Random Forest, Naïve Bayes, eXtreme Gradient Boosting, and Support Vector Machines) and model performance was evaluated by calculating the area under the receiver operating characteristic curve (AUC-ROC). Chemical structure-based models showed reasonable predictive power for DILI (best AUC-ROC = 0.75±0.03) and DICT (best AUC-ROC = 0.83±0.03), while Tox21 assay data alone only showed better than random performance. DILI and DICT prediction models built using a combination of assay data and chemical structure information did not have a positive impact on model performance. The suboptimal predictive performance of the assay data is likely due to insufficient coverage of an adequately predictive number of toxicity mechanisms. The Tox21 consortium is currently expanding coverage of biological response space

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L.Y. developed computational models and helped to write the manuscript. D.K.N. helped with data analysis and wrote the manuscript. T.X. helped with data analysis and modeling. Z.L. helped with human toxicity data collection. J.Z., S.S. and L.Z. conducted the in vitro assays. T.Z. helped with data analysis. R.H. designed and directed the study. R.H., M.X. and A.S. managed the project. All authors reviewed the manuscript.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

with additional assays that probe toxicologically important targets and under-represented pathways that may improve the prediction of *in vivo* toxicity such as DILI and DICT.

Keywords

Liver injury; Hepatotoxicity; Cardiotoxicity; *In vitro* assay; High-throughput screening; Tox21

Introduction

Modern drug development is aimed at producing compounds that maximize therapeutic benefits while also minimizing adverse effects. Despite promising results from preclinical studies in animal and cell models, more than 30% of small molecules fail in clinical trials because they are found to be harmful to human health.[1] Furthermore, 80% of drug candidates fail in human clinical trials due to unmanageable toxicity or lack of clinical efficacy.[1] Drug-induced liver injury (DILI) and cardiotoxicity (DICT) are major concerns during the safety profile evaluation of current drugs and development of novel therapeutics. The liver is responsible for a wide range of functions, including xenobiotic detoxification, protein synthesis, storage and synthesis of glucose, production of the bile necessary for digestion, and regulation of blood cholesterol and triglycerides. Due to its central role in biotransformation and excretion of foreign compounds, the liver represents a primary target for adverse drug reactions. Many drugs and environmental chemicals can evoke some degree of liver injury,[2,3] making DILI the single most common adverse indication, thus leading to drug candidate failure and/or withdrawal from the consumer market. However, DILI is largely unpredictable due to complex factors that give rise to liver damage, hence making prevention difficult.[4] Moreover, current *in vivo* toxicological studies are insufficient to assess the hepatotoxic potential of compounds early in the drug development process, thereby presenting an urgent need for alternative DILI prediction strategies.[5]

Likewise, DICT is another major safety concern and common cause of drug withdrawals from the consumer market.[6] The NIH National Cancer Institute (NCI) broadly defines cardiotoxicity as “toxicity that affects the heart”,[7] and major cardiovascular adverse effects may include tachycardia, hypertension, and electrocardiographic abnormalities such as prolonged QT interval. Many antineoplastic agents have been linked to cardiovascular toxicity with extended use.[8] In addition, cardiotoxicity has been observed in a diverse range of drug classes such as antipsychotics, antidepressants, and antibiotics.[9] Like DILI, the mechanisms that underly DICT are not fully understood, and effective predictive approaches are critical for reducing chemical-induced cardiotoxicity.

Quantitative structure-activity relationship (QSAR) modeling and machine learning methods have become increasingly popular for predicting compound properties such as toxicity.[10] To date, QSAR modeling has been widely used in liver toxicity research to study hepatotoxicity and predict DILI. While a number of hepatotoxicity prediction models have recently been developed, they often suffer from imbalanced and/or limited training/testing datasets that produce unsatisfactory predictive performance.[11] Consequently, the applicability of these models is often limited due to their insufficient coverage of the chemical space. The

etiopathogenesis of DILI is complex and involves the interaction of several factors, so the limited DILI data available in humans is a major drawback for QSAR modeling.[12] Similarly, QSAR approaches have been conducted to predict DICT compounds. However, the performance from these cardiotoxicity models varies widely because they are heavily influenced by the type of molecules and techniques used for model building.[13] Most previously published models use in-house data or proprietary data obtained from industry that are not publicly available, while a limited number of other studies that use data extracted from public databases often suffer from a certain amount of experimental uncertainty.[14] Collectively, these factors compromise the practical use of prior models for reliable assessment of cardiotoxicity. Small model training datasets and lack of proof of validation are additional factors that limit the usefulness of prior models.

To improve toxicity testing and prediction, the Toxicology in the 21st Century (Tox21) consortium was established as a federal collaboration between the National Center for Advancing Translational Sciences (NCATS), the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences (NIEHS), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA).[15–17] The aim of Tox21 is to develop alternative toxicity assessment methods to quickly and efficiently predict potential adverse effects of chemicals on human health. Tox21 employs quantitative high-throughput screening (qHTS), an automated robotic process in which each compound of a large chemical library is tested at multiple concentrations, to test large collections of chemicals in multiple cell-based assays. A Tox21 screening library comprised of approximately 10,000 chemical samples, also known as the "Tox21 10K compound library",[18] has been screened for potential biological pathway disruptions that may result in toxicity.[19,20] The Tox21 10K compound library contains industrial and consumer products, food additives, drugs, and chemical mixtures, and also includes the NCATS Pharmaceutical Collection (NPC),[21,22] a collection of approximately 3,000 small molecule drugs approved for clinical use or investigational purposes by the U.S., European, Japanese, Australian, and Canadian authorities. The Tox21 10K compound library has been screened in more than 70 cell-based assays in qHTS format related to nuclear receptor and stress signaling pathways, and also a smaller number of assays that probe for genotoxicity, developmental toxicity, G-protein coupled receptors, and cell-death signaling. To date, the screening has generated nearly 102 million data points that are publicly available to the scientific community.[23,24]

In this study, we aim to build machine learning models to predict DILI and DICT. To develop robust models for DILI and DICT prediction, a comprehensive set of human *in vivo* toxicity data is essential for model training and testing. We compiled reference lists of DILI and DICT compounds by incorporating *in vivo* toxicity data integrated from a diverse set of literature sources. Both chemical structure and assay data were used as descriptors to build the DILI and DICT prediction models. These two types of descriptors were tested both independently and in combination, and the resulting model performances were assessed. Accordingly, we identified assay targets and pathways as well as chemical features that contributed the most to model performance. The chemical structural features and targets that are significantly associated with liver toxicity and/or cardiotoxicity can be further investigated as potential indicators to help us better understand the underlying pathways and mechanisms of these toxicities.

Materials and methods

In vivo toxicity data

To compile a comprehensive list of reference compounds for DILI and DICT, data were integrated from six sources including ChemIDplus (DILI and DICT), Pharmapendium® (DILI and DICT), U.S. Food and Drug Administration (FDA)'s Center for Drug Evaluation and Research (CDER) (DICT) and National Center for Toxicological Research (NCTR) (DILI and DICT),[25] Enzo Life Sciences cardiotoxicity library (DICT), and the Side Effect Resource (SIDER) database[26] via the European Molecular Biology Laboratory (EMBL) (DICT). ChemIDplus is a database produced by the U.S. National Library of Medicine (NLM) that collects compound records (chemical nomenclature, properties, toxicity, and structures) from more than 100 sources. Pharmapendium® is a curated database containing extensive information on adverse drug effects extracted from the FDA and European Medicines Agency (EMA) drug approval documents. The Enzo Life Sciences cardiotoxicity library is a collection of 130 diverse compounds with known cardiotoxicity, including ion channel blockage, mitochondrial toxicity, arrhythmia, and fibrosis. SIDER contains information on marketed medicines and the associated adverse drug reactions that were extracted from public documents and package inserts. A total of 474 drugs that did not have any cardiotoxicity reports (244 overlapped with the Tox21 10K compound library) were collected from the SIDER database, and these compounds were included as negative controls in our DICT reference list.

In vivo toxicity data scoring and cutoff for DILI and DICT

For the reference lists collected for DILI and DICT, each compound was assigned a toxicity score according to the frequency in which it was identified as "toxic" in the literature. First, within each data source, the compounds were each assigned a value between 0 and 1 as specified in the following. For compounds retrieved from Pharmapendium® and ChemIDplus, different types (e.g., via different mechanisms or manifestations of toxicity) of DILI and/or DICT were normally reported. Compounds with 10 or more such reports were assigned a value of 1 and others were assigned the value N/10, where N is the number of toxicity reports. For the DILI and DICT lists provided by NCTR, compounds with the most toxicity concern were assigned a value of 1, compounds with less toxicity concern were assigned a value of 0.5, and compounds with no toxicity concern were assigned a value of 0. For compounds from the other data sources, compounds listed as toxic were assigned a value of 1 and 0 otherwise. Finally, the values from all data sources were averaged for each compound to yield the final DILI or DICT score, such that a larger score indicates a higher likelihood of toxicity and 0 means non-toxic.

A total of 1,407 compounds were collected from three different sources for the DILI reference list (Supplementary Table 1). The best model performance for DILI was obtained when using 0.4 as the cutoff value to separate the data into toxic and non-toxic binary classes, i.e., a compound was considered toxic if its DILI score was > 0.4. Other compounds on the list were considered non-toxic. A total of 1,160 compounds were collected from six sources for the DICT reference list (Supplementary Table 2). Different DICT score cutoffs were tested, and the following conditions produced the best model performance for

DICT prediction. Compounds with a DICT score of 0 were considered non-toxic, while compounds with a score ≥ 0.5 were considered toxic. Any compounds with a DICT score between 0 and 0.5 were considered inconclusive and removed from the modeling set.

In vitro assay data

qHTS data used for modeling in this study were generated by screening the Tox21 10K compound library, including 70 assays with 203 readouts. All *in vitro* assay data and detailed assay descriptions used in this study are publicly available on the NCATS website (https://tripod.nih.gov/tox21/pubdata/) and PubChem.[27,28] The following cell lines were used for the qHTS assays: human cell lines (80.88%), murine embryo fibroblast (7.35%), Chinese hamster ovary cell lines (5.88%), and other (5.88%). The majority of these assays cover several pathways related to nuclear receptor signaling (NR, 55.90%), stress response (SR, 11.80%), cytotoxicity (8.80%), and other targets/pathways related to toxicity (23.50%). Curve rank is a value between −9 and 9 that was used as a measure of compound activity, such that a positive number represents activation while a negative number represents inhibition.[29] For modeling purposes, compounds with absolute curve rank > 0.5 were labeled as active (1), and inactive (0) otherwise.

Structure data

To build classification models in this study, two structure-based fingerprint sets were used: ToxPrint and ECFP4. Publicly available Toxprint chemotypes (v2.0_r711, https://toxprint.org/) generated within the associated ChemoTyper application (https://chemotyper.org/) consists of 729 uniquely defined chemical features.[30] Extended-Connectivity Fingerprints (ECFP4) is a 1024-bit fingerprint set that was generated using the CDK package in KNIME v.4.0.2.25.[31] Each bit is representative of a structural feature and was designated a value of 1 for the presence of a particular feature and 0 otherwise.

Feature selection

To identify assays significantly related to DILI or DICT, each assay was used to predict hepatotoxicity and cardiotoxicity, and these assays can be viewed as single-descriptor models. ToxPrint chemotypes were used to identify chemical structural features significantly enriched in DILI and DICT compounds. Statistical significance was measured by the Fisher's exact test with $p < 0.05$ considered as significant.

Feature selection using the Fisher's exact test was used to further optimize model performance and determine features (chemical features or assays) significant for DILI and DICT prediction. Ten independent p-values ranging from 0.01 to 0.1 were used to select features at different p-value cutoffs to train models. The best performing feature sets were selected to build the final model. The top feature sets were further combined to rebuild the model to determine if the combined feature set achieved better model performance than that of the structure or assay feature set alone.

Prediction modeling

In this study, a total of 1,407 unique compounds were used to build the DILI prediction models, including 831 non-toxic compounds and 578 toxic compounds, for chemical

structure-based modeling (ToxPrint or ECFP4, Supplementary Table 1). To obtain a balanced modeling set, a subset of toxic compounds with a size roughly equal to the set of non-toxic compounds was randomly selected from the original dataset. The modeling set was further randomly split into two sets, 70% for training and 30% for model validation, and this process was repeated 100 times. For assay activity-based models (assay-only), compounds with complete activity profiles in the 70 assays (213 readouts), including 663 non-toxic compounds and 466 toxic compounds, were used for model training and validation. The same dataset and modeling procedure were repeated for the chemical structure (structure-only) and assay activity combined (structure + assay) models for DILI.

A total of 646 unique compounds were used to build DICT prediction models, including 244 non-toxic compounds and 402 toxic compounds, for chemical structure-based modeling (ToxPrint or ECFP4, Supplementary Table 2). For assay activity-based models, compounds with complete activity profiles in the 70 assays (213 readouts), including 209 non-toxic compounds and 335 toxic compounds, were used for model training and validation. The same dataset and procedure were repeated for the structure + assay combined models for DICT.

Four different machine learning classification algorithms were applied: Random Forest (RF), Naïve Bayes (NB), eXtreme Gradient Boosting (XGBoost), and Support Vector Machines (SVM). Models were built and tested using R version 4.1.2, with the “Random Forest” package for the RF classifier, the “e1071” package for the NB and SVM classifiers, and the “xgboost” package for the XGBoost classifier. The implementation of the NB classifier was adapted with the settings of Laplace smoothing, and the Gaussian Radial Basis Function kernel was used for the SVM classifier. In addition, the optimal parameters for the SVM and RF classifiers were selected using the “e1071” package. The R codes used in this study are publicly available on the GitHub repository at https://github.com/TX-2017/machine-learning.

Model performance was evaluated by calculating the area under the receiver operating characteristic curve (AUC-ROC). The ROC curve is a graphical plot that illustrates the predictive ability of a binary classification model across different thresholds. The ROC curve is created by plotting true positive rates against false positive rates at various thresholds. The area under the curve (AUC) provides an aggregate measure of model performance. A larger AUC value indicates better classifier performance. A perfect predictive model would have an AUC score of 1, while an AUC score of 0.5 indicates a random classifier. For feature selection, assays with AUC-ROC scores greater than 0.5 were considered to be predictive of toxicity and retained for further analysis.

To assess the applicability domain (AD) of the structure-based models, the closest structural neighbor in the training set was found for each of the compounds to be predicted, e.g., the Tox21 10K library. Structural similarity was determined by calculating the Tanimoto coefficient using ECFP4 fingerprints, which measures the similarity between two compounds based on the number of shared structural features. It is calculated by taking the number of structural features in common by both compounds and dividing it by the total number of structural features in either compound. The Taminoto coefficient ranges

from 0, where the compounds have no chemotypes in common, to 1, when the compounds are identical. A compound that has a close structural neighbor in the training set with a Tanimoto similarity (Tmax) ≥ 0.4 was considered to be within the model's AD.

Results

Model performance

The modeling results for both DILI and DICT are summarized in Table 1.

The DILI modeling results showed that liver toxicity could be reasonably predicted by chemical structure, with ECFP4 demonstrating the best predictive performance. While the AUC-ROC scores of the ToxPrint-based models ranged from 0.65±0.03 to 0.68±0.03, ECFP4-based models performed better as shown by the scores ranging from 0.71±0.03 to 0.75±0.03. The AUC-ROC scores of the assay-based models ranged from 0.59±0.02 to 0.61±0.04, which were much lower than both chemical structure-based models. The AUC-ROC scores (0.63±0.03 to 0.72±0.03) of the models built on both chemical structure (Toxprint or ECFP4) and assay data showed that the addition of assay data did not improve model performance compared to chemical structure-based models alone.

Likewise, the application of chemical structure could also be used to predict cardiotoxicity as shown by the modeling results from DICT prediction. The AUC-ROC score of the ECFP4-based model ranged from 0.73±0.04 to 0.83±0.03, while the AUC-ROC score of the ToxPrint-based model ranged from 0.70±0.04 to 0.75±0.02. Similarly, the AUC-ROC scores of the assay-based models were relatively low, scoring within the range of 0.56±0.04 to 0.58±0.04. The AUC-ROC of the models built using both chemical structure (Toxprint or ECFP4) and assay data revealed that the addition of assay data did not improve the DICT model performance, suggesting that chemical structure alone was the better predictor of hepatotoxicity and cardiotoxicity. In both DICT and DILI models, ECFP4 fingerprints produced better model performance than ToxPrint fingerprints.

Feature selection was used to optimize model performance. Ten different p-value cutoffs between the range of 0.01 and 0.1 were used for feature selection, and the best cutoff value was selected for each method. Comparing the model performance before (Supplementary Table 3) and after (Table 1) application of feature selection, we observed that the impact of feature selection on model performance varied across the different descriptor types and machine learning methods. For DILI prediction, feature selection for NB with ECFP4 exhibited the greatest AUC-ROC score increase of 0.15, improving from 0.60±0.03 to 0.75±0.03. Likewise, feature selection for NB with ECFP4 substantially improved DICT prediction by an AUC-ROC score by 0.13, increasing from 0.70±0.03 to 0.83±0.03 (Table 1; Supplementary Table 3). Overall, feature selection helped to boost model performance for both DILI and DICT prediction. We observed modest improvements for RF after application of feature selection, which is likely due to the built-in feature selection already implemented in this method.

Toxic structural feature identification for DILI and DICT compounds

The ToxPrint chemotypes were used to identify chemical features that are significantly (Fisher's exact test) enriched in toxic compounds. Several distinct features were present in hepatotoxic compounds (Figure 1), particularly aromatic rings such as bond:N(=O)_nitro_aromatic (4.1×10^{-2}), ring:hetero_[6]_N_piperidine (4.95×10^{-6}), and ring:hetero_[4]_N_beta_lactam (1.60×10^{-3}).

Moreover, small chemical fragments such as bond:CX_halide_alkyl-X_trihalo_(1_1_1-) (6.22×10^{-3}) and bond:CN_amine_aliphatic_generic (2.00×10^{-12}) were found enriched in DILI compounds. For cardiotoxic compounds (Figure 2), we also observed the presence of six-membered rings including ring:hetero_[6]_Z_1- (4.35×10^{-6}), ring:hetero_[6]_N_piperazine (3.49×10^{-4}), ring:hetero_[6]_N_pyridine (2.60×10^{-2}), and bond:CC(=O)C_quinone_1_4-benzo (2.75×10^{-2}).

In addition, the double-ring structure ring:hetero_[6_6]_N_quinoline (1.62×10^{-2}) was identified in cardiotoxic compounds. The most significant chemotypes identified for both hepatotoxic and cardiotoxic compounds are presented in Supplementary Tables 4 and 5, respectively.

Assays predictive of DILI and DICT

We examined the performance of each assay within the Tox21 assay panel in predicting DILI and DICT. The top five assays that are the most predictive of DILI measured by their AUC-ROC scores, in descending order of significance, are: tox21-p450-2c9-p1_ratio (0.60), tox21-ror-cho-antagonist-p1_ratio (0.58), tox21-ar-bla-agonist-p1_ratio (0.57), tox21-gr-hela-bla-antagonist-p1_ratio (0.57), and tox21-er-bla-antagonist-p1_ch2 (0.57). Likewise, the top five assays for predicting DICT and their corresponding AUC-ROC scores, in descending order of significance, are as follows: tox21-pparg-bla-agonist-p1_ratio (0.60), tox21-casp3-cho-p1_viability (0.60), tox21-ahr-p1_viability (0.59), tox21-erb-bla-antagonist-p1_ratio (0.59), and tox21-casp3-hepg2-p1_viability (0.59). A full list of the top 20 most predictive assays for DILI and DICT are shown in Tables 2 and 3, respectively.

Predicting the DILI and DICT potential of the Tox21 10K compound library

We applied the ECFP-based RF, NB, XGBoost, and SVM models, which achieved good performance during model training and testing, to predict the DILI and DICT potential of the compounds in the Tox21 10K compound library (Supplementary Table 6). Each compound was assigned a toxicity probability based on model predictions, and the majority of compounds in the 10K library were found to be non-toxic for both DILI and DICT. The ROC curve was used to determine the optimal probability cutoff for toxicity. With respect to hepatotoxicity, the RF model predicted 2,807 compounds (35%) as toxic. We observed a similar trend using the NB model, which identified 3,745 compounds (47%) as potentially toxic. The XGBoost model predicted 2,384 compounds (30%) as toxic, while the SVM model predicted 2,065 hepatotoxic compounds (26%). With respect to cardiotoxicity, the RF model predicted 4,420 compounds (55%) as toxic. Similarly, the NB model predicted 3,075 toxic compounds (38%). The XGBoost model predicted 2,532 compounds (32%) as toxic, while the SVM model identified 2,163 potentially cardiotoxic compounds (27%). All four

methods predicted a consensus 1,374 hepatotoxic compounds (17%) and 1,469 cardiotoxic compounds (18%), resulting in smaller numbers of predicted toxic compounds.

The top 10 toxic compounds for DILI and their average predicted probabilities, in order of descending probability, are: floxacillin sodium salt (0.91), floxacillin sodium hydrate (0.91), dicloxacillin sodium salt monohydrate (0.91), cloxacillin sodium (0.91), oxacillin sodium salt (0.91), nafcillin sodium monohydrate (0.90), piperacillin sodium salt (0.89), cefixime (0.89), azlocillin sodium (0.89), and methicillin sodium hydrate (0.89). All compounds are reported as toxic according to our DILI reference list (Supplementary Table 1), and this finding validates our models' robustness to detect known liver toxicants.

The top 10 toxic compounds for DICT that fell within the model AD (Tmax ≥ 0.4) ranked in descending order by the average of their predicted probabilities are: cisapride (0.87), orphenadrine dihydrogen citrate (0.85), roxithromycin (0.85), tamoxifen citrate (0.84), everolimus (0.83), dirithromycin (0.82), azithromycin (0.81), erythromycin ethylsuccinate (0.81), sirolimus (0.81), and doxepin hydrochloride (0.81). Six out of these ten compounds (cisapride, orphenadrine dihydrogen citrate, roxithromycin, tamoxifen citrate, sirolimus, and doxepin hydrochloride) were known toxicants in our DICT reference list (Supplementary Table 2). The remaining four compounds (everolimus, dirithromycin, azithromycin, and erythromycin ethylsuccinate) have literature evidence potentially linking them to cardiotoxicity as detailed below, so these compounds may warrant further investigation.

Everolimus (CAS# 159351-69-6) is an antineoplastic agent used to treat various types of cancer including kidney, pancreas, breast, and brain cancer. Everolimus is an mTOR inhibitor that has been reported to have a direct cardiotoxic effect by blocking signaling for blood vessel formation which may contribute to coronary artery disease (CAD) risk factors such as hyperglycemia, hyperlipidemia, and hypertension.[32] Dirithromycin (CAS# 62013-04-1), azithromycin (CAS# 83905-01-5), and erythromycin ethylsuccinate (CAS# 1264-62-6) are all antibiotics used to treat several different types of bacterial infections. Specifically, they belong to the macrolides class of antibiotics, which are commonly used to treat both acute and chronic infections. Macrolides are also known to cause QT prolongation and cardiac arrhythmias. Dirithromycin was discontinued in the United States; however, it is still available in many European countries.[33] In 2013, the FDA warned azithromycin to be linked to arrhythmia-related adverse cardiac events especially in patients who are at high risk for cardiovascular events.[34] Despite these cautions, azithromycin continues to be a popular broad-spectrum antibiotic. In a previous study, azithromycin was found to have low hERG liability based on *in vitro* assay results.[35] Erythromycin ethylsuccinate is an antibiotic that carries the highest risk of cardiotoxicity among the more commonly used macrolides.[36] Specifically, it is known to cause significant prolongation of the QT interval and potentially a fatal ventricular tachyarrhythmia called *Torsade de Pointes* (TdP).

Overall, the RF, NB, XGBoost, and SVM models predicted that the majority of compounds in the 10K library were not likely to be toxic. The DILI and DICT prediction results using RF, NB, XGBoost, SVM, and consensus models are summarized in Table 4.

Furthermore, we presented the distributions of the predicted toxicity probabilities of compounds in the Tox21 10K library generated from the four machine learning methods (RF, NB, XGBoost, and SVM) in Supplementary Figure 2. These results reiterate our findings that the majority of compounds in the Tox21 10K library were non-toxic regarding both DILI and DICT.

The reliability of the predictions obtained from structure-based models is often limited by the model AD. We assessed the similarity of each compound in the prediction set to the compounds in the model training set (see Methods for details). When considering only compounds that fall within the model AD (Tmax ≥ 0.4), we observed an overall reduction in the predicted number of toxic compounds. For DILI models, the NB method predicted the most toxic compounds (1,838; 23%), while SVM identified the least number of compounds (1,268; 16%) as toxic. For DICT models, the RF method picked up the most toxic compounds (2,357; 29%). Similarly, SVM predicted the least number of cardiotoxic compounds (1,357; 17%). The consensus of all four methods further reduced the total number of predicted toxic compounds for both DILI and DICT, resulting in the prediction of 845 (11%) hepatotoxic compounds and 953 (12%) cardiotoxic compounds.

As observed in Table 4, the use of the AD narrowed the predicted number of toxic compounds by removing compounds that are not structurally similar to the model training set. Defining the AD greatly reduced the number of both hepatotoxic and cardiotoxic compound predictions in the Tox21 10K compound library, with predicted compounds decreasing by at least 10% using RF, NB, XGBoost, and SVM methods separately. The compounds in our training set mostly consists of drugs while the Tox21 10K compound library contains many environmental chemicals that do not have drug-like structures, thus they may fall outside of the model AD. Our QSAR models were limited by the chemical space of the training set that was used to build the models. Nonetheless, similar modeling approaches to predict hepatotoxicity and/or cardiotoxicity have been utilized in previous studies to fill in data gaps related to human organ toxicity.[37,38] The applicability of these models could be improved by expanding the training dataset to cover a wider range of chemical structural space beyond drug-like molecules.

We next examined compounds by consumer product use categories[39,40] in the Tox21 10K compound library that were predicted to be hepatotoxic or cardiotoxic. The distribution of DILI and DICT compounds in each use category was calculated and the significance of the enrichment was determined using the Fisher's exact test. The top ten categories enriched with predicted hepatotoxic compounds (in order of descending significance) are: "drug" (95.5%; 1.00×10^{-20}), "discontinued" (36.4%; 1.00×10^{-20}), "antibiotic" (9.2%; 6.55×10^{-20}), "food" (3.5%; 6.75×10^{-20}), "orphan" (1.5%; 1.63×10^{-17}), "pediatric" (5.1%; 1.20×10^{-9}), "diuretic" (3.7%; 1.57×10^{-9}), "anticancer" (6.3%; 4.14×10^{-9}), "anti-inflammatory" (5.5%; 6.13×10^{-9}), and "antihypertensive" (4.1%; 2.86×10^{-7}). The categories "antibiotic", "diuretic", "anticancer", "anti-inflammatory", and "antihypertensive" refer to pharmaceutical-related compounds. "Drug" refers to any drug product or compound related to the manufacturing of drugs, whereas "food" applies to products designated for human consumption, excluding food additives, as well as manufacture and facilities contaminants related to food.

Furthermore, the top ten categories enriched with potential cardiotoxic compounds along with the corresponding statistical significance p-value (in order of descending significance) are: “drug” (89.9%; 1.00×10^{-20}), “discontinued” (23.7%; 4.05×10^{-13}), “food” (31.8%; 1.51×10^{-12}), “pediatric” (5.6%; 2.00×10^{-9}), “anti-HIV” (4.3%; 1.35×10^{-8}), “antipsychotic” (3.3%; 2.45×10^{-7}), “orphan” (9.6%; 6.82×10^{-6}), “biomarker” (3.5%; 7.39×10^{-5}), “dopamine antagonist” (1.8%; 7.21×10^{-4}), and “angiotensin” (1.3%; 0.0011). The following categories refer to pharmaceutical-related compounds: “drug”, “discontinued”, “pediatric”, “anti-HIV”, “antipsychotic”, “orphan”, “biomarker”, “dopamine antagonist”, and “angiotensin”. Compounds in the “food” category can potentially induce cardiotoxicity through food consumption and/or contaminants. For instance, sorbitan monooleate (CAS # 1338-43-8) is a food additive with emulsifying properties. More recently, the European Food Safety Authority (EFSA) Panel on Food Additives and Nutrient Sources Added to Food (ANS) re-evaluated the safety of various sorbitan esters (including sorbitan monooleate) as food additives and concluded that while there is no safety concern at the reported uses and use levels, their recommendation is that the authorities consider lowering the current limits for sorbitan esters to ensure that these compounds will not be a significant source of toxic elements (arsenic, cadmium, lead, and mercury) in food. Another compound albendazole (CAS # 54965-21-8) belongs to an anthelmintic class of drugs that is widely used for the treatment of gastrointestinal, parasitic infections in animals. It has been approved for farmed ruminants and recently considered as a regulatory treatment against parasites for fish in aquaculture.[41] Sucralose (CAS # 56038-13-2) is an artificial sweetener and sugar substitute. In recent decades, there has been increased attention in the safety concerns of low/no-calorie sweeteners such as sucralose. A recent rat study revealed that a 10-week consumption of sucralose mixture resulted in a significant vascular endothelial dysfunction.[42] Xylazine (CAS # 7361-61-7) is an adrenergic agonist used in veterinary medication for sedation, analgesia, and muscle relaxation in animals. Food safety concerns may arise from xylazine and metabolite residue in animal-derived food products.[43] Five out of ten use categories (“drug”, “discontinued”, “food”, “pediatric”, and “orphan”) overlapped between DILI and DICT compounds, suggesting that these product use categories contain compounds that may contribute to both hepatotoxicity and cardiotoxicity. The majority of the compounds identified by our models are categorized as pharmaceutical-related compounds. Since the training data consisted mostly of drugs, application of our structure-based models is likely to identify other drug-like compounds that are potentially toxic for human consumption.

Discussion

To improve the prediction of DILI and DICT, we compiled a comprehensive dataset that incorporates human *in vivo* hepatotoxicity and cardiotoxicity data from a diverse set of data sources. The resulting DILI and DICT reference compound lists were used to develop and train the models in this study for the prediction of hepatotoxicity and cardiotoxicity. In addition, assays and chemical structure features that contributed the most to DILI and DICT were identified. The top 20 DILI predictive assays are listed in Table 2. Some of these assays measure targets or pathways that have known connections to hepatotoxicity such as nuclear factor kappa B (NF-κB)[44], pregnane X receptor (PXR)[45], Nrf2/ARE[46], P450[47], and

mitochondria toxicity[48]. These assays can serve as a validation for our methods' utility in identifying hepatotoxicity-related targets and pathways.

NF-κB represents a family of transcription factors that is involved in several aspects of the immune and stress/inflammatory responses. Basal NF- κB activity is essential for cell survival at different stages of development, particularly for protection of the liver against apoptosis. Inactivation of the NF- κB pathway can lead to adverse effects, including liver failure and apoptosis induction. Moreover, PXR is a member of the orphan nuclear receptor family that is highly expressed in the liver where it serves as a master xenobiotic sensor and plays a critical role in drug metabolism.[49] PXR tightly regulates the gene expression involved in the detoxification and elimination of drugs in the liver (hepatic drug-clearance system); however, its undesired activation plays an integral role in DILI and has been reported to induce liver injury by increasing the expression of drug metabolizing enzymes.[50]

The nuclear factor erythroid 2-related factor 2 (Nrf2) is a transcription factor that binds to the antioxidant response element (ARE), and activation of the Nrf2/ARE pathway induces cellular defense against oxidative stress and inflammation. Loss of Nrf2 broadly increases the sensitivity of hepatotoxic chemicals, suggesting that Nrf2 is a pleiotropic transcriptional factor that may serve as a modifier in the development and progression of chronic diseases, including liver injury. The tox21-are-bla-p1 assay detects compounds that can induce the Nrf2/ARE pathway resulting in toxicity. In addition, cytochrome P450 is a superfamily of enzymes that contributes to the metabolism of a wide range of xenobiotic and endogenous compounds. CYP-mediated metabolism of drugs to toxic reactive metabolites leads to the pathogenesis of DILI.[51] The Tox21 P450 assays identify CYP inhibitors and substrates which could interfere with the CYP activities producing toxic metabolites. Lastly, some drugs can cause liver injury through mitochondrial toxicity. Drug-induced mitochondria dysfunction can interfere with drug metabolism and renal excretion, ultimately leading to hepatotoxicity.[52,53] The tox21-mitotox-p1 assay detects compounds that can disrupt the mitochondria membrane potential resulting in mitochondria dysfunction. Other assay targets identified in our analysis to be predictive of DILI can improve our understanding of the underlying pathways and mechanisms that may lead to hepatotoxicity.

Furthermore, the top 20 predictive assays for DICT based on our analysis are listed in Table 3. Some assay targets were well linked to cardiotoxicity, while others may begin to bridge the gap between understanding cardiotoxicity mechanisms and the first stages of drug development. For example, Tox21 assays that measure the peroxisome proliferator-activated receptor gamma (PPARγ), estrogen receptor beta (ER-β), and vitamin D receptor (VDR) signaling pathways as well as mitochondrial membrane potential, which is an indicator of mitochondrial toxicity, were found to be predictive of cardiotoxicity. PPARs are lipid-activated transcription factors that are critically involved in the regulation of lipid and glucose homeostasis. PPARs are highly expressed in cardiac cells, so activation of PPARγ can exhibit cardiovascular risks through both PPARγ-dependent (on-target) and -independent (off-target) mechanisms. The Tox21 PPARγ assays run in agonist and antagonist modes detect PPARγ agonists and antagonists, respectively. Both modes were found to be predictive of DICT based on our analysis (Table 3). PPARγ agonists such as thiazolidinediones (TZDs), are commonly used as antidiabetic drugs, with two widely used

TZDs – pioglitazone and rosiglitazone – being reported to increase the risk of heart failure and other cardiovascular events in diabetic patients.[54,55] Nonetheless, the role of PPARγ and the heart remains elusive, so there exists a continual need to better understand the role of PPAR in the physiology and pathology of cardiovascular-related diseases.

ER-β is a receptor that is expressed on cardiomyocytes, where it exerts a protective effect on cardiac function by initiating signaling pathways essential for the growth, repair, and survival of cardiomyocytes.[56] Modulation of estrogen receptors by potential therapeutic agents is currently being considered for the prevention and treatment of various pathological conditions, including cancer and cardiovascular diseases.[57] For breast cancer patients, however, cardiotoxicity is one of the most concerning side effects of treatments which inhibit ER-β. There is a strong association between the drug tamoxifen, an antineoplastic agent that is also an ER antagonist, and an increased risk for cardiotoxicity manifested in several forms (e.g., thrombotic/thromboembolic events and pulmonary embolism).[58] The tox21-erb-bla-antagonist-p1 assay that identifies ER-β antagonists is among the top 20 predictive assays of DICT.

VDR, the molecular mediator for Vitamin D signaling is present in almost all tissues, including vascular smooth muscle cells, cardiomyocytes, and endothelial cells. High doses of vitamin D in humans have been strongly associated with extensive arterial calcium phosphate deposits, with both activation and inhibition of VDR promoting different types of calcification. The tox21-vdr-bla-antagonist-p1 assay detects compounds that inhibit VDR activity and is among the top 20 DICT predictive assays. Vascular calcification is a disorder commonly linked with cardiovascular mortality in which calcium mineralization occurs along vascular walls, often leading to vessel stiffening and reduced compliance.[59] Vascular calcification is complex, and the exact impact of VDR signaling on this process is not well understood.

The mitochondrial membrane potential is an indicator for normal cell function that results from the electron transport and oxidative phosphorylation process, an essential component for ATP production.[60] Given that the mitochondria is abundantly found in cardiac muscle cells, it plays a critical role in maintaining myocardial tissue homeostasis as well as regulating cardiac muscle contraction and heartbeat.[61,62] As a result, cardiomyocytes are more susceptible to mitochondrial dysfunction and oxidative stress due to the mitochondria-rich environment and low levels of antioxidants compared to other cells.[61] Many drugs, such as antineoplastic, antiangiogenic, antiviral, and antidiabetic drugs, have been linked to adverse cardiovascular effects through interference with mitochondria-related signaling pathways.[62] Three common mechanisms of mitochondrial toxicity are the ROS/Redox system, calcium homeostasis system, and endoplasmic reticulum stress signaling.[63]

While inhibition of the hERG channel is known to elicit QT interval prolongation and potentially lead to TdP, the Tox21 hERG inhibition assay[64] was not among the top 20 most predictive assays for cardiotoxicity, with an AUC-ROC score (0.52) slightly above random. To investigate the cause of this miscorrelation, we checked the activity of the compounds on the DICT reference list in the hERG assay. Of the 441 reference compounds tested in the hERG assay, 241 compounds (54.6%) did not inhibit the hERG channel but were classified

as cardiotoxic (toxicity score > 0.5), 106 compounds (24.0%) inhibited the hERG channel but were not classified as cardiotoxic, and only 94 compounds (21.3%) both inhibited hERG activity and were cardiotoxic (Supplementary Figure 2). These findings demonstrate that the hERG channel inhibition is not the only mechanism for DICT and does not necessarily lead to clinical cardiotoxicity, with a notable example being the antiarrhythmic drug verapamil. This calcium-channel blocker inhibits the hERG channel but does not cause QT prolongation or TdP, which could likely be attributed to the inhibition of multiple channels that mitigates the hERG-blocking effect.[65] In the Tox21 hERG assay, verapamil showed hERG inhibition with IC_{50} values between 4–11 μM. Additionally, verapamil was assigned a toxicity score of 0.17 on our DICT reference list, indicating that this compound was seldomly identified as cardiotoxic in the literature. Drug-induced blockage of the hERG channel can lead to a variety of cardiovascular ailments, so it crucial to recognize the hERG liabilities of compounds.[35]

Other compounds, such as the antihypertensive drug alfuzosin, may not inhibit hERG channels but still lead to QT prolongation through interactions with other ion channels.[66] Alfuzosin is used to treat benign prostatic hyperplasia by increasing the sodium current, rather than blocking the hERG potassium current, to delay cardiac repolarization. The Tox21 qHTS data revealed that alfuzosin was inactive in the hERG assay; however, the toxicity score of 0.4 on the DICT reference list indicates that ~40% of the literature sources reported this drug as cardiotoxic.

Lastly, we found that 65% of the top 20 cardiotoxicity predictive assays are cell viability assays. The cell viability assay serves as the counter screen for cell-based pathway assays by assessing the integrity of cells in which a loss in signal indicates cell death or cytotoxicity interference. It is not surprising that cytotoxicity could lead to *in vivo* toxicity such as cardiotoxicity, but the exact toxicity mechanism could not be inferred from generic cytotoxicity assays. Since most of the Tox21 assays that correlated with cardiotoxicity were cell viability assays rather than target- or pathway-specific assays, it suggests that the current Tox21 assay panel has insufficient coverage of the targets/pathways that detect cardiotoxicity. To address this limitation, the Tox21 program is currently screening additional assays to improve the coverage of the toxicity-related biological response space.

In vitro assays are valuable tools for studying a compound's mechanism of action and can be used to screen tens of thousands of compounds in a short timeframe when compared to *in vivo* studies. One of the limitations of *in vitro* assays is that they are missing some functional physiological machinery, such as drug metabolism enzymes (e.g., CYPs), which may fail to identify the chemicals that need metabolic activation. To overcome this limitation, the Tox21 partners have developed an *in vitro* assay that incorporates metabolic components[67] to mimic real physiological conditions. This type of assays will be good candidates to be adapted for regulatory use in the future.

Our models also identified chemical features that are significantly enriched in hepatotoxic and cardiotoxic compounds. Examples of compounds that contain significant hepatotoxic chemotypes include oxacillin, flutamide, entacapone, maprotiline, and desloratadine (Figure 1; Supplementary Table 4). Oxacillin (ring:hetero_[4]_N_beta_lactam) is a penicillin

antibiotic used to treat a wide variety of bacterial infections.[68] It has been linked to two forms of hepatotoxicity: (1) an acute elevation of hepatic enzymes (e.g. alanine aminotransferase) and (2) a more prolonged idiosyncratic liver injury similar to the hepatotoxicity describe with other second-generation penicillins (e.g. dicloxacillin).[68] Flutamide (bond:CX_halide_alkyl-X_trihalo_(1_1_1-)) belongs to a class of drugs known as anti-androgens, and it is used in the management and treatment of prostate cancer. Drug-induced elevation of serum aminotransferase levels have led to several instances of acute liver injury, thereby making flutamide a potent hepatotoxin in certain patients. Entacapone (bond:N(=O)_nitro_aromatic) is a peripheral catechol-O-methyltransferase (COMT) inhibitor often used as a beneficial adjunct to treat Parkinson's disease. Although rare, acute liver dysfunction and low rates of serum enzyme elevations have been linked to entacapone therapy.[68,69] The antidepressant drug maprotiline (bond:CN_amine_aliphatic_generic) has been reported in both clinical case reports and *in vitro* animal studies to cause hepatic damage after prolonged use over the course of several years. Specifically, abnormally high elevations of liver enzymes and jaundice were reported in patients.[70,71] Finally, desloratadine (ring:hetero_[6]_N_piperidine) is the major metabolic derivative of loratadine, a second-generation antihistamine used widely to treat allergy symptoms. In rare cases, desloratadine has been connected to instances of clinically apparent acute liver injury.[72]

Moreover, compounds that contain structure features enriched in cardiotoxic compounds include daunorubicin, ranolazine, bosutinib, abiraterone acetate, and mitomycin C (Figure 2; Supplementary Table 5). Daunorubicin (ring:hetero_[6]_Z_1-) is an antibiotic that belongs to the anthracyclines class of drugs that is widely used in the treatment of a variety of malignancies. Anthracycline use has been associated with cardiotoxicities such as arrhythmia, systolic dysfunction, long QT interval, and in some cases hypertension, myocardial ischemia, and thromboembolism.[73] Ranolazine (ring:hetero_[6]_N_piperazine) is an antianginal agent that is primarily indicated for the treatment of patients with coronary artery disease and chronic stable angina. It inhibits the sodium ion current in cardiac cells, thus interfering in transmembrane cardiac action potential.[74] TdP has not been identified as a side effect of this drug, however, the risk for developing this condition may increase in patients that are also administered other QT-prolonging medications.[75] Bosutinib (ring:hetero_[6_6]_N_quinoline), a BCR-ABL1 tyrosine kinase inhibitor (TKI), has been available for several years as a treatment for chronic-, accelerated-, and blast-phase chronic myeloid leukemia (CML) for patients with resistance or intolerance to prior therapy. While the incidence of bosutinib-induced cardiotoxicity is relatively rare, cardiovascular adverse events such as hypertension, palpitations, and even cardiac failure were found to be associated with bositinib.[76] Abiraterone acetate (ring:hetero_[6]_N_pyridine) is often used in combination for the treatment of metastatic castration-resistant prostate cancer. Patients with prostate cancer that were treated with abiraterone acetate reported the highest incidence of cardiotoxic effects.[77] Specifically, hypertension and cardiac disorders were prominently observed in Phase III of a placebo-controlled trial that evaluated the risk ratio of abiraterone acetate-related adverse events.[78,79] Mitomycin C (bond:CC(=O)C_quinone_1_4-benzo), an anti-cancer drug exhibiting antibiotic properties, is used in chemotherapy treatment. Several studies dating back from the 1970s suggests that cardiotoxicity induced by mitomycin C is

dose dependent, and these adverse effects are enhanced in patients that are also treated with doxorubicin.[80,81] The chemical features collectively identified from this study can serve as structural alerts of hepatotoxicity or cardiotoxicity and should be avoided when designing chemicals for new drug development to minimize their toxicity potential.

Overall, the assays previously discussed can serve as a starting point to better understand the underlying biological mechanisms that lead to DILI and DICT. The adverse outcome pathway (AOP) is an analytical construct that organizes biological interactions over the span of multiple levels and toxicity mechanisms to promote more meaningful analysis of toxicological effects that can advance human health risk assessment of chemicals.[82] AOPs are useful for linking molecular initiating events (MIEs), the initial chemical-induced disruption in a biological system, with key events (KEs), the intermediate progression of toxicity, that ultimately leads to a specific adverse outcome (AO). The Tox21 *in vitro* assay targets that contributed the most to DILI and/or DICT could be MIEs or KEs within an AOP, thus complementing the AOP concept. For example, the assay "tox21-pxr-p1_ratio" was found to be one of the most relevant assays to DILI (AUC-ROC = 0.57) in our study (Table 2), and pregnane X receptor (PXR) plays a critical role in the detoxification of xenobiotics and bile acid homeostasis in liver.[83] PXR was recorded as the MIE in an AOP concept that is related to hepatic steatosis (https://aopwiki.org/aops/60). The assay "tox21-p450-2c9-p1_ratio" was found to be one of the top relevant assays to DILI (AUC-ROC = 0.55) in our study (Table 2), and CYP2C9 is a liver enzyme responsible for the metabolism of a wide range of clinical drugs (e.g., warfarin).[84] However, there is no record of CYP2C9 being involved in an AOP framework. The assay "tox21-pparg-bla-agonist-p1_ratio" was found to be the most relevant to DICT (AUC-ROC = 0.60) in our study (Table 3), and PPARγ has been reported to be closely associated with cardiotoxic regulation.[85] However, PPARγ was only documented as the MIE in a few AOP concepts that are unrelated to DICT, such as lung fibrosis (https://aopwiki.org/aops/206), sarcomas in rats, mice, and hamsters (https://aopwiki.org/aops/163), and pulmonary fibrosis (https://aopwiki.org/aops/347).

In this study, we found that the addition of assay data to chemical structure does not always improve the model performance; these results are not surprising and similar results have been reported in previous studies.[37] Hepatotoxicity and cardiotoxicity are complex and multifactorial, whereas *in vitro* assay typically only represents a single toxicity mechanism that covers only a small number of compounds in the modeling set, such that assay results are often biased with much more inactives than actives. Hence, the contribution of an individual assay tends to be small in a global model (e.g., a model for a complex toxicity endpoint) and the relevance of this assay becomes negligible during the modeling process. On the other hand, assay data could play a more significant role when developing a local model (e.g., a specific toxicity pathway model). Although our model performance did not benefit from the addition of assay data, recent studies have demonstrated successful use of assay data for modeling and validating QSAR predictions.[86–88] In cases where structure-based models cannot be achieved (e.g., a defined chemical structure is not available), assay data can be used to build models for toxicity prediction and elucidation of the biological mechanisms of drug-induced toxicity. QSAR models, such as the models constructed in this study, are often limited by the extent of chemical space coverage. Our models were trained using a compound collection enriched with drug-like molecules, therefore making them less

suitable for making predictions on other types of chemicals (e.g., environmental chemicals). Presently, Tox21 is focusing on the expansion of pathway coverage by adding assays that probe under-represented pathways in the current portfolio to improve the predictivity of Tox21 data for adverse drug effects as observed in DILI and DICT.

Conclusion

In this study, we compiled reference compound lists for DILI and DICT to build human *in vivo* hepatotoxicity and cardiotoxicity prediction models. The chemical structure-based models showed good performance in identifying hepatotoxic or cardiotoxic compounds, while Tox21 assay data-based models only showed better than random performance. The optimal models were applied to predict potential hepatotoxic and cardiotoxic compounds within the Tox21 10K compound library. These models can be applied to make predictions on other large compound libraries regarding DILI and DICT. In addition, we identified significant hepatotoxic and cardiotoxic chemical structure features and assays that were most predictive of DILI and DICT. The findings uncovered in this study can be used to better understand the underlying pathways and mechanisms for drug-induced toxicity as it relates to the liver and heart as well as highlight structural features that may alert us of these toxicities. Tox21 is currently expanding the coverage of biological response space with additional assays that probe toxicologically important targets and under-represented pathways to improve the prediction of *in vivo* toxicity, including DILI and DICT.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Reference compound lists for DICT and DILI were compiled to build human *in vivo* hepatotoxicity and cardiotoxicity prediction models
- Chemical structure-based models showed reasonable predictive power for DILI and DICT prediction
- Significant chemical features and assays for predicting hepatotoxicity and cardiotoxicity were identified
- Predicting the DILI and DICT potential of the Tox21 10K compound library revealed that most compounds were non-toxic

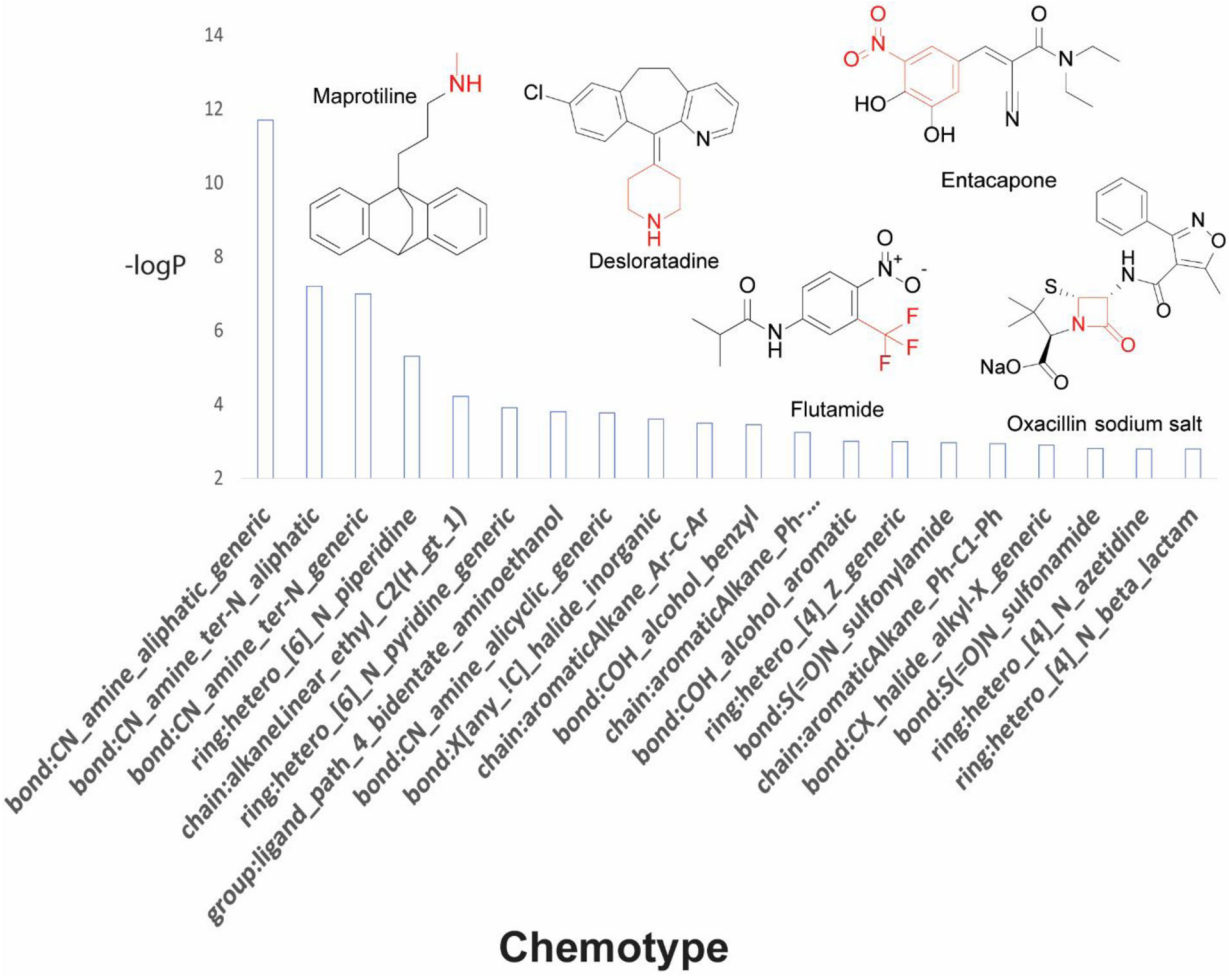

Figure 1.

Chemotypes significantly enriched in compounds active for DILI. Chemotypes are sorted by significance based on p-values. Representative drugs that contain these structural fragments are displayed with significant chemotypes highlighted in red.

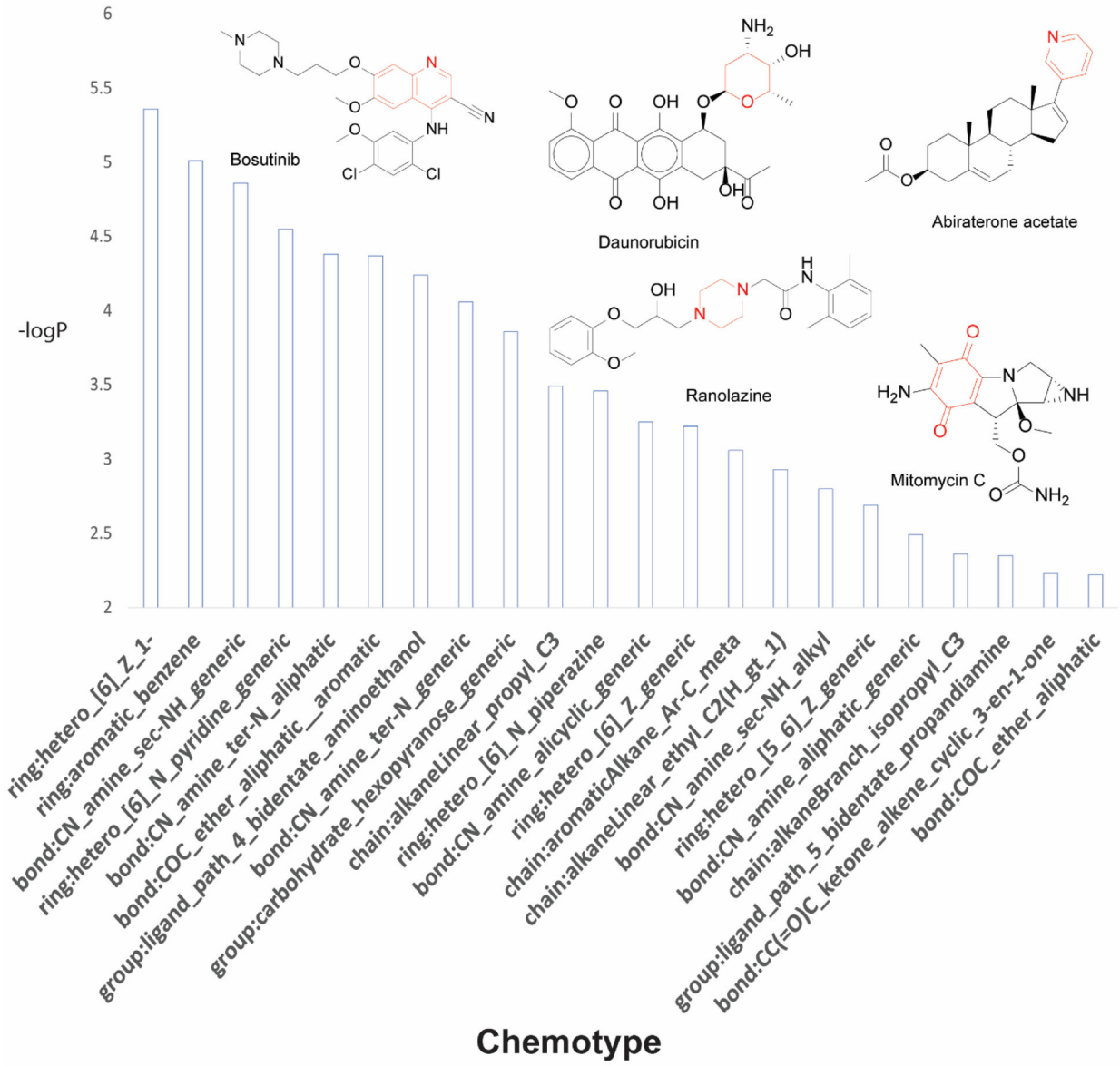

Figure 2.

Chemotypes significantly enriched in compounds active for DICT. Chemotypes are sorted by significance based on p-values. Representative drugs that contain these structural fragments are displayed with significant chemotypes highlighted in red.

Table 1.

Performance of four classification models (RF, NB, XGBoost, and SVM) for each toxicity endpoint (DILI and DICT) with feature selection.

In vivo Endpoints	Descriptor	RF	NB	XGBoost	SVM
DILI	ToxPrint	0.68±0.03	0.66±0.03	0.67±0.03	0.65±0.03
	Assay	0.59±0.02	0.61±0.04	0.59±0.03	0.59±0.03
	ToxPrint + Assay	0.67±0.02	0.63±0.03	0.66±0.03	0.65±0.02
	ECFP4	0.75±0.03	0.74±0.02	0.71±0.03	0.72±0.03
	ECFP4 + Assay	0.72±0.03	0.72±0.02	0.68±0.03	0.70±0.03
DICT	ToxPrint	0.72±0.03	0.75±0.02	0.70±0.04	0.71±0.04
	Assay	0.58±0.04	0.58±0.04	0.57±0.04	0.56±0.04
	ToxPrint + Assay	0.69±0.04	0.66±0.04	0.67±0.04	0.68±0.04
	ECFP4	0.79±0.04	0.83±0.03	0.73±0.04	0.78±0.03
	ECFP4 + Assay	0.73±0.03	0.71±0.06	0.71±0.03	0.74±0.04

Table 2.

Top 20 Tox21 assays that are predictive of DILI.

#	DILI Assay	Target	AUC
1	tox21-er-bla-antagonist-p1_ch1	ER-BLA antagonist viability	0.57
2	tox21-pxr-p1_ratio	PXR agonist	0.57
3	tox21-err-p1_agonist	ERR	0.56
4	tox21-mitotox-p1_rhodamine	Mitochondria toxicity	0.56
5	tox21-err-p1_ratio	ERR	0.56
6	tox21-are-bla-p1_ratio	ARE	0.55
7	tox21-casp3-cho-p1_viability	Caspase-3/7 cytotoxicity counter screen	0.55
8	tox21-mitotox-p1_ratio	Mitochondria toxicity	0.55
9	tox21-pr-bla-antagonist-p1_ch2	PR-BLA antagonist	0.55
10	tox21-are-bla-p1_ch2	ARE	0.55
11	tox21-p450-2c9-p1_ratio	CYP2C9	0.55
12	tox21-rxr-bla-agonist-p1_ch1	RXR-BLA control viability	0.55
13	tox21-pgc-err-p1_ratio	PGC-ERR	0.54
14	tox21-cre-antagonist-p1_ch2	CRE antagonist	0.54
15	tox21-ar-bla-antagonist-p1_ratio	AR-BLA antagonist	0.54
16	tox21-car-antagonist-p1_ratio	CAR antagonist	0.54
17	tox21-pparg-bla-agonist-p1_ratio	PPARγ agonist	0.54
18	tox21-ahr-p1_ratio	AhR	0.54
19	tox21-cre-agonist-p1_ch1	CRE control viability	0.54
20	tox21-cre-antagonist-p1_ratio	CRE antagonist	0.54

Table 3.

Top 20 Tox21 assays that are predictive of DICT.

#	DICT Assay	Target	AUC
1	tox21-pparg-bla-agonist-p1_ratio	PPARγ agonist	0.60
2	tox21-casp3-cho-p1_viability	Caspase-3/7 cytotoxicity counter screen	0.60
3	tox21-ahr-p1_viability	AhR cytotoxicity counter screen	0.59
4	tox21-erb-bla-antagonist-p1_ratio	ER-β antagonist	0.59
5	tox21-casp3-hepg2-p1_viability	Caspase-3/7 cytotoxicity counter screen	0.59
6	tox21-err-p1_viability	ERR cytotoxicity counter screen	0.58
7	tox21-elg1-luc-agonist-p1_viability	ATAD5 cytotoxicity counter screen	0.58
8	tox21-pparg-bla-antagonist-p1_ratio	PPARγ antagonist	0.58
9	tox21-gh3-tre-antagonist-p1_viability	TR-β antagonist cytotoxicity counter screen	0.58
10	tox21-dt40-p1_100	Cell viability	0.58
11	tox21-pxr-p1_viability	PXR agonist cytotoxicity counter screen	0.58
12	tox21-shh-3t3-gli3-agonist-p1_viability	Hedgehog agonist cytotoxicity counter screen	0.58
13	tox21-shh-3t3-gli3-antagonist-p1_viability	Hedgehog antagonist cytotoxicity counter screen	0.57
14	tox21-aromatase-p1_viability	Aromatase cytotoxicity counter screen	0.57
15	tox21-mitotox-p1_rhodamine	Mitochondria toxicity	0.57
16	tox21-pr-bla-antagonist-p1_ratio	PR-BLA antagonist	0.57
17	tox21-vdr-bla-antagonist-p1_ratio	VDR-BLA antagonist	0.57
18	tox21-ppard-bla-agonist-p1_viability	PPAR-δ-BLA agonist cytotoxicity counter screen	0.57
19	tox21-pgc-err-p1_viability	PGC-ERR cytotoxicity counter screen	0.57
20	tox21-pparg-bla-antagonist-p1_ch1	PPAR-δ-BLA antagonist control viability	0.57

Table 4.

Distribution of model-predicted DILI and DICT compounds in the Tox21 10K library with and without the application of the applicability domain (AD) using Random Forest (RF), Naïve Bayes (NB), eXtreme Gradient Boosting (XGBoost), Support Vector Machines (SVM), and a consensus of all four methods.

		RF	NB	XGBoost	SVM	Consensus
DILI	**without AD**	2,807 (35%)	3,745 (47%)	2,384 (30%)	2,065 (26%)	1,374 (17%)
	with AD *	1,536 (19%)	1,838 (23%)	1,389 (17%)	1,268 (16%)	845 (11%)
DICT	**without AD**	4,420 (55%)	3,075 (38%)	2,532 (32%)	2,163 (27%)	1,469 (18%)
	with AD *	2,357 (29%)	1,698 (21%)	1,502 (19%)	1,357 (17%)	953 (12%)

*Tanimoto coefficient ≥ 0.4