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REVIEW

Getting the most out of PubChem for virtual screening

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ABSTRACT

Introduction: With the emergence of the ‘big data’ era, the biomedical research community has great interest in exploiting publicly available chemical information for drug discovery. PubChem is an example of public databases that provide a large amount of chemical information free of charge.

Areas covered: This article provides an overview of how PubChem’s data, tools, and services can be used for virtual screening and reviews recent publications that discuss important aspects of exploiting PubChem for drug discovery.

Expert opinion: PubChem offers comprehensive chemical information useful for drug discovery. It also provides multiple programmatic access routes, which are essential to build automated virtual screening pipelines that exploit PubChem data. In addition, PubChemRDF allows users to download PubChem data and load them into a local computing facility, facilitating data integration between PubChem and other resources. PubChem resources have been used in many studies for developing bioactivity and toxicity prediction models, discovering polypharmacologic (multi-target) ligands, and identifying new macromolecule targets of compounds (for drug-repurposing or off-target side effect prediction). These studies demonstrate the usefulness of PubChem as a key resource for computer-aided drug discovery and related area.

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1. Introduction

Thanks to high-throughput screening (HTS) [1,2] technology and combinatorial chemistry [2,3], small research laboratories in academic institutions now can generate bioactivity data for a large number of molecules at a low cost. Through data mining and manual curation, many groups can also collect a substantial amount of chemical information from various sources, including scientific articles and patent documents. In addition, funding agencies have introduced data sharing policies for studies that they support, and journal publishers require the authors of papers to make underlying data publicly accessible. All these recent trends have led to a rapid growth in chemical information available in the public domain. With the emergence of the big data era, there is a great interest from the biomedical research community in exploiting this public information for virtual screening (VS) [4,5], which uses computational techniques to explore a large compound library to select a small subset of potentially bioactive molecules that are tested in subsequent *in vitro* and *in vivo* experiments. VS is an essential part of modern drug discovery and has been reviewed in many articles [4,5].

PubChem [6–8] is a public chemical information archive developed and maintained by the U.S. National Institutes of Health (NIH). PubChem collects chemical substance descriptions and their biological activities from hundreds of data sources and provides them to the public free of charge. With receiving millions of requests from tens of thousands users per day, PubChem serves as a key resource for biomedical science

communities in many areas, including cheminformatics, chemical biology, medicinal chemistry, and drug discovery. Detailed information on PubChem is given elsewhere [6,7].

There have been great interest in using PubChem for VS. In some studies [9,10], 3-D structures of compounds downloaded from PubChem were used for molecular docking. In other studies [11,12], PubChem was searched for molecules structurally similar to known active compounds using similarity search [11] or for compounds with a particular scaffold through substructure search [12]. PubChem was also screened using various predictive models to identify compounds with desired bioactivity [13–20]. Importantly, many studies [21–35] used bioactivity data archived in PubChem to develop bioactivity or toxicity prediction models [24–35]. In addition, PubChem data were used to build computational models to predict adverse drug reactions [36,37]. Recently, ‘dark chemical matter’ [38] in PubChem, defined as compounds that have never shown bioactivity after being tested repeatedly in many HTS experiments, has attracted much attention as a promising starting point for discovering lead molecules.

The present paper reviews important aspects of PubChem in the context of its application for VS. A brief overview of PubChem is given, including data organization as well as data contents relevant to VS. Information on chemical vendors and patents for compounds in PubChem is also discussed, which helps prioritize hit compounds for subsequent *in vitro* or *in vivo* screenings. In addition, this paper describes programmatic access to PubChem data, which is critical for building

Article Highlights

- PubChem is the largest source of publicly available chemical information, collected from more than 400 data sources.
- In addition to bioactivity data generated through high-throughput screenings, PubChem contains a substantial amount of bioactivity information extracted from scientific articles.
- Chemical vendor and patent information for compounds in PubChem helps prioritize hit compounds for further screening.
- PubChem supports programmatic access to its data, allowing for building an automated virtual screening pipeline.
- PubChemRDF allows users to download PubChem data on a local computing facility and integrate them with their own data.
- PubChem data can be used for developing computational prediction models for bioactivity or toxicity of molecules.

This box summarizes key points contained in the article.

automated VS pipelines, and PubChemRDF, which facilitates integration of PubChem data with other resources useful for VS. A review of some important publications is given to explain how PubChem resources are used for developing bioactivity and toxicity prediction models, discovering polypharmacologic (multitarget) ligands, and identifying new macromolecule targets of chemicals (for drug repurposing or off-target side-effect prediction). Other relevant topics, such as dealing with active/inactive compound imbalances in PubChem's bioactivity data and developing benchmarking data sets for VS from PubChem data, are also discussed.

2. An overview of PubChem as a resource for VS

2.1. Data organization and chemical space coverage in PubChem

PubChem [6,7] contains chemical substance descriptions and biological activity information, contributed by more than 400 data contributors [39]. While PubChem's data are primarily

about small molecules, they also include other molecular entities, such as small-interfering RNAs and micro-RNAs, peptides, lipids, carbohydrates, chemically modified macromolecules, and many others.

PubChem organizes these data into three primary databases: Substance, Compound, and BioAssay (Figure 1) [6,7]. The Substance database [6,40] archives chemical substance descriptions submitted by individual data contributors. The Compound database [6,41] stores unique chemical structures extracted from the Substance database through the PubChem standardization process. The BioAssay database [7,42] contains the descriptions of biological assay experiments and bioactivity data for substances tested in the assays. The records in the Substance, Compound, and BioAssay databases are called substances, compounds, and assays, respectively. Similarly, SID (Substance ID), CID (Compound ID), and AID (Assay ID) are used as the record identifiers for the Substance, Compound, and BioAssay databases, respectively. As of May 2016, PubChem contains more than 219 million substances, 89 million compounds, and 230 million bioactivity outcomes from more than 1 million assays covering around 10,000 unique protein sequences.

There has been much interest in analyzing the chemical space covered by molecules in PubChem. Especially, many studies [43–47] have compared PubChem's chemical space with those of other public databases of known molecules, such as DrugBank [48] and ChEMBL [49], as well as those of databases of 'virtual' molecules, such as chemical universe databases GDB-11 (26.4 million molecules with up to 11 atoms of C, N, O, and F) [43], GDB-13 (977 million molecules with up to 13 atoms of C, N, O, S, and Cl) [44], and GDB-17 (166.4 billion molecules up to 17 atoms of C, N, O, S, and halogens) [45]. As shown in Figure 2, 68.7 million compound records in PubChem (77% of the total) are drug-like compounds that satisfy Lipinski's rule of 5 [50]. Among them, 10.3 million (12% of the total) are fragment-like ones, which satisfy Congreve's rule of 3 [51].

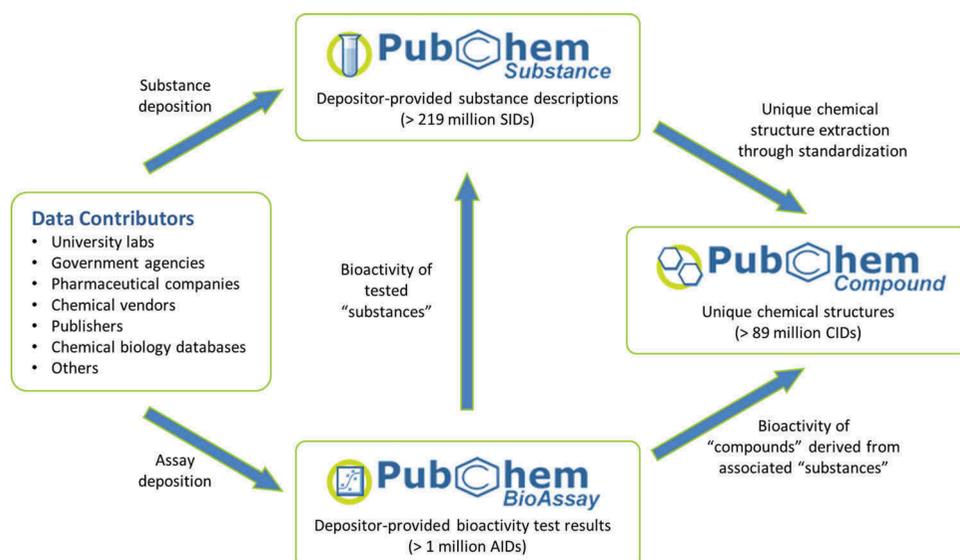


Figure 1. Data organization in PubChem. Chemical information deposited by more than 400 data contributors is organized into three primary databases: substance, compound, and Bioassay.

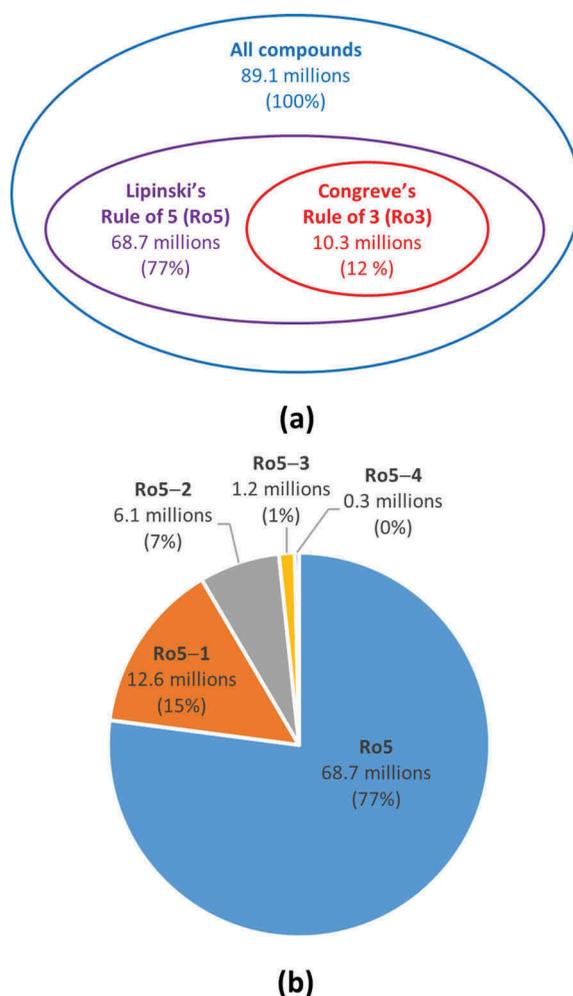


Figure 2. Chemical space covered by PubChem. Panel (a) shows the proportion of compounds that satisfies Lipinski's rule of 5 (Ref [50].) and Congreve's rule of 3 (Ref [51]). Panel (b) shows the proportion of compounds that satisfy all criteria of Lipinski's rule of five (Ro5), and those that violate one, two, three, and four criteria of the rule of five (Ro5-1, Ro5-2, Ro5-3, and Ro5-4), respectively.

2.2. Bioactivity data in PubChem

Figure 3 shows the distribution of PubChem compounds according to the availability of bioactivity data. Currently, 2.1 million compounds in PubChem have been tested in any assay in PubChem, corresponding to 2.4% of all 89.1 million compound records. About half of these tested compounds (1 million compounds, 1.1% of all compounds) have been declared to be active in at least one assay. About 509,000 compounds had an activity concentration between 1 nM and 1 μ M, and 39,000 compounds had an activity concentration of 1 nM or below.

The majority of bioactivity data contained in PubChem were generated from HTS. Because HTS campaigns aim to identify hit molecules from a large compound library, HTS data typically contain a large number of inactive compounds with only a handful of active compounds, which are tested further in low-throughput experiments. Although advanced HTS technologies such as quantitative HTS (qHTS) [52] allow for getting bioactivity data at multiple compound concentrations in a single experiment, HTS is often run at a single concentration, and therefore, there is no guarantee that hit molecules from such HTS experiments would perturb the

biological system in a dose-response way. In addition, HTS data may contain false hits, for example, due to aggregators [53], which nonspecifically bind to multiple unrelated proteins, or autofluorescent compounds [54], which can emit light in the absence of artificial fluorescent markers used in fluorescence-based HTS assays. For these reasons, HTS data are considered to have low qualities in general.

However, PubChem also contains a substantial amount of high-quality bioactivity data extracted from scientific articles through manual curation or data mining [55], which complement the HTS data contained in PubChem. These data are contributed by various PubChem depositors, including ChEMBL [49], PDBbind [56], BindingDB [57], and IUPHAR/BPS Guide to Pharmacology [58]. Data from these contributors cover different chemical domains from each other [55]. For example, ChEMBL [49] manually extracts bioactivity data from peer-reviewed papers published in journals in the medicinal chemistry and natural product domains. PDBbind [56] collects experimentally measured binding affinity data for biomolecular complexes in the Protein Data Bank (PDB) [59]. BindingDB [57] provides binding affinities, focusing chiefly on the interactions of protein considered to be

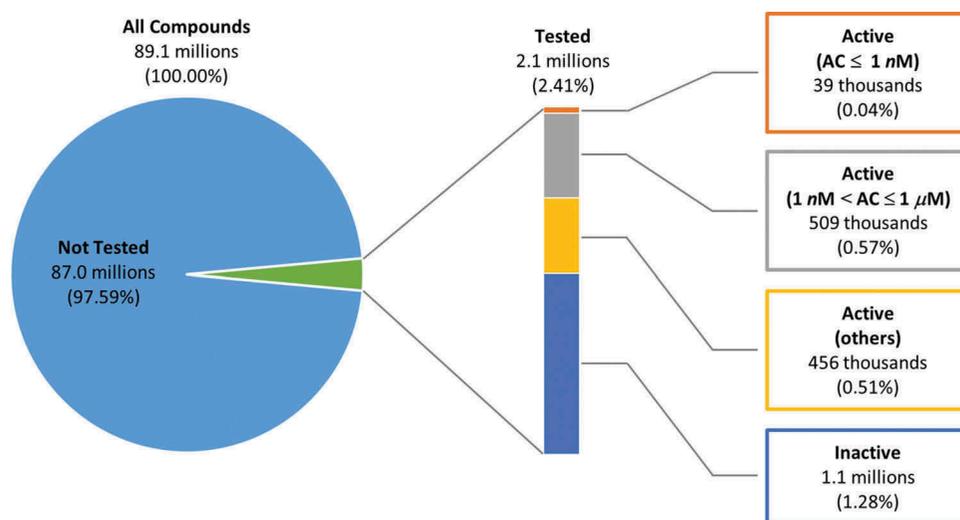


Figure 3. Distribution of tested, active, and inactive compounds in PubChem. Tested compounds are those tested in at least one assay experiment archived in PubChem. Active compounds are those which are declared as active in at least one assay in PubChem. Inactive compounds are those which are not declared as active in any assay in PubChem.

drug targets with drug-like small molecules. The Guide to Pharmacology [58], which collects a wide range of information on important drug targets (e.g. G-protein-coupled receptors [GPCRs], ion channels, and nuclear hormone receptors), provides information on these proteins and their ligands.

2.3. Annotations available in PubChem

In addition to bioactivity data, PubChem contains a great deal of compound information that is useful for VS. For example, thanks to data integration with DrugBank [48], PubChem provides users with comprehensive information on U.S. FDA-approved and investigational drugs, including their drug indications, mechanisms of action, target macromolecules, interactions with proteins and genes, ADMET (absorption, distribution, excretion, metabolism, and toxicity) properties, and many others. PubChem also contains toxicological information on chemicals that are of interest in environmental and human health, contributed by the Hazardous Substances Data Bank (HSDB) [60].

Experimentally determined 3-D structures of small molecules are also available in PubChem. The Molecular Modeling Database [61] contributes to PubChem experimentally determined protein-bound ligand structures, derived from PDB [59]. In addition, PubChem provides links to crystal structures available at the Cambridge Structural Database [62].

PubChem also collects chemical information from important regulatory agencies, such as the FDA and the U.S. Environmental Protection Agency (EPA). For example, information on drug products and ingredients from the FDA Orange Book [63] is integrated in PubChem. PubChem also contains FDA's Unique Ingredient Identifiers [64] and Pharmacologic Classes [65] for drug ingredients. In addition, drug labeling information is available in PubChem, through NLM's DailyMed [66]. EPA Substance Registry Services provides PubChem with information on chemical substances tracked or regulated by EPA. Chemical data collected under the Toxic Substance

Control Act and the Clean Air Act are also available in PubChem.

2.4. Availability of compounds for subsequent experiments

Because the primary goal of VS is to select a list of compounds to test in subsequent experiments, the availability of compounds is an important consideration. That is, they should be either synthesizable or purchasable. It is noteworthy that PubChem does not include 'virtual' molecules. For each compound in PubChem, there are one or more data contributors who claim that they have the compound and/or information about it. Importantly, some of these contributors are chemical vendors from which one can purchase the compound.

Two important characteristics of PubChem records are worth mentioning, with respect to the availability of compounds in PubChem. First, PubChem records may become nonlive, meaning that the records are not searchable, although they do exist in the database. Data contributors to PubChem can revoke their substance information in PubChem for various reasons, for example, when they realize that they mistakenly submitted substances that they do not have, when they find incorrect information about the substance, or when they choose not to share their information with others. As an archive, PubChem does not remove the revoked substance information, but makes it nonlive (i.e. not searchable). When a compound record does not have any live substance records associated, it becomes nonlive. The compound can become live again if a live substance record associated with it appears in PubChem.

The second issue concerning the compound availability is that some information in PubChem is no longer maintained by data contributors (e.g. because they do not have continued funding). Especially, some chemical vendors are out of business, and compounds that were purchasable from them in the past are not available any more. To address this issue, PubChem introduced a 'legacy' designation for collections that are not regularly updated. This legacy designation applies to projects/contributors that

appear to no longer be active, as well as to their individual substance records. Legacy records will not be shown in the 'Chemical Vendors' section of the Compound Summary page. Instead, they will only be found under 'Legacy Depositors' in the 'Substances by Category' section of the Compound Summary page. This designation will help PubChem users quickly identify records that may have out-of-date information and/or hyperlinks.

2.5. Patentability of compounds for intellectual property protection

In drug discovery programs, it is critical to make sure that identified drug candidates are patentable. PubChem currently offers links between about 6 million patent documents and more than 16 million unique chemical structures, with over 336 million chemical substance-patent links covering U.S., European, and World Intellectual Property Organization (WIPO) patent documents published since 1800. This information is contributed by various organizations, including IBM [67], SureChEMBL (formerly known as SureChem) [68,69], NextMove Software [70], SCRIpDB [71], and BindingDB [57].

When a compound has patent information, its Compound Summary page displays the patents associated with it in a tabular format. In addition, the compound record is annotated with WIPO International Patent Classification (IPC) [72] information for the associated patents to. IPC is a hierarchical classification system used to classify patent documents according to the technical fields they pertain to. The IPC information is displayed under the Classification section of the Compound Summary page.

Users can search the Compound database for those associated with a particular patent document or retrieve all compounds that have patent information. Programmatic access to patent information is also possible through PUG-REST [73], which will be discussed later in this paper.

2.6. PubChem 2-D and 3-D neighbors

A disparity of available information exists among compounds contained in PubChem. Some compounds such as approved drug molecules have a great deal of information, including bioactivity data, therapeutic use, mechanism of action, metabolism, literature and patents associated, and so on. However, many other structures (e.g. synthesized for HTS purposes) do not have much information other than their chemical structures. When a compound does not have desired information, it can be inferred from information available to similar compounds. PubChem assists users in finding similar chemical structures, by providing a precomputed list of structurally similar molecules, called 'neighbors,' for each compound in PubChem [74,75].

PubChem neighbors come in two flavors: 2-D neighbors and 3-D neighbors (also known as 'Similar Compounds' and 'Similar Conformers', respectively). Two compounds are defined as 2-D neighbors of each other when they have a 2-D molecular similarity score of 0.9 or greater, which is computed using the Tanimoto coefficient [76] with the PubChem subgraph binary fingerprints [77]. Computation of 3-D neighbors uses two Rapid Overlay of Chemical Structures-based [78] 3-D similarity measures: the shape-Tanimoto (ST), which quantifies the 3-D steric shape overlap between molecules, and the color-Tanimoto (CT),

which evaluates the similarity in 3-D orientation of feature atoms between molecules. When one or more pairs of conformers of two compounds have an ST score of ≥ 0.80 and a CT score of ≥ 0.50 , the two compounds are defined as 3-D neighbors of each other. For practical reasons, PubChem 3-D neighboring currently uses up to nine conformers per compound, although compounds in PubChem may have up to 500 conformers [74]. In addition, 3-D neighboring only considers compounds with computationally generated 3-D conformer models, covering $\sim 90\%$ of all compounds in PubChem [74]. Whereas much slower than 2-D neighboring, 3-D neighboring often identifies structural similarity that traditional 2-D graph-based structural similarity methods fail to recognize [74]. Therefore, 3-D neighboring may offer complementary views on structural similarity between molecules with similar biological activities.

One may consider that PubChem neighboring is ligand-based VS against the entire PubChem Compound database with each compound as a query. The 2-D and 3-D neighbors of a compound can be accessed either through its Compound Summary page or programmatically through PUG-REST [73]. They are also available in PubChemRDF [79], allowing users to import them into local computing resources and to take advantage of semantic web technologies (to be discussed later).

3. Automation of VS pipelines

3.1. Programmatic access to PubChem for automated VS pipelines

PubChem provides multiple programmatic access routes to its data [73], which allows one to build an automated VS pipeline that exploits PubChem data. These access routes include Entrez Utilities (also called E-Utilities or E-Utills) [80], Power User Gateway (PUG) [81], PUG-SOAP [82], and PUG-REST [83]. The characteristics of these methods are summarized in Table 1, and more detailed information is given in our recent paper [73].

Among the four access routes in Table 1, PUG-REST is the simplest to use and learn, because almost all information

Table 1. Programmatic access routes to PubChem data. See Ref. [73]. for more detail.

Entrez Utilities (E-Utilities or E-Utills) [80]
<ul style="list-style-type: none"> • Used for programmatic access to information contained in the Entrez system. • Suitable for accessing text-fielded or numeric-fielded data. • Cannot handle data types specific to PubChem (e.g. chemical structures and tabular bioactivity data).
Power User Gateway (PUG) [81]
<ul style="list-style-type: none"> • A common gateway interface (CGI) that serves as the central gateway to several PubChem services. • Suitable for low-level programmatic access to PubChem. • Exchanges data through a complex XML schema. • Requires some expertise to use.
PUG-SOAP [82]
<ul style="list-style-type: none"> • Uses the simple object access protocol (SOAP). • Exchanges information using SOAP-formatted message envelopes. • Suitable for SOAP-aware GUI workflow applications and most programming/scripting languages.
PUG-REST [83]
<ul style="list-style-type: none"> • Uses a Representational State Transfer (REST)-like interface. • Does not require the overhead of XML and SOAP envelopes. • Information necessary to make a PUG-REST request can be encoded into a single URL. • The simplest to use and learn.

necessary to make a PUG-REST request can be encoded into a one-line Uniform Resource Locator (URL). In addition, it provides convenient access to information on PubChem records that are not accessible through the other programmatic interfaces. Importantly, PUG-REST supports various chemical structure searches commonly used in ligand-based VS, such as 2-D and 3-D similarity searches, substructure search, superstructure search, and identity search.

It should be noted that PubChem has a standard time limit of 30 s per web service requests. In addition, users should limit their web requests to no more than three per second, and violation of usage policies may result in the user being temporarily blocked from accessing PubChem (or NCBI) resources. See the NCBI policies and disclaimers [84] for more information.

3.2. PubChemRDF for data exchange and integration

One may want to use PubChem data for building a new in-house VS library or annotating an existing one. PubChemRDF [79], which is Resource Description Framework (RDF)-formatted PubChem data, can be used for this purpose. RDF [85] is a World Wide Web Consortium (W3C) standard model for data interchange on the web. RDF breaks knowledge into so-called triples, each of which consists of the subject, object, and predicate. In essence, RDF expresses knowledge into a directed, labeled graph.

PubChemRDF is downloadable through the File Transfer Protocol (FTP). The RDF data on the PubChem FTP site is arranged in such a way that one can download only the desired type of information, instead of getting all RDF data. The downloaded data can be imported into a triplestore, such as Apache Jena TDB and OpenLink Virtuoso, and searched using an SPARQL query interface. Alternatively, one can load them and use the graph traversal algorithms to query the RDF graphs. In addition, PubChem provides a REST-ful interface for programmatic access to PubChemRDF data (not to be confused with PUG-REST). The PubChemRDF REST interface supports simple SPARQL-like query capabilities for grouping and filtering relevant resources.

PubChemRDF harnesses ontological frameworks to help facilitate PubChem data sharing, analysis, and integration with resources external to the National Center for Biotechnology Information (NCBI) and across scientific domains. Importantly, PubChemRDF enhances cross-integration by providing direct links to available authoritative RDF resources within applicable subdomains, including reference, synonym, and InChIKey [86] to MeSH RDF [87]; protein to UniProt RDF [88]; protein and substance to PDB RDF [89]; Biosystem to Reactome RDF [88]; substance to ChEMBL RDF [88]; and compound to WikiData RDF [90].

4. Dealing with data imbalance issues in PubChem data

4.1. Imbalance in HTS data

Bioactivity data from HTS typically contain only a handful to a few hundred hits (active compounds) with many folds of inactive compounds. This imbalanced nature of HTS data presents a great challenge for developing an accurate prediction model from them [91–94]. This issue may be addressed by generating a balanced data set through resampling of the

original HTS data set. Several studies [33,91–94] have applied different resampling techniques for analysis of HTS data in PubChem. They are broadly categorized into two classes: undersampling of the majority class (inactive compounds) and oversampling of the minority class (active compounds).

Li et al. [91] applied the granular support vector machines (SVMs) with repetitive undersampling (GSVM-RUS) [95] to develop an SVM from a highly imbalanced HTS data (with an active-to-inactive compound ratio of 1/377 and 1/379 for the training and blind test sets, respectively). The underlying idea of this method is that, because only support vectors (SVs) are important for SVM model classification, removal of non-SV samples does not substantially affect the model performance. In essence, this method enables one to extract important compounds from the data set and to eliminate unimportant ones. The best SVM model constructed in this study showed a sensitivity of 86.60% and a specificity of 88.89% for the blind test set. In some studies [33], inactive compounds were selected into the modeling set only if it had a relatively high similarity to active compounds, leading to a data set that is more challenging to establish robust prediction models.

In a study of Chang et al. [92], the simple oversampling technique was used to develop SVM models that classify compounds according to predicted cytotoxicity against the Jurkat cell line. It was demonstrated that oversampling of the minority class (toxic compounds) leads to SVM models with better predictive ability for both the training and external test sets compared to results reported in previous studies. More recently, Hao et al. [93] applied the synthetic minority oversampling technique (SMOTE) [96] to tackle the HTS data set imbalance issue. Unlike the traditional oversampling method, SMOTE oversamples the minority class by creating ‘synthetic’ samples along the line segments connecting the original minority-class samples with their *k*-nearest neighbors (kNN). Predictive models developed from the oversampled data set through the SMOTE algorithm were found to have better accuracies than those from simple oversampling.

Based on analysis of several common strategies for imbalanced data modeling with PubChem’s HTS data, Nicklaus and coworkers [94] proposed a hybrid method that combines undersampling approach with cost-sensitive learning [97], which takes the misclassification costs into account by imposing penalties for misclassifications. The proposed method was shown to provide more accurate prediction results than other methods considered in their study [94].

4.2. Imbalance in literature-extracted data

As mentioned previously, PubChem contains not only HTS data, but also high-quality bioactivity data extracted from literature through data mining and/or manual curation [55]. Most of scientific articles typically contain data for active compounds but do not report much information on inactive compounds. As a result, use of literature-derived bioactivity data for VS presents a data imbalance issue, which may be considered to be opposite to the imbalance in HTS data. Whereas HTS data are predominated by inactive compounds, literature-derived data have little to no inactive compounds.

In theory, resampling techniques may be used to balance literature-derived data set. However, this is not a viable option if the data set does not have any inactive compounds. In such cases, 'putative' inactive compounds (also called putative negatives) may be generated to balance the data set, as proposed in a study by Han et al. [98] This approach involves grouping all compounds in PubChem into clusters according to their molecular descriptors, followed by randomly selecting compounds from clusters that do not contain any known active molecules against the target. Because this method does not require known inactive compounds, it enables more expanded coverage of the inactive chemical space in case of little or no knowledge of inactive compounds. Of course, undiscovered active compounds may be included in the inactive space, leading to a reduced ability of computational models to identify novel active compounds. However, such an adverse effect is expected to be relatively small, as demonstrated in the study by Han et al. [98] Many studies [15,98–101] have shown that computational models derived from putative negatives can perform reasonably well in VS.

5. Computational toxicity prediction models from PubChem bioactivity data

Because experimental determination of toxicity of a large number of compounds is expensive and time-consuming, use of computational models is considered as an alternative or complement approach that can reduce the cost of experimental toxicity assessment in the early stage of drug discovery. This section summarizes computational toxicity prediction models that use PubChem's bioactivity data.

5.1. hERG-related cardiotoxicity prediction

The human Ether-a-go-go-Related Gene (hERG) protein [102] is a tetrameric potassium ion channel that plays an important role in cardiac action potential. Its blockage by drug molecules is believed to be a major cause of drug-induced acquired long QT syndrome and cardiac arrhythmia called Torsades de Pointes, which are considered as electrocardiac symptoms of cardiotoxicity. Because undesirable hERG-related cardiotoxicity is a major problem in clinical studies of drug candidates and often results in withdrawal of approved drugs from the market, it is important to identify potential hERG blockers early in the drug discovery process.

Many computational predictive models for hERG blockers have been proposed, as summarized in a recent review by Villoutreix and Taboureau [102]. Several studies [24–27] used PubChem BioAssay data as a test or training set for developing classification models that distinguish hERG blockers from hERG nonblockers. An early example is a study by Li et al. [24], in which an SVM-based hERG classification model was developed using a training set of 495 compounds obtained from literature. The model was tested on a set of 1948 compounds whose hERG activities were available in the PubChem BioAssay database (i.e. 248 actives and 1700 inactives in AID 376), resulting in a 73% accuracy (sensitivity = 57% and specificity = 75%).

In a study of Su et al. [25], the compound set from AID 376 was reduced into a set of 876 compounds that are smaller, more condensed, and more applicable for lead optimization against the hERG receptor, by removing compounds that violate Lipinski's rule of five [50] and discarding actives with logP values of <4.1 and inactives with logP values of >2.8. These hydrophobicity constraints employed were based on the observation that the hydrophobicity of drugs tends to increase the hERG blocking effect, while hydrophilic molecules tend to decrease the hERG blocking effect. When this test set was used to evaluate binary hERG classification models derived from a continuous partial least-squares hERG binding model, the best model showed an improved accuracy of 83% (sensitivity = 97% and specificity = 82%).

Wang et al. [26] developed binary hERG classification models by employing Naive Bayesian (NB) classification and recursive partitioning (RP) techniques in conjunction with several sets of molecular descriptors. It was found that the NB classifier outperformed the RP-based model. When applied on a test set derived from AID 376, the best Bayesian classifier at a threshold of 40 μ M resulted in an accuracy of 76% (with 37% sensitivity and 82% specificity).

Whereas the PubChem hERG assay data from AID 376 were used as an external test set in all three studies [24–26] mentioned earlier, these data have also been used as a training set for model building. For example, Shen et al. [27] derived a training set of 1668 compounds from AID 376 to build SVM-based binary hERG classification models, the best of which had accuracies of 95% (with 90% sensitivity and 96% specificity) for the training set and 87% (90% sensitivity and 74% specificity) for the external set of 356 compounds.

5.2. Prediction of cytochrome P450s inhibition

The cytochrome P450s (CYPs) are a superfamily of heme-containing enzymes that catalyze the metabolism of a variety of endogenous and xenobiotic compounds. They are major enzymes involved in drug metabolism, which affects the bioavailability of drug molecules. In addition, the broad substrate specificity of CYPs often leads to unexpected drug–drug interactions, which is an important issue in drug discovery and development as well as in their clinical applications.

The PubChem Compound database provides manually curated information on the metabolism for more than 5000 compounds, collected from data contributors such as HSDB [60] and DrugBank [48]. Moreover, more than 9000 compounds have links to the corresponding records in the Human Metabolome Database (HMDB) [103], which offers comprehensive information on metabolites.

The PubChem BioAssay database also contains a large amount of experimental bioactivity data for compounds tested against CYPs. While some of them were extracted from scientific articles, others were determined through HTS. Using these bioactivity data, several groups [28–32] have developed computational prediction models for CYP inhibition of small molecules. For example, Cheng et al. [28] constructed inhibitor prediction models for five major CYP

isoforms, namely 1A2, 2C9, 2C19, 2D6, and 3A4, which account for more than 90% of drug metabolism. Their model used an algorithm that combines a back-propagation artificial neural network with other machine learning methods including kNN, SVM, NB, and C4.5 decision tree. Using a rule-based C5.0 decision tree algorithm with several molecular descriptors, Su et al. [29] developed an improved prediction model, which can classify CYP inhibitors and noninhibitors with an 81.4–93.0% accuracy.

5.3. Toxicity prediction models from cellular toxicity

When toxicity of a chemical arises from its interaction with a particular target protein, gene, or pathway, one can build a computational model that predicts whether a compound is toxic by virtue of its interaction with the target. However, chemical toxicity often comes from much more complex processes that involve many different proteins and genes in multiple pathways. In this case, a computational toxicity prediction model can be developed from cellular toxicity data generated in cell proliferation assays that do not address any specific target or underlying mechanism.

PubChem's toxicity data have been used to develop computational toxicity prediction models. In a study of Zhu et al. [33], HTS data for cell viability of 1408 compounds tested against six cell lines were used to construct a kNN quantitative structure–activity relationship (QSAR) model that predicts rodent carcinogenicity of chemicals. This study demonstrated that, when cell viability data were used together with chemical descriptors, the resulting kNN QSAR model had a better accuracy than those developed using chemical descriptors only.

Guha and Schürer [34] built computational models to predict cell toxicity based on cell proliferation HTS data contained in PubChem. To reduce the impact of the imbalanced nature of the data set employed, their prediction models were developed using an ensemble of 30 random forest models, each of which was constructed from a training set with equal distributions of toxic and nontoxic compounds sampled from the original set. These models resulted in correct classification rates between 70% and 85% against the test sets, depending on the nature of the data sets and the descriptors employed. However, when applied to predict *in vivo* animal toxicity, they showed a significantly reduced accuracy, although there were cases where cell toxicity strongly relates to *in vivo* animal toxicity.

Zhang et al. [35] proposed a method to predict acute animal toxicity of compounds (represented by the LD₅₀ values of rats), using bioactivity profiles of compounds extracted from bioassay data in the PubChem BioAssay database. Sedykh et al. [104] demonstrated that the use of dose–response data from qHTS assays as biological descriptors can improve the accuracy of QSAR models for *in vivo* toxicity prediction when combined with chemical descriptors. More recently, a prediction model for oxidative stress-induced hepatotoxicity of chemicals [105] was generated from HTS data archived in PubChem. The use of HTS data for chemical toxicity prediction is well reviewed in a recent article by Zhu et al. [106]

6. Application of PubChem data for polypharmacology

The term 'polypharmacology' [107] is used to describe a new drug development paradigm, which aims to develop a drug or a combination of drugs that simultaneously act on multiple drug targets. This multitarget approach is considered as an alternative to the traditional single-target paradigm, particularly in the treatment of complex diseases such as cancer and central nervous system disorders. Polypharmacology is also very closely related to drug repurposing, which identifies new indication for existing drugs, as well as predicting off-target adverse drug reactions (side effects), which are caused by interaction of drug molecules with unintended proteins.

PubChem data have been used in several studies that developed computational methods for identifying multitarget ligands [101,107–110]. Some of these studies [108] employed a combinatorial approach in which predictive models were separately constructed for each target and subsequently used for parallel screening against each target to find compounds that simultaneously bind to multiple targets. This approach may also be used for identifying selective ligands for structurally related protein targets.

Alternatively, several studies used network-based approaches for finding multitarget compounds. Chen et al. [107] performed cross-assay analyses to investigate the polypharmacological nature of bioactivity data contained in PubChem. With 602 bioassays that had information on target proteins at that time, they constructed a network of assays, by representing each assay with a node and connecting nodes with an edge if the assays corresponding to the nodes have one or more common active compounds. Through bipartite mapping, this assay network was merged with other networks, such as drug–target network, protein–protein interaction network, and pathway. The resulting bipartite networks helped identifying compounds that are active against multiple targets, as well as interesting protein pairs that can be targeted simultaneously under the polypharmacological drug development paradigm.

Because the bipartite mapping approach used in the study by Chen et al. [107] requires knowledge of the assay targets, it was not applicable to assays that have no target information (such as phenotypic assays). An alternative network-based approach has also been developed which allows for analysis of both target-based and phenotypic assays. In a study by Swamidass et al. [109], a network of 1581 assays with at least 5000 tested compounds was constructed based on similarity between two assays in terms of correlation between bioactivity scores of the compounds tested in both assays. The bioactivity score correlation was quantified with the promiscuity-adjusted correlation, which downweights promiscuous compounds that were tested active in many assays. The underlying assumption in this study is that if many molecules have similar bioactivities in two assays, there is likely a strong relationship between the assays (e.g. having similar protein targets or closely related biological pathways). This approach allows one to deduce the target and underlying biology of a phenotypic assay from information available for target-based assays connected to that assay.

7. Benchmark data sets for VS derived from PubChem data

Because many VS methods have been developed, it is not easy to decide which method will be best for a particular drug discovery project. Therefore, the objective evaluation of these VS methods is an important issue. This evaluation involves a retrospective validation of VS methods using benchmark data sets that consist of known active compounds against a target protein as well as inactive compounds or untested decoys. Examples of such data sets are the Directory of Useful Decoys (DUD) set [111], and its enhanced version (DUD-E) [112], Virtual Decoy Sets [113], the Demanding Evaluation Kits for Objective in Silico Screening [114,115], the GPCR ligand library [116] and GPCR decoy database [116], the Unbiased Ligand Set [117], and Unbiased Decoy Set [117].

In several studies [118–121], HTS data in the PubChem BioAssay database were used to construct benchmark data sets for VS validation. For example, Rohrer and Baumann [119] developed the Maximum Unbiased Validation (MUV) benchmarking data sets from the HTS data for 17 protein targets. The MUV sets were designed to minimize the 'benchmark data set bias,' [119] which is caused by two critical issues in many benchmark data sets: artificial enrichment [122] and analog bias [123]. As a result, the MUV sets enable a more accurate and impartial evaluation of VS methods.

Although some of the 17 targets covered in the MUV sets have experimentally determined 3-D structures in PDB [59], the design focus of the MUV sets was primarily on validation of ligand-based VS methods, not structure-based ones. Lindh et al. [121] developed validation data sets suitable for validation of both structure-based and ligand-based VS methods, based on PubChem's HTS data for seven protein targets whose crystal structure has been reported in PDB. Importantly, these data sets were designed to have a higher ratio of the number of inactive to active compounds than other benchmark data sets in order to reflect typical drug discovery scenarios in which hit compounds from VS are subsequently tested in an HTS experiment. Therefore, these data sets would give more realistic measures of the performance of different VS methods.

8. Conclusion

PubChem provides comprehensive chemical information collected from more than 400 data sources. It contains experimental bioactivity data as well as other valuable information relevant to drug discovery, including pharmacology, toxicology, mechanisms of action, ADMET properties, 3-D structures, and so on. Especially, information on chemical vendors and patents helps prioritize hit compounds from VS for further screening. In addition, the precomputed PubChem 2-D and 3-D neighboring relationships enable quick access to structurally similar compounds for a given compound.

Because information contained in PubChem can be programmatically accessed (through several methods including E-Utilities, PUG, PUG-SOAP, and PUG-REST), it is possible to build an automated VS pipeline that exploits information

contained in PubChem. In addition, through PubChemRDF, users can integrate PubChem's data into their own in-house data on a local computing machine.

PubChem data have been used in many drug discovery studies. For example, PubChem's bioactivity data were used to build computational models for bioactivity or toxicity prediction or to discover polypharmacologic multitarget ligands. In some studies, they were used to develop benchmark data set, which allows for objective evaluation of different VS methods.

When using PubChem's bioactivity data to construct a prediction model, one should keep in mind that they are highly imbalanced. HTS data are predominated by inactive compounds with only a few active compounds, and literature-derived data often contain only active compounds without any inactive compounds. This data imbalance issue should be addressed to develop an accurate prediction model.

9. Expert opinion

PubChem is the largest source of publicly available chemical information, with more than 219 million substances, 89 million compounds, and 230 million bioactivity outcomes from more than 1 million assays covering around 10,000 unique protein target sequences. Therefore, the biomedical research community has great interest in exploiting PubChem's data for drug discovery.

PubChem contains a large amount of chemical information that is useful for VS. In addition to HTS data generated by NIH's Molecular Libraries Program and other HTS projects, PubChem contains a substantial amount of literature-extracted bioactivity information contributed by ChEMBL [49], Guide to Pharmacology [58], BindingDB [57], PDBbind [56], and so on. Moreover, through data integration with other databases such as DrugBank [48], HSDB [60], and HMDB [103], PubChem provides a broad range of annotated information on small molecules, including pharmacology, toxicology, drug target, metabolism, safety and handling, and many others. PubChem also hosts data from important regulatory agencies, such as the FDA and EPA.

PubChem provides information on chemical vendors and patents for compounds. Currently, it offers links between about 6 million patent documents and more than 16 million unique chemical structures, with over 329 million chemical substance-patent links covering U.S., European, and WIPO patent documents published since 1800. Chemical vendor and patent information for compounds in PubChem would be useful for prioritizing hit compounds for further screening.

A large variation in the amount of available information exists among compounds contained in PubChem. For example, as shown in Figure 3, about 98% of PubChem compounds have never been tested in any assays archived in the BioAssay database. Inevitably, biological activities of these molecules need to be inferred from their structurally similar molecules that have biological activity data. PubChem helps users quickly identify similar chemical structures, by providing a precomputed list of 2-D and 3-D neighbors [74,75] for each compound.

To assist users in automating VS pipelines, PubChem provides multiple programmatic access routes, including E-Utilities, PUG, PUG-SOAP, and PUG-REST. In addition,

PubChemRDF [79] allows users to download PubChem data on a local computing facility and integrate them with their in-house data, facilitating data sharing and integration with other information resources. Importantly, PubChemRDF enhances cross-integration by providing direct links to available authoritative RDF resources within applicable subdomains.

When bioactivity data in PubChem are used to develop a prediction model, the data imbalance issue needs to be taken care of. Typically, HTS data in PubChem are strongly imbalanced, containing a small number of active compounds with a very large number of inactive compounds. When such imbalanced data sets are used to build computational models that predict bioactivity of molecules, they need to be balanced by undersampling inactive compounds or oversampling active compounds. Several studies proposed various sampling techniques to address the issue of data set imbalance. On the other hand, because scientific articles primarily report data for active compounds, literature-extracted bioactivity data in PubChem often lack information on inactive compounds, creating another type of data set imbalance. To use these literature-extracted data for model building, putative inactive compounds may be generated to balance the data set.

PubChem contains a large amount of toxicity data generated from HTS assays, as well as those extracted from literature through manual curation or data mining. These toxicity data have been used in many studies to construct computational models that predict toxicity of molecules. Some of these studies aimed to predict target-specific toxicities, such as cardiotoxicity due to hERG inhibition, and drug-induced liver damage due to CYP inhibition. Other studies developed prediction models for cellular toxicity, carcinogenicity, *in vivo* animal toxicity, which arise from much more complex mechanism involving multiple genes, targets, and pathways. These prediction models can be used for structure alerts for potentially toxic molecules during VS.

A large amount of target information for compounds in PubChem can be used to find multitarget ligands for polypharmacologic drug development. In addition, it can be used to find new targets for a compound, which allows one to predict off-target side effects of drug molecules that cause adverse drug reaction as well as to repurpose existing drug molecules for a new indication. These areas can be harnessed by using PubChemRDF, which presents a promising opportunity to exploit public chemical information not only in PubChem, but also in other chemical and biological databases.

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Declaration of interest

S Kim is an employee of the National Library of Medicine. He has no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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