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# Drug-induced liver injury severity and toxicity (DILIst): binary classification of 1279 drugs by human hepatotoxicity



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**Drug-induced liver injury (DILI) is of significant concern to drug development and regulatory review because of the limited success with existing preclinical models. For developing alternative methods, a large drug list is needed with known DILI severity and toxicity. We augmented the DILIrank data set [annotated using US Food and Drug Administration (FDA) drug labeling)] with four literature datasets ($N > 350$ drugs) to generate the largest drug list with DILI classification, called DILIst (DILI severity and toxicity). DILIst comprises 1279 drugs, of which 768 were DILI positives (increase of 65% from DILIrank), whereas 511 were DILI negatives (increase of 65%). The investigation of DILI positive–negative distribution across various therapeutic categories revealed the most and least frequent DILI categories. Thus, we consider DILIst to be an invaluable resource for the community to improve DILI research.**

## Introduction

The current paradigm of drug development faces major challenges in cost, time, and failure. On average, it takes ~10 years at a cost of ~US$2.6 billion to bring a drug to market; recent years have seen a decline in the Phase I to launch rate from 8.3% to 4.7% [1–3]. Many failures during early clinical trials are attributed to safety concerns [3,4]. Specifically, DILI has been identified as a significant cause of drugs being either withdrawn from the market or terminated, particularly during the late stages of development [5–7]. DILI-related safety concerns and the increasing cost of drug development are issues that have persisted for over a decade [8,9].

Several studies have demonstrated a low concordance between animal models used for testing hepatotoxicity and human outcomes; this might be one reason for the prevalence of hepatic injury noted during late-stage drug development [10]. To add more complexity to addressing DILI-related safety concerns, it is well established that alternative diagnosis are frequent in DILI studies and result in the confounding results. These observations have driven significant efforts to evaluate alternative methods to identify DILI at an earlier stage by emerging methodologies, such as high-throughput screening, high-content assays, and toxicogenomics, along with advanced computational modeling techniques [11]. This move towards animal-free and high-throughput methodologies marks a paradigm shift for 21st-century risk assessment [12,13]. Some of these approaches have already been evaluated for regulatory decision-making [14]. For example, the European Union (EU) has made significant progress in REACH/3Rs [15], whereas Tox21 [16] and ToxCast [17], both initiated by US Government agencies, have been underway for several years. These initiatives have focused specific attention on efforts to evaluate high-throughput approaches, especially those based on in vitro data [18–23], *in silico* approaches [24–27], and toxicogenomics for risk assessment [7,23,28]. These methodologies often use various statistical approaches to develop predictive models, for which a large database of compounds with known toxic outcome is essential.

Overall, the probability of success of high-throughput alternative methods in DILI research would be enhanced by an extensive drug list, systematically classified for DILI potential in humans. Thus, we developed such an approach to classify DILI potential of

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drugs using FDA-approved drug labeling documents [29]. We first reported a benchmark dataset of 287 drugs [29] and then expanded the list to generate a larger DILI drug list (DILIrank) [30], which comprised 775 drugs classified into three categories of DILI concern (most-, less-, and no-DILI concern), plus 254 drugs for which the DILI potential was undetermined (lack of DILI-related causal evidence). Both data sets have been extensively used to develop in silico predictive models [24,31], in vitro biomarkers [32,33], and in other applications [34,35]. Of note, the current study is based on a retrospective analysis.

DILIrank utilized only FDA drug-labeling information, potentially missing three rich sources of data. First, drugs approved by other countries, such as those in Europe and Japan, might not be included in the FDA labeling. Second, many drugs withdrawn from the world market are not necessarily available in the current labeling system, particularly if they were removed from the market a long time ago. Finally, the labeling documents might not provide sufficient information to identify some drugs as hepatotoxic. For example, there are 254 drugs in DILIrank that were classified as 'ambiguous'. However, some of these drugs have been studied extensively and reported in the literature. For example, four large DILI data sets ($N >= 350$) were described based on different approaches, including a clinical evidence-based approach (LiverTox data set) [36,37], a literature-based approach (Greene data set) [38], a case registry-based approach (Suzuki data set) [39], and an approach based on curating data from the FDA Adverse Event Reporting System (FAERS) (Zhu data set) [40]. These large literature data sets offer an opportunity to generate a larger DILI reference list that goes beyond DILIrank. To capitalize on this opportunity, we developed an integrative approach to merge the four literature data sets (LiverTox, Greene, Suzuki, and Zhu) into DILIrank to generate DILIst, a data set with nearly twice the number of drugs and a broader range of therapeutic categories. This augmented data set is the largest database of drugs ($N = 1279$) with positive or negative DILI classification currently available.

## Development of DILIst via incremental augmentation

We augmented DILIrank with drugs from other large data sets where human DILI data were readily available. To identify candidate data sets suitable for augmentation, a literature search was performed to select only those with >350 drugs with a human DILI classification. Four data sets were selected: LiverTox [36,37], Suzuki [39], Greene [38], and Zhu data sets [40] (Fig. 1). We calculated the percentage overlap of these four data sets with DILIrank (LiverTox 64%; Suzuki 65%; Greene 41%; and Zhu 36%) and started the augmentation with the highest overlapping data set that had both DILI positives and DILI negatives (LiverTox). The augmentation process was incremental; one data set was considered at a time for the concordance analysis. Extreme caution was used when including the LiverTox, Suzuki, Greene, and Zhu data into the new classification scheme, as specified below.

Given the fact that each data set used a different source of information for DILI classification, we conducted a concordance analysis between each of the four literature data sets and DILIrank for both DILI positive and negative categories, respectively. If the

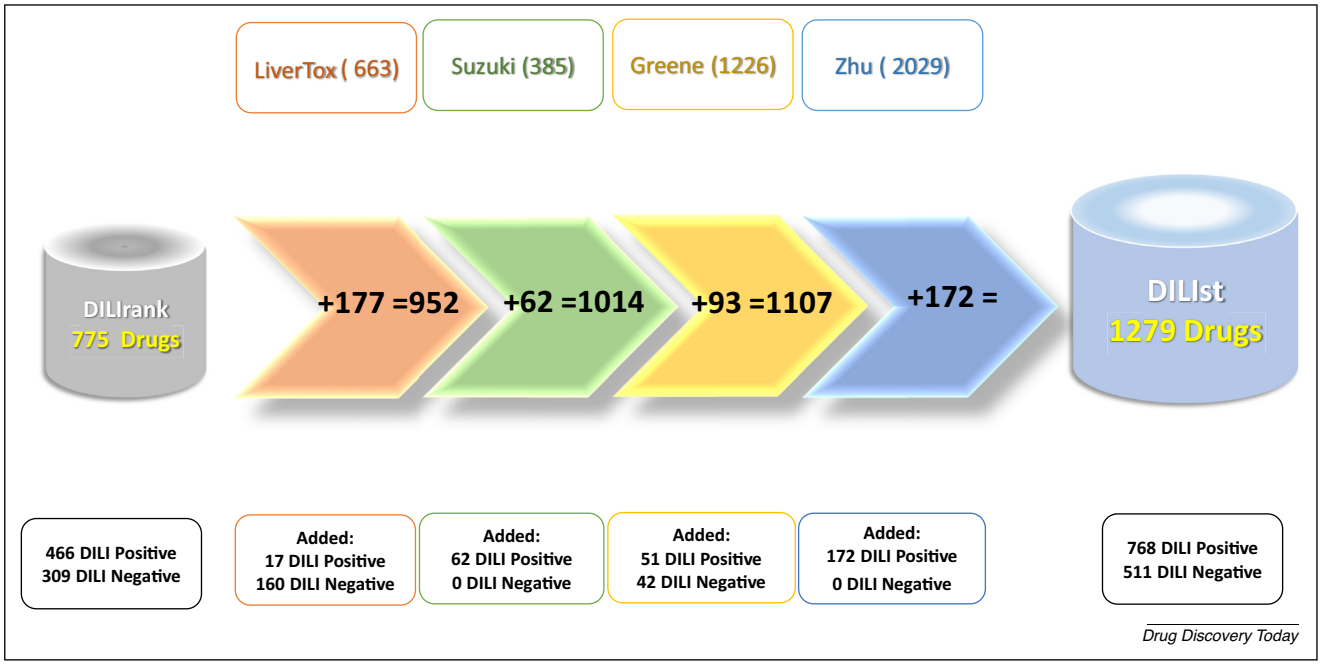


**FIGURE 1**

Generation of Drug-Induced Liver Injury Severity and Toxicity (DILIst): four literature data sets (>350 drugs) were selected to augment DILIrank to generate DILIst. First, the overlap between DILIrank and each of the four data sets was assessed to determine the sequence of augmentation. Then, for each appended data set, the concordance in each respective DILI severity category (e.g., DILI positive or negative) was calculated (see Figure S1 in the supplemental information online). Where there was >75% concordance for a severity category, the additional drugs from that data source in that category were added to become a part of DILIst. For subsequent augmentation, the updated list was used for concordance assessment. Most new DILI-negative drugs were from LiverTox, whereas the Suzuki and Zhu data sets contributed only DILI-positive drugs. The detail information is available from Table S1.

concordance was >75% for a specific category (i.e., DILI positive or negative), the drugs from the literature data set that were not present in DILIrank were incorporated. This concordance analysis for augmenting the drugs from the literature data set into DILIst improved the overall accuracy. Of note, DILIrank categorized drugs into three categories (No-, Less-, and Most-DILI-concerns) [1]. For generating DILIst, the drugs categorized as Less- and Most-DILI-concerns were considered DILI positives and drugs categorized as No-DILI-concern was considered as DILI negative. For the description of the augmentation process and statistics, please see Supplementary Material in the supplemental information online. The complete drugs list generated for DILIst including classification information is provided in Table S2 in the supplemental information on online.

## DILI landscape in the context of therapeutic and chemical classes

The creation of the largest list of drugs with a well-defined DILI classification offers an opportunity to consider DILI profiles across various therapeutic and chemical classes. Maintained by the WHO, the Anatomical, Therapeutic, Chemical (ATC) Classification System is an internationally accepted classification system for drugs [41] and was used for this purpose. In the ATC Classification System, drugs are divided into five levels [42]: the highest level is the main anatomical group, the second level is the therapeutic subgroup, the third level is the pharmacological subgroup, the fourth specifies chemical subgroup, and the fifth level denotes chemical substance [43,44]. We examined trends and patterns in the DILI landscape at multiple levels of ATC classification, with a focus on anatomical, pharmacological, and chemical features. By comparing DILIst to DILIrank, an enrichment analysis was conducted to identify the 'enhanced' categories (where the number of drugs was significantly increased from DILIrank to DILIst) at the various levels of ATC classification. Enrichment analysis focused not only on the increase in the number in a category at all levels of ATC, but also on the identification of categories where the DILI positive/negative ratio was altered.

Upon analyzing the anatomical group (the top level of ATC), as represented in Fig. 2a, both DILIrank and DILIst covered all 14 anatomical categories with no new categories introduced into

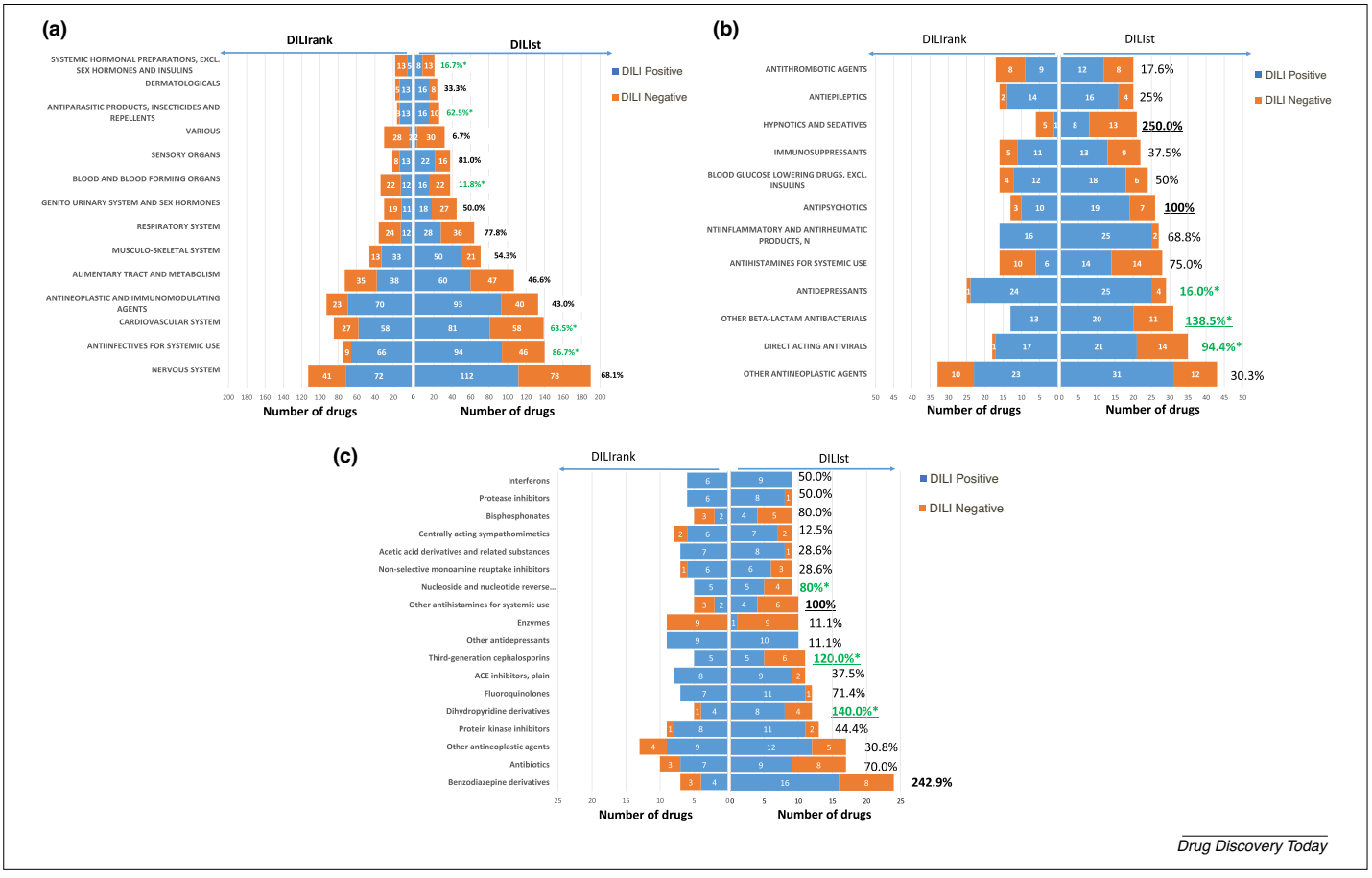


**FIGURE 2**

Anatomical, Therapeutic, Chemical (ATC) distribution and comparison in Drug-Induced Liver Injury (DILI)rank and DILI Severity and Toxicity (DILIst). A comparison of DILI-negative drugs (orange bars) and DILI-positive drugs (blue bars) in DILIst versus DILIrank depicted by three levels of ATC hierarchy [**(a)** anatomical; **(b)** pharmacological; and **(c)** chemical], showing the number of positive or negative drugs. Although all the ATC anatomical categories are presented in both DILI lists **(a)**, only the most frequent subgroups are depicted in **(b)** (pharmacological subgroups with ≥20 drugs) and **(c)** (chemical subgroup with ≥9 drugs). The percentages depict the increase in the number of drugs in DILIst versus DILIrank. Categories and/or subgroups enriched in actual numbers of drugs by ≥100% are underlined. * depicts categories and/or subgroups where there was a statistically significantly different positive/negative ratio ($P < 0.05$) in DILIst compared with DILIrank (also highlighted in green text) calculated using Fisher's exact test for each category.

DILIst (Table 1, column 4). Overall the ATC anatomical categories that were abundant in DILIrank remained abundant in DILIst, but with an increased number of drugs, suggesting concordance between the two lists in the context of overall coverage across various organ systems. Drugs related to the nervous system, anti-infective for systemic use, cardiovascular system, antineoplastic and immunomodulating agents, and alimentary tract and metabolism were predominant in DILIst. Notably, these anatomical categories contained drugs well known for their association with hepatotoxicity, as noted in multiple publications [45,50]. The nervous system anatomical subgroup contributed the highest number of drugs to DILIst (190; 14.5% of the total drugs) overall and the highest number of DILI-positive drugs in the group (112) (Fig. 2a and Table 1). One notable change was that, if we considered only the DILI-positive drugs, antineoplastic and immunomodulating agents (93) were elevated in the rankings (Table 1). Overall, the drugs present in the top five ATC anatomical categories contributed ~55% of the drugs in DILIst. The number of drugs in all categories was increased in DILIst compared with DILIrank (Fig. 2a). Five of the high-frequency anatomical subgroups had a statistically different ratio of DILI-positive to DILI-negative drugs in DILIst compared with DILIrank (Fig. 2a; Table 1, Column 5); in two cases, this was because of the addition of only DILI-positive drugs to DILIst and in three cases this was because of the addition of both DILI-positive and negative drugs but in differing proportions.

Upon analyzing the pharmacological subgroups represented in DILIst (the first subgroup in ATC), there was a total of 172, an addition of 16 new subgroups (Table 1, Column 4). The top five most-frequent pharmacological subgroups collectively contributed a total of 10% of the drugs to DILIst; these top five drugs have already been associated with DILI causality [45–50]. In comparing DILIrank with DILIst, there were three subgroups where there was a ≥100% increase in the number of drugs in the category (hypnotics and sedatives; beta-lactam antibacterials; and antipsychotics) (Fig. 2b). Three of the high-frequency pharmacological subgroups (direct acting antivirals, other beta lactam antibacterials, and

**TABLE 1**

**DILIst in the context of the ATC Classification System[a]**

| ATC category | DILIst | | | DILIst versus DILIrank |
|---|---|---|---|---|
| | Top 5 categories | Top 5 DILI-positive categories | Top 5 DILI-negative categories | Statistically significant categories ($P < 0.05$) |
| Anatomical (total DILIst: 14 categories; newly added: 0 categories) | Nervous system (190) | Nervous system (112/190) | Nervous system (78/190) | Anti-infectives for systemic use |
| | Anti-infectives for systemic use (140) | Anti-infectives for systemic use (94/140) | Cardiovascular system (58/139) | Cardiovascular system |
| | Cardiovascular system (139) | Antineoplastic and immunomodulating agents (93/133) | Alimentary tract and metabolism (47/107) | Blood and blood-forming organs |
| | Antineoplastic and immunomodulating agents (133) | Cardiovascular system (81/139) | Anti-infectives for systemic use (46/140) | Antiparasitic products, insecticides, and repellents |
| | Alimentary tract and metabolism (107) | Alimentary tract and metabolism (60/107) | Antineoplastic and immunomodulating agents (40/133) | Systemic hormonal preparations |
| | Other antineoplastic agents (43) | Other antineoplastic agents (31/43) | Direct-acting antivirals (15/35) | Direct-acting antivirals |
| Pharmacological (total DILIst: 172 categories; newly added: 16 categories) | Direct-acting antivirals (35) | Antidepressants (25/29) | Antihistamines for systemic use (14/28) | Other beta-lactam antibacterials |
| | Other beta-lactam antibacterials (31) | Antiinflammatory and antirheumatic products (25/28) | Hypnotics and sedatives (13/21) | Antidepressants |
| | Antidepressants (29) | Direct-acting antivirals (21/35) | Other antineoplastic agents (12/43) | Lipid-modifying agents, plain |
| | Antihistamines for systemic use (28) | Other beta-lactam antibacterials (20/31) | Other beta-lactam antibacterials (11/31) | Drugs for treatment of TB |
| | Benzodiazepine derivatives (24) | Benzodiazepine derivatives (16/24) | Enzymes (9/10) | Third-generation cephalosporins |
| Chemical (total DILIst: 381 categories; newly added: 66 categories) | Antibiotics (17) | Other antineoplastic agents (12/17) | Benzodiazepine derivatives (8/24) | Nucleoside and nucleotide reverse transcriptase inhibitors |
| | Other antineoplastic agents (17) | Protein kinase inhibitors (11/17) | Antibiotics (8/17) | Angiotensin II antagonists, plain |
| | Protein kinase inhibitors (13) | Fluoroquinolones (11/12) | Drugs for urinary frequency and incontinence (7/7) | |
| | Dihydropyridine derivatives (12) | Other antidepressants (10/10) | Selective serotonin (5HT1) agonists (7/7) | |

[a] The DILIst drugs were organized by ATC hierarchical categories (anatomical, pharmacological, and chemical; column 1) with information about the total number of categories and/or subgroups and the newly added subgroups in DILIst versus DILIrank. Colum 2 highlights the top 5 subgroups containing the most drugs (column 2), along with the top 5 DILI-positive subgroups (column 3) and the top 5 DILI-negative subgroups (column 4).

antidepressants) had a statistically different ratio of DILI-positive to DILI-negative drugs in DILIst compared with DILIrank (Fig. 2b; Table 1, Column 5) driven by the addition of 14, 11, and three new DILI-negative drugs to the three categories, respectively. This is valuable given that there were few DILI-negative drugs in these categories in DILIrank. This change gives us a revised perspective on these pharmacological categories. Two further categories (lipid-modifying enzymes and drugs for TB) also had a statistically different ratio of DILI-positive to DILI-negative drugs, but are not shown in Fig. 2b because they were below the cut-off (>20 drugs) for inclusion.

Upon analyzing the chemical subgroups (the fourth subgroup in ATC), there were 66 additional subgroups in DILIst compared with DILIrank (Table 1, Columns 1–4). The most frequently occurring chemical subgroups in DILIst were the benzodiazepine derivatives, antibiotics, other antineoplastic agents, protein kinase inhibitors, and dihydropyridine derivatives (Table 1, Column 2). There were four chemical categories (benzodiazepine derivatives, dihydropyridine derivatives, third-generation cephalosporins, and other antihistamines for systemic use) where there was a ≥100% increase in the number of drugs in the category (Fig. 2c). These observations are consistent with the prior DILI causality knowledge from multiple sources [51,52]. Three of the high-frequency chemical subgroups (third-generation cephalosporins, nucleoside and nucleotide reverse transcriptase inhibitors, and dihydropyridine derivatives) had a statistically different ratio of DILI-positive to DILI-negative drugs in DILIst compared with DILIrank. There were several notable changes between the drug profiles in DILIrank and DILIst. Six DILI-negative third-generation cephalosporins were added against a background of zero in DILIrank. Also, there were one or two DILI-negative drugs added to three chemical categories (protease inhibitors, ACE inhibitors, and fluoroquinolones) where there were no DILI-negative drugs listed previously in DILIrank. The antibiotics chemical category contained primarily DILI-positive drugs in DILIrank, but this profile was altered in DILIst. The additional perspective offered by DILIst provides an opportunity for future DILI predictive model building.

## DILI-related hotspot identification

DILIst offers great potential for data-driven safety predictions, which are invaluable in developing new approaches based on high-throughput technologies and *in silico* methodologies. In support of this, we analyzed the distribution of DILI-positive drugs among the anatomical categories to determine hot spots of risk within pharmacological (Fig. 3a) and chemical (Fig. 3b) subgroups. 'Hot-spot' analysis aims to understand and identify the over-represented subgroups of ATC pharmacological and chemical subgroups over ATC anatomical categories. Hot spots for DILI risk (75–100% DILI positive) in drugs targeting the nervous system included antidepressants (25/29; 86% DILI positive) and antiepileptics (16/20; 80% DILI positive) (Fig. 3a). Antidepressants drugs are one of the most commonly prescribed drugs and are well known for causing liver injury, contributing to 2–5% of clinical DILI-related cases. Other hot spots for DILI risk (75–100% DILI

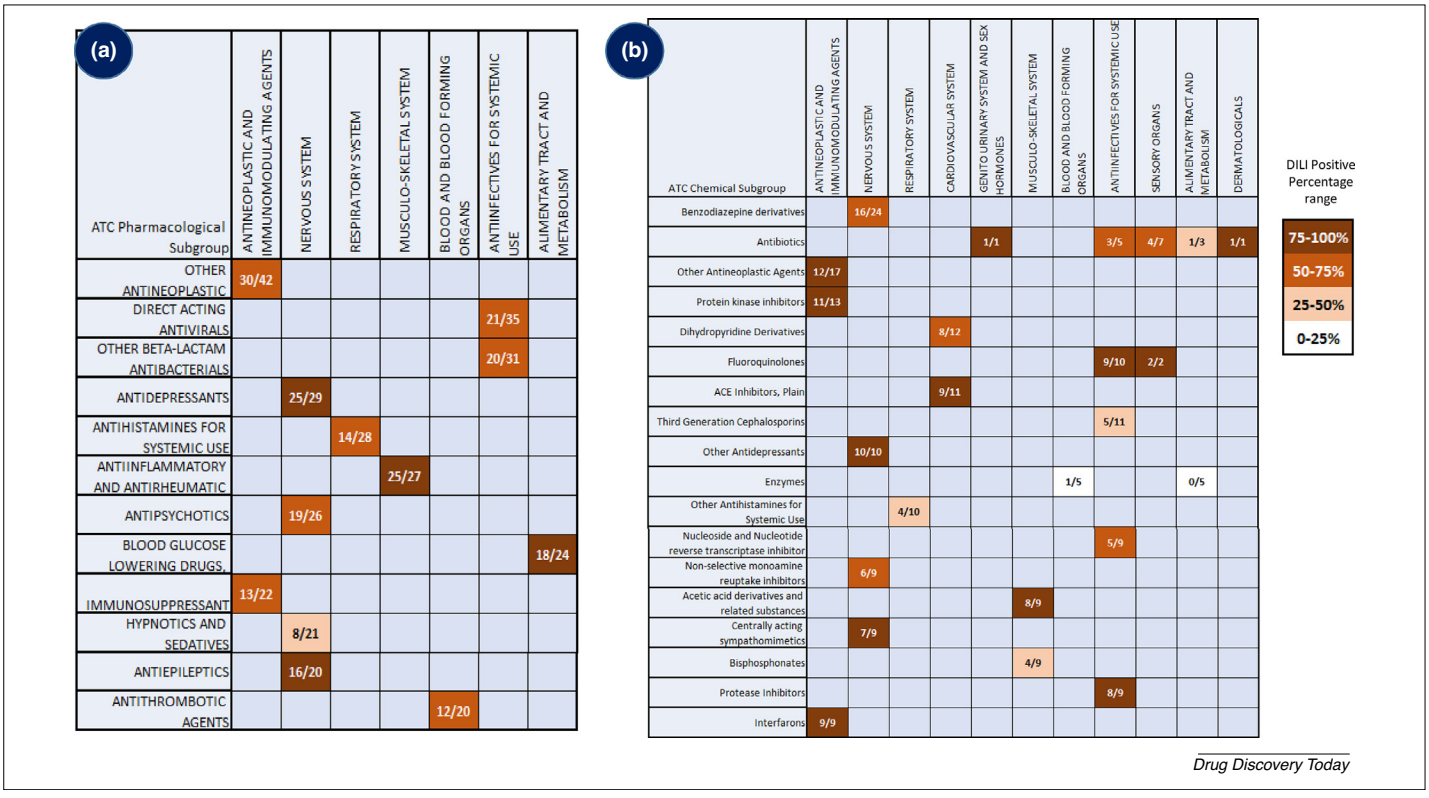


**FIGURE 3**

'Hot-spot' analysis of over-represented subgroups of Anatomical, Therapeutic, Chemical (ATC) pharmacological **(a)** and chemical **(b)** subgroups by ATC anatomical categories. The number in each cell is the total number of DILI-positive drugs (nominator) over the total number of drugs (denominator) in that 'intersection' and its color indicates the percentage of DILI-positive drugs in that 'intersection'.

positive) were in anti-inflammatory and antirheumatic drugs targeting the musculoskeletal system, where 25/27 (>90%) were DILI positive, and in blood glucose-lowering drugs, where 18/24 (75%) were DILI positive (Fig. 3a).

Looking at the chemical subgroups (Fig. 3b), there were several notable findings. Within the antineoplastic agents, kinase inhibitors and the interferons were in the high category for DILI, with all nine interferons being DILI positive. In the cardiovascular system, ACE inhibitors showed a high risk of DILI (9/11; 81%). The antidepressant chemical class 'monoamine oxidase inhibitors' was 66% DILI positive (6/9), whereas 'other antidepressants' exhibited a 100% incidence of DILI positivity (10/10). Also notable in the analysis of DILI-positive drugs among the chemical (Fig. 3b) subgroups was that enzymes carried a very low DILI risk.

In analyzing the data in DILIst for patterns that could be useful in understanding DILI prediction, we noted five pharmacological groups (antidepressants, antiepileptics, anti-inflammatory and antirheumatic products, and blood glucose-lowering drugs) that were >75% DILI positive (Fig. 3a,4). The pharmacological subgroups antidepressants and antiepileptics had multiple chemical subgroups with high numbers of DILI-positive drugs. As highlighted earlier, this could reflect the observation that the nervous system anatomical subgroup contributed the highest number of DILI drugs to DILIst (190; 14.5% of the total drugs) overall and the highest number of DILI positive drugs (Fig. 2a and Table 1). The other two anatomical groups in Fig. 4 (musculoskeletal and alimentary tract metabolism) are present because of only one pharmacological subgroup each; anti-inflammatory and antirheumatic products in the case of the musculoskeletal system, and blood glucose-lowering drugs in the case of alimentary tract metabolism. Many of the chemical subgroups in these two anatomical groups were 100% DILI positive.

## Discussion

In this study, we augmented DILIrank by incorporating DILI information from multiple data sources to give a comprehensive DILI database containing 1279 drugs with 381 chemical subgroups. This offers the opportunity to analyze the largest number of drugs classified by their human hepatotoxicity profile. Thus, DILIst represents a valuable resource to support the current paradigm shift towards alternative predictive toxicological methods based on high-throughput technologies by providing a large

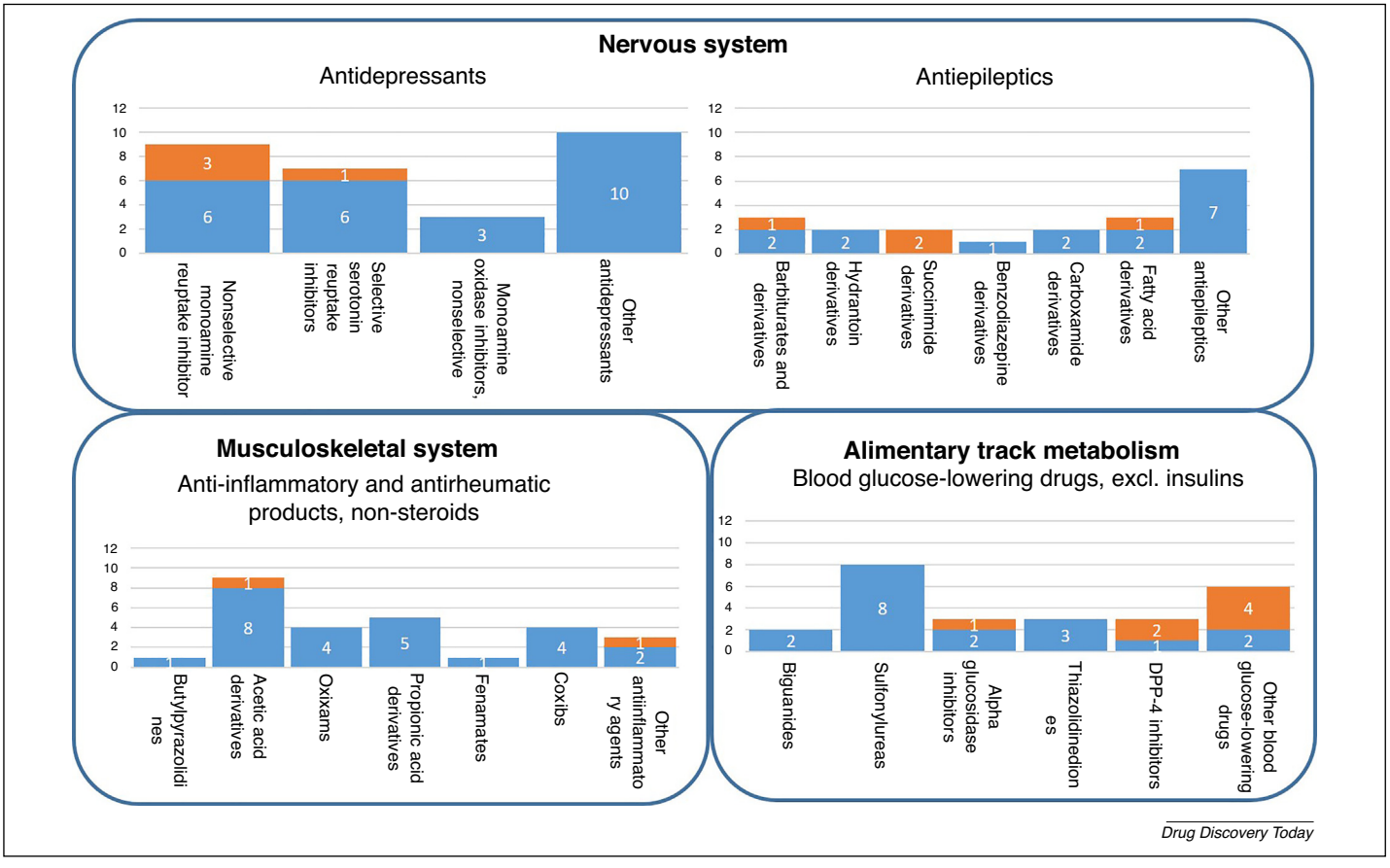


**FIGURE 4**

Analysis of the drug-induced liver injury (DILI) potential of different drug classes. The most DILI-enriched pharmacological subgroups (>75% DILI-positive percentage in Figure 3a in the main text) were selected as an upper threshold to assess the possibility of developing certain drug classes with less DILI concern in the most DILI concern pharmacological categories. Four pharmacological subgroups (i.e., antidepressants, antiepileptics, anti-inflammatory and antirheumatic products non-steroids, and alimentary track metabolism) met the criteria, which are from three anatomical categories (i.e., nervous system, musculoskeletal system, and alimentary track metabolism). The histograms depict the frequency of DILI-positive (blue) and DILI-negative drugs (orange) for each chemical subgroup. The actual number of drugs in that category is shown in white.

reference drug list that is well classified for human DILI information and compound information.

DILI is a multifactorial endpoint with multiple schemes and approaches for its classification. The assignment of DILI risk to drugs can be challenging especially when DILI occurs with a low incidence but with a wide range of severity and with multiple injury patterns. In addition, the causality of liver injury can be hard to ascertain for various reasons, such as co-medication, alcohol consumption, pre-existing liver disease, or other confounding factors. DILIst addresses this by creating a single database that incorporates different data sources, such as the number of case reports [36,37,39], the FDA approved-drug labeling [29,30], FAERS [40], drug registry [39], and literature evidence [38], to provide consolidated and comprehensive DILI information.

Using DILIst, we were able to identify DILI-related patterns in chemical subgroups that were not apparent in DILIrank. For example, the fluoroquinolones are primarily DILI positive (>75%) and span two anatomical subgroups (anti-infective for systemic use and sensory organs). Other prominent DILI positive subgroups (>75% DILI positive) are ACE inhibitors, protease inhibitors, and other antidepressants. It is challenging not only to determine DILI-positive drugs, but also to be definitive about drugs being DILI negative, especially because DILI is idiosyncratic. Through DILIst, we were able to identify many additional DILI-negative drugs in all the categories, with which, we were able to identify DILI-negative spots. For example, the enzyme chemical subgroups were comparatively safer, with a <25% DILI-positive rate. Another chemical subgroup where DILI-negative drugs predominate was the bisphosphonates.

It is important to point out the potential limitations in this study. First, we adopted a binary classification (positive versus negative), which is useful for predictive modeling and assessing high-throughput technologies in drug development, but is less realistic for clinical practice because we are all aware that a 'black-or-white' DILI risk is oversimplistic. Second, the augmentation generated an opportunity to work with a large data set, which is crucial in the era of big data and artificial intelligence, but also encounters a challenge of reducing the noise from diverse data sets because they have different classification schema. To mitigate the risk of bringing noise, we implemented a statistical approach in the augmentation process. Specifically, we started with DILIrank data and applied a statistical criterion to augment DILIst with drugs from other sources. This statistical criterion ensured that the incorporated data were consistent with DILIrank including causality. In other words, we did not blindly merge all the drugs from all the literature data sets to DILIrank. We only took those meeting the statistical criteria for inclusion. For example, exclusion/inclusion criteria for LiverTox is illustrated in Figure S1a in the supplemental information online. Third, using literature data sets, particularly for some of these data sets that were published some years back, they could introduce both false positives and false negatives, and the data collected at the time of publication might not now be up to date. Thus, DILIst is not meant to be the final classification and it will be updated periodically. In fact, the DILI classification of some of the drugs was updated in DILIst based on new evidence.

## Concluding remarks

DILIst is relevant to many interested parties, including regulators, researchers, and others involved in drug discovery and development. DILIst will be an invaluable resource for the community to improve DILI research in the areas of elucidation of mechanisms, predictive model development, and biomarker identification, and provides additional opportunities to exploit the potential of emerging technologies. This enriched information has the potential to support the next generation of toxicological developments from drug development and regulatory decision making to epidemiologically associated applications. Using this comprehensive DILIst, researchers and regulators will be empowered to exploit the new technologies and new data streams to provide new insights.

## Disclaimer

The views presented in this paper do not necessarily reflect current or future opinion or policy of the U.S. Food and Drug Administration. Any mention of commercial products is for clarification and not intended as endorsement.

## Appendix A. Supplementary data

'Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.drudis.2019.09.022.

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