The Application of the Open Pharmacological Concepts Triple Store (Open PHACTS) to Support Drug Discovery Research

Joseline Ratnam1*, Barbara Zdražil2, Daniela Digles2, Emilio Cuadrado-Rodriguez1, Jean-Marc Neefs3, Hannah Tipney5, Ronald Siebes6, Andra Waagmeester7, Glyn Bradley5, Chau Han Chau5, Lars Richter2, Jose Brea1, Chris T. Evelo7, Edgar Jacoby3, Stefan Senger5, Maria Isabel Loza1, Gerhard F. Ecker2, Christine Chichester4

1. Universidade de Santiago de Compostela, Grupo BioFarma-USEF, Departamento de Farmacología, Campus Universitario Sur s/n, 15782 Santiago de Compostela, Spain, 2. University of Vienna, Department of Pharmaceutical Chemistry, Althanstrasse 14, 1090 Vienna, Austria, 3. Janssen Research & Development, Turnhoutseweg 30, Beersel, Belgium, 4. Swiss Institute of Bioinformatics, CALIPHO Group, CMU – Rue Michel-Servet 1, 1211 Geneva 4, Switzerland, 5. GSK Medicines Research Centre, Gunnels Wood Road, Stevenage, Hertfordshire, SG1 2NY, United Kingdom, 6. Vrije Universiteit, Faculty of Sciences, division of Math. and Computer Science, De Boelelaan 1081a, 1081 HV Amsterdam, The Netherlands, 7. Department of Bioinformatics – BIGCaT, Maastricht University, Maastricht, The Netherlands

*joselinepriya.ratnam@usc.es

Abstract

Integration of open access, curated, high-quality information from multiple disciplines in the Life and Biomedical Sciences provides a holistic understanding of the domain. Additionally, the effective linking of diverse data sources can unearth hidden relationships and guide potential research strategies. However, given the lack of consistency between descriptors and identifiers used in different resources and the absence of a simple mechanism to link them, gathering and combining relevant, comprehensive information from diverse databases remains a challenge. The Open Pharmacological Concepts Triple Store (Open PHACTS) is an Innovative Medicines Initiative project that uses semantic web technology approaches to enable scientists to easily access and process data from multiple sources to solve real-world drug discovery problems. The project draws together sources of publicly-available pharmacological, physicochemical and biomolecular data, represents it in a stable infrastructure and provides well-defined information exploration and retrieval methods. Here, we highlight the utility of this platform in conjunction with workflow tools to solve pharmacological research questions that require interoperability between target, compound, and pathway data. Use cases presented herein cover 1) the comprehensive identification of chemical matter for a
Introduction

While the approval rates for new drugs may be somewhat stable, pharmacological data of increasing size, dimensionality and complexity is being housed in public and proprietary databases [1], [2]. Within these separate data pools resides valuable scientific information that can help in the design of novel drugs, for example by predicting protein interactions with novel compounds [3], [4], [5], suggesting novel molecules with better properties or by finding existing chemical matter to test against a newly identified target. However, gathering relevant and comprehensive information from diverse sources is complicated; differences in data formats, the need for separate interfaces and query mechanisms, the lack of consistency between descriptors and identifiers in different resources and the absence of a simple mechanism to link them make this task non-trivial [6], [7].

Manual searches across different databases are tedious and time consuming, and thus often limited to individual compounds or targets only. The manual collation of data can be error prone and incomplete, of variable quality, and may not routinely capture the provenance of the original data sources. Moreover, for the effective and systematic combination and integration of complex data, the scientist analyst is required to possess an in-depth knowledge of the data models and licensing for each of a large set of systems. In addition, the need for bio- and chemo-informatics expertise and the ability to post-process any data retrieved makes this approach less accessible for a large majority of users. It is clear that many members of the drug discovery community will benefit greatly from accessible and well-structured data combined with useful analytics. For example, an integrated and comprehensive interface to publicly available pharmacology, physicochemical and biomolecular data could support initial drug screening stages and limit expensive late-stage trial failure. Such tools would also be invaluable to academia and small to medium enterprises (SMEs), which have historically enjoyed little access to proprietary integrated platforms.

A recent approach to address these issues is the integration of data from different sources by means of semantic web technologies [8], [9], [10]. The Open Pharmacological Concepts Triple Store (Open PHACTS) is an Innovative Medicines Initiative Knowledge Management project (IMI - 2nd call 2009) focusing on the application of semantic web technologies to overcome data access
and knowledge integration challenges which can hinder current drug discovery efforts. The Open PHACTS Discovery Platform offers solutions for access to multiple, disparate and heterogeneous information sources, lack of standards and common identifiers for domain entities, and provides a means to interrogate the system with complex research questions [6], [7]. By drawing together multiple sources of publicly-available biomolecular, pharmacological and physicochemical data, Open PHACTS offers a state of the art platform that responds to structured, well defined queries in a meaningful and reproducible way (see S1 Table for currently available resources). An important functionality to maximise usefulness, especially in the pharmaceutical industry, is the ability to offer secure access to the Open PHACTS Discovery Platform. Presently, a robust security policy has been developed with a commercial triple store provider, Open Link (an Open PHACTS consortium partner), to supply the requisite privacy mechanisms.

As a collaboration between multiple European universities, the European Federation of Pharmaceutical Industries and Associations (EFPIA), and various SMEs (http://www.openphacts.org/partners/consortium), the Open PHACTS project benefits from a wealth of market experience and technical expertise. Development of the Open PHACTS Discovery Platform is driven in an agile, stepwise fashion focused on scientific competency questions and use cases for analysis of underlying data concepts and associations [11]. This approach ensures delivery of a platform ready and able to support drug discovery and development in both the public and private sector. A drug discovery focused Open PHACTS ‘Researchathon’ event (attended by 18 scientists from 8 academic institutions and 2 EFPIA companies) in 2013 identified critical requirements in terms of the specific datasets, functionalities and Application Programming Interface (API) calls which have shaped the Open PHACTS Discovery Platform development necessary to answer the specific questions presented here. The complete list of participants can be found here: http://www.openphacts.org/documents/events/130424_Researchathon_London_Participant%20List.pdf.

The aim of the present work is to highlight how the Open PHACTS Discovery Platform has been used by academic and pharmaceutical industry drug discovery scientists for the integration of public and proprietary pharmacology resources to i) identify target-specific chemical compounds, ii) support pathway-driven drug discovery. We describe how the platform can be used to solve common queries that require linkage of the entities of targets, compounds, and pathways, using the examples of a single target, Dopamine Receptor D2, and two well curated pathways of therapeutic interest from the public resource WikiPathways [12], ErbB signaling and Vitamin D metabolism (for detailed pathway selection criteria see S1 Method). As the platform is designed to be easily accessible from computational workflow systems, we show how the modularization of tasks using the Open PHACTS API [7] as well as full integration with pipelining tools can create workflows to answer complex queries around the selected examples. The workflow tools used herein are KNIME [13], a widely used, open-source graphical workbench to create and run workflows between executable ‘nodes’ and Pipeline Pilot [14], a proprietary workflow tool built on the Accelrys Enterprise Platform.
that similarly uses configurable ‘components’ to automate the process of accessing, analyzing and reporting scientific data.

Here, we demonstrate the utility of Open PHACTS in early drug discovery project through the development and application of workflows based on the Open PHACTS API and pipelining software, thereby allowing scientists to find answers to complex research questions requiring a wide range of data sources.

Methods

Open PHACTS API, databases, and workflow tools

All use case workflows utilized the Open PHACTS API version 1.3 (https://dev.openphacts.org/docs/1.3, Accessed 2014 Nov 30) to query across integrated public data sources: ChEMBL [15], [16], ChEBI [17], [18], [19], Drugbank [20], Chemspider [21], Gene Ontology (GO) [22], [23], WikiPathways [12], Uniprot [24], ENZYME [25] and ConceptWiki [26] (S1 Table). These data are available for download from the different data providers, under licensing models, such as Creative Commons Attribution (CC-BY), which require mostly citation and attribution for their reuse. The Open PHACTS consortium has endeavored to clarify and align data and software licenses to remove any barriers to use. The current resources, discussion of issues, and help documents are available on the Open PHACTS support site: http://support.openphacts.org/support/home (Accessed 2014 Nov 30). Proprietary databases used in Use Case A are: GVKBio GOSTAR (www.gostardb.com), Thomson Reuters (integrity.thomson-pharma.com) and in-house pharmacology databases from Janssen.

Use case workflows were constructed in the following manner: 1) entities of interest (targets, compounds, pathways, bioactivities, etc.) needed for the specific step in the workflow were identified, 2) URIs for the entities of interest were determined, 3) Open PHACTS API calls were executed, 4) results were parsed, 5) the steps were repeated multiple times if answers to previous cycles were needed to reach the final question. For each use case, the tasks were automated using the two most common cheminformatics workflow tools, namely Pipeline Pilot (http://accelrys.com/products/pipeline-pilot/) and KNIME version 2.9 (http://www.knime.com).

A custom Pipeline Pilot component library was co-developed with Accelrys to access the Open PHACTS API calls and parse the output. These components were used for the Use Case A workflow and are available on the Open PHACTS page on the Accelrys community website at (https://community.accelrys.com/docs/DOC-6473. Accessed 2014 Nov 30).

A series of generic KNIME utility nodes (https://github.com/openphacts/OPS-Knime, Accessed 2014 Nov 30) were created to incorporate the Open PHACTS services into the KNIME workbench. These nodes use two-dimensional tables, such as named rows and columns, as input and generate equivalent output. Since the Open PHACTS API services produce nested output (e.g. JSON or XML), a KNIME ‘unfolding’ algorithm was implemented as a node, transforming the Open
PHACTS output into a KNIME table. The Open PHACTS API services are described in the Swagger REST service description format, enabling automatic generation of templates in KNIME. The result of running this utility node is a URL that represents the desired service call within a workflow. These nodes were used to construct workflows for Use Cases B and C.

An overview of the API calls used to construct workflows for all use cases is represented in Fig. 1.

Internal dictionaries for standardizing target, compound, and bioactivity nomenclature in proprietary databases

Use Case A required prior resolution of non-standard identifiers for compounds, targets and bioactivities present in proprietary pharmacology databases. As such, tautomeric SMILES nomenclature was selected for compounds, human gene symbols for targets, and log-transformation for bioactivity data, as these standards are stable and offer possibilities for integration with additional data types. To align external databases with EFPIA in-house data that traditionally use legacy gene symbols and not community accepted standard identifiers, a mapping table was created to link pharmacology database fields with HUGO gene symbols. An internal dictionary was created for each database to map the drug target keywords to HUGO gene symbols, and this information was added back to target information when necessary.

We also ensured that results from Open PHACTS would map to the different database fields by strictly adhering to target dictionaries and field mappings in a Pipeline Pilot protocol.

Generating a list of related targets (gene names)

In order to expand pharmacology data to related proteins, three strategies are possible: finding targets linked to the same GO concept in Open PHACTS (the ‘Target Classifications’ API call), using the target protein sequence in a BLAST alignment to obtain UniProt identifiers of related proteins (by sequence), or by manual collection of protein identifiers from literature or protein family databases. In all cases, Open PHACTS can be used to obtain gene names correlated with UniProt identifiers. The related proteins retrieved from these methods may represent splice variants, orthologues or homologous paralogues. In the following use cases the distinction between these cases were not investigate, although they could potentially have some influence on the number of pharmacological records retrieved from Open PHACTS. In the case of a well-studied target like the human dopamine receptor 2, with numerous pharmacology records, target similarity searches were not performed.
Generating a merged list of compounds active against a target, ranked by bioactivity

A Pipeline Pilot workflow was created to provide a collection of targets, assay numbers, activity data, and chemical structure information from the databases mentioned above. The final steps of the workflow merge information per assay and data source, and sort the tabular results to present a ranked list of chemical compounds and their activities. In a facultative step, the workflow can also be programmed to search for similar chemical compounds and their pharmacological effects. This returns a complete activity profile for a comprehensive list of compounds of interest. A schematic representation of the workflow is shown in Fig. 2.
Returning data for free text
Free text entered in the ‘Free Text to Concept’ API call can be used to find all corresponding concept URIs to enable usage of other API calls.

Finding orthologues for a given target using free text
URLs for all orthologues of a given target were obtained using the ‘Free Text to Concept for Semantic Tag’ API call. The name of the target was used as free text
Returning data for a pathway

After choosing the pathway of interest on the WikiPathways website, the pathway can be used as input for queries with the Open PHACTS API in several different ways. Either the URI of the pathway is used directly (e.g. in the format of http://www.wikipathways.org/index.php/Pathway:WP1531) or the title or identifier of the pathway can be used in the ‘Free Text to Concept’ API call to retrieve a URI. Here, the branch parameter can be set to return concepts of WikiPathways only.

General information for the pathway such as the version of the data, the pathway title, and its description can be returned with the ‘Pathway Information’ API call.

A list of proteins and genes present in a pathway can be retrieved directly with ‘Pathway Information: Get Targets’. The API call results reflect the WikiPathways data, which can be either gene or protein URIs. The results can be used without further processing as input for target based API calls.

Pathways containing specific targets can be retrieved using ‘Pathways for Target: List’ API call. Either gene or protein URIs can be used as input.

Creating heat-map and overlap representations of pharmacology data

To provide a better distribution for visualization, the activity values (for Potency, IC_{50}, EC_{50}, AC_{50}, K_i and K_d endpoints) were transformed into their negative logarithmic Molar values (‘-logActivity values [molar]’). The same activity endpoints are available as ‘pCHEMBL values’ from the ChEMBL database, but in addition we also kept values with a relation different from ‘=’, but discarded the relation information for the following steps. For a binary representation (active: 1, inactive:0), a cutoff value of ‘-logActivity values [molar]’ of at least six was applied to determine active molecules.

A pivot table was generated to display bioactivities of compounds against multiple targets using the ‘Pivoting’ node in KNIME grouping rows by ‘Compound name’ and columns by ‘Target Name’. If several activity values are given for the same compound-target pair, only one value can be kept (e.g. a mean value or the most active value). In the case of the binary representation, ‘1’ (active) is chosen if an ambiguous classification is made. The resulting heat-maps were visualized with the HeatMap (JFreeChart) node in KNIME.

In order to detect compound specificity for single versus two or more targets within the pathway, an overlap table was generated. From the pivot table generated as above, the number of times a compound ‘hits’ a target was counted using the node ‘Column Aggregator’. The ‘Numeric row splitter’ node splits...
compounds hitting more than one target from those hitting just one. The former set was used to generate an overlap table.

Retrieving pharmacology data for a target/compound and filtering options
The ‘Target Pharmacology: List’ API and ‘Compound Pharmacology: List’ API calls can be used to retrieve pharmacology data from ChEMBL for single protein targets and protein complexes containing the target. If only single protein targets are sought, the type is specified as target_type = single_protein in the API parameters. The pharmacology output is always filtered to exclude records where compound activity is unspecified. Values larger than 10^8 are also removed to avoid potential data errors. The data can be filtered in many different ways, for example to return data for a specific activity (eg. IC_{50}) or assay type (eg. binding or functional assays) or to only return agonists/activators or inhibitors/antagonists. Several different values can be requested in one call (e.g. IC_{50}|EC_{50}|AC_{50}|K_i|K_d|Potency). Activity values can be limited by different cut-off parameters, for example by setting max-activity_value=2000. The number of results for a given query can be retrieved with the ‘Target Pharmacology: Count’ or ‘Compound Pharmacology: Count’ API calls.

The data can be returned in one piece by using the parameter _pageSize=all. In cases which might return too many data points (e.g. several ten thousands), a smaller _pageSize parameter can be used, in combination with a loop overall result sets with the _page parameter.

Finding Approved Drugs for an individual target or all targets in a pathway
The first approach uses the ‘Target Information’ API call where target URIs (gene or protein) are used as input. Compounds targeting this protein are derived from the DrugBank dataset where each molecule is labeled according to its type (‘approved’, ‘biotech’, ‘experimental’, ‘illicit’, ‘investigational’, ‘nutraceutical’, ‘small Molecule’, ‘withdrawn’). The resulting data are filtered for ‘Drug type=approved’. The second approach uses the ‘Target Pharmacology: List’ API call to find all compounds active against a given target based on ChEMBL records. These compound URIs are then used in the ‘Compound Information’ API call and results filtered for approved drugs as before. The search retrieves all approved drugs that have bioactivity against a given target, even if not approved for that target in DrugBank. The results from both approaches are merged.

Retrieving Chemical Entities of Biological Interest (ChEBI) terms associated with a compound
ChEBI terms for a molecule are retrieved with the ‘Compound Classifications’ API call setting the tree parameter to ‘chebi’. The resulting data was restricted to
classifications of the type “has role”, which includes the three sub-categories: ‘chemical role’, ‘biological role’, and ‘application’.

Retrieving GO terms associated with a target
GO terms for a target can be retrieved using the ‘Target Classifications’ API call by setting the tree parameter to ‘go’. This returns classifications from the three branches of GO (cellular component, molecular function, and biological process). The resulting data was filtered for ‘biological process’.

Retrieving positive and negative regulators of a pathway via GO terms
GO terms associated with the term ‘regulation of Vitamin D’ were obtained with the ‘Free text to Concept’ API call, the resulting data was restricted to ‘alternative’ exact match type, to include only GO terms. Children of these terms were retrieved using ‘Hierarchies: Child’ API call to enable separation of positive and negative regulators. Gene products associated with these GO terms were obtained using ‘Target Class Member: List’ API call

Results
Three use case workflows were implemented to highlight different applications of the integrated Open PHACTS data. Use case A assembled a ranked list of compounds targeting the dopamine receptor D₂ (DRD2) and then found related targets in both public and proprietary pharmacology databases to aid in the design of a new compound library for the dopamine receptor drug discovery program. Use case B identified compounds active against all targets in the Epidermal growth factor receptor (ErbB) signaling pathway that have a relevance to disease. Use case C evaluated established targets in the Vitamin D metabolism pathway and then expanded the scenario to view these targets in other contexts.

Use case A: Comparison of existing public and proprietary pharmacology data for DRD2
The mesolimbic dopamine system is a central component of the brain reward circuit [28]. Pharmacological agents targeting dopaminergic neurotransmission have been clinically used in the management of several neurological and psychiatric disorders, including Parkinson’s disease, schizophrenia, bipolar disorder, Huntington’s disease, attention deficit hyperactivity disorder (ADHD), and Tourette’s syndrome (reviewed by [29]). The physiological actions of dopamine are mediated by five distinct but closely related G protein-coupled receptors that are divided into two major groups: the D1-like (D₁ and D₅) and D2-like (D₂, D₃, D₄) classes of dopamine receptors (DARs) on the basis of their structural, pharmacological, and biochemical properties [30], [31]. Of the five
DARs and their variants, the DRD2 and its properties continue to be the most actively investigated because it is the main clinical target for antipsychotics and for the dopamine agonist treatment of Parkinson’s disease [32]. Despite being one of the most validated targets for neuropsychiatric disorders, truly selective drugs for the DRD2 subtype have been hard to obtain due to high conservation of orthosteric binding sites among DARs and other GPCRs, leading to undesirable side-effects. As such, there has been tremendous effort to identify novel DRD2-selective ligands that will be useful not only as improved pharmacotherapeutic agents, but also to help define the function of D2-like receptor subtypes and as *in vitro* and *in vivo* imaging agents. We aimed to rank existing compounds known to target the DRD2 to aid in the design of a novel DRD2-targeted screening library.

**Ranked list of public and proprietary compounds targeting DRD2**

Our workflow (Fig. 2) for finding DRD2-targeted chemical matter (run in February 2014), identified 2278 ‘active’ organic compounds in Open PHACTS public repositories showing either % activity or IC$_{50}$ values against the DRD2 ([S1 File](#)). Considering a cut-off of $>$50% for % activity values and $-\log$(IC$_{50}$) values $>$6, we identified 6194 bioactivity values; an additional 164 ‘inactive’ compounds are found with activity values below 50% or $-\log$(IC$_{50}$) values below 6 ([Table 1](#)). The same protocol identified 3148 organic compounds in patent reporting databases: Thomson Reuters Integrity monthly updates, World Drug Index quarterly reports, and PharmaProjects monthly updates were licensed from Thomson Reuters. 8959 additional compounds with over 50,000 activity and $-\log$ (IC$_{50}$) data points are found in the in-house proprietary pharmacology screening database. The total number of compounds found is the sum of those found in the different sources as there is little overlap between them. This is because Open PHACTS/ChEMBL uses public information, Thomson Reuters uses patent information (often not published), and the in-house pharmacology databases use internal information (often not patented). Our workflow provides 2278 compounds that would have been missed altogether or difficult to find using approaches independent of Open PHACTS. In a facultative step, the workflow can also search for similar chemical compounds and their pharmacological effects, to present a complete activity profile for a comprehensive list of compounds of interest. Thus, using Open PHACTS we were able to produce a cohesive list of interesting DRD2-targeting compounds derived from heterogeneous data stored in multiple databases.

The most interesting compounds have a high activity, or are reported in patent literature to act on the target of interest. They must also have little reported activity on other targets. Conversely, the least interesting compounds have low or no reported activity on targets of interest and have higher reported activity on other targets. This sorting allows a more efficient processing of tables that sometimes contain data on several hundreds of compounds. A Pipeline Pilot script running all the steps described above automatically produces a relevant listing of compounds, activity data, and target information in under an hour, making the process of looking for compounds for new targets and target families a
simple and reproducible task. The above script allows control of the different process steps, and has been successfully used at Janssen to support various drug discovery projects.

Finally, programmatic access to the individual data sources previously required a specific case by case approach: for example, access to biological activity data from ChEMBL was via a locally installed MySQL database, from DrugBank from a copy of the XML, from GVKBio GOSTAR from a remotely installed Oracle database, from Thomson Reuters from a tab-delimited text file, and from the in-house pharmacology database from a local server-based Oracle database. Searching the different databases for target information was done mostly manually, where information had to be carefully assembled for each target in each database and the process repeated for each request for new target information. By using Open PHACTS, data from ChEMBL and DrugBank could be retrieved from a single source, reducing the effort needed for data integration. The custom Pipeline Pilot Open PHACTS component library enabled access to the databases in Open PHACTS, on par with components already in use for proprietary databases, thereby allowing a true integration of all available pharmacology data in one protocol. The workflows for retrieving the data from the different data sources are depicted in a Pipeline Pilot screenshot S1 Fig.

This example illustrates the benefit of accessing the Open PHACTS data in the competitive Pharmaceutical research environment, even for well-known targets that have already been extensively studied.

### Use case B: Compounds active against targets in the ErbB signaling pathway and their disease relevance

Epidermal growth factor receptors (known as ErbB) are receptor tyrosine kinases consisting of four members: ErbB1/EGFR, ErbB2/HER2, ErbB3 (HER3), and ErbB4 (HER4). Members of the EGF family of growth factors (e.g. EGF, neuregulins), are natural ErbB receptor ligands which upon binding induce homo- or heterodimerization of the receptor and subsequent activation of intrinsic kinase activity [33]. Different ErbB heteromers activate different downstream signaling pathways (http://www.wikipathways.org/index.php/Pathway:WP673): mitogen-activated protein kinase (MAPK) signaling and phosphatidylinositol 3-kinase (PI3K)-AKT pathway, SRC tyrosine kinase pathway, signal transducer and activator of transcription proteins (STATs), and

| Activity Data Source                  | Number of Compounds found |
|--------------------------------------|---------------------------|
| Open PHACTS                          | 2278 (active) +164 (inactive) |
| Patent Reporting Databases           | 3148                      |
| Janssen Compound Screening Databases | 8959                      |

Active compounds have % activity values $>50\%$ or $-\log(\text{IC}_{50})$ values $>6$.

doi:10.1371/journal.pone.0115460.t001
mammalian target of rapamycin (mTor) pathway [33]. Upon activation of different branches of the ErbB signaling network, different responses are triggered ranging from cell division to death, motility to adhesion. Insufficient ErbB signaling in humans is associated with the development of neurodegenerative diseases, such as multiple sclerosis and Alzheimer’s disease [34]. ErbB-1 and ErbB-2 are found in many human cancers and [35], [36] their excessive signaling is associated with the development and malignancy of these tumors. Accordingly, the ErbB receptor family with their most prominent members EGFR and HER-2 represent validated targets for anti-cancer therapy, and anti-ErbB monoclonal antibodies (e.g. cetuximab, panitumumab, and trastuzumab) and tyrosine kinase inhibitors (gefitinib, erlotinib, and lapatinib) have now been approved for the treatment of advanced colorectal cancer, squamous cell carcinoma of the head and neck, advanced non-small-cell lung cancer, as well as pancreatic and breast cancer [33].

However, current therapy treats only a subset of patients carrying specific mutations and even within this population, tumor resistance is common. Identification of specific protein targets involved in ErbB-mediated cancer development is confounded by the multiplicity of pathways activated by ErbB receptors and the existence of more than 100 potential protein binding partners identified by large-scale phosphoproteomic screening [37]. As members of the ErbB receptor family cooperate in signal transduction and malignant transformation, the concurrent inhibition of two or more receptors or specific heteromeric ErbB family receptor complexes may yield the next generation targeted therapies. However, only a small proportion of publicly available bioactivity data reports on the activation of ErbB oligomers. In many cases, the exact mechanism of ligand-protein binding and protein activation is simply not known and bioactivity of small molecules is tested on single proteins only. This leads to challenges for structure-based drug design and interpretation of pharmacological data. As such, understanding the role of receptor oligomers in the ErbB signaling pathway is invaluable for the purpose of drug discovery.

Pathway targets and pharmacology
In total, 54 NCBI Gene IDs were retrieved as targets from the ErbB signaling pathway. Of those, only 35 single proteins returned pharmacological data with the applied bioactivity filters. Additionally, data for 12 protein families, 5 protein complexes, 2 protein-protein interactions and one chimeric protein containing a target from the pathway were retrieved, increasing the total number of targets to 55. While a pharmacology query without any filters would retrieve nearly 150,000 data points, filtering reduced the data to 108,014 bioactivities and 65,780 unique compounds (see Fig. 3). Using the pChEMBL values to filter bioactivities led to a significantly lower number of records as compared to -logActivity values: 53 targets, 65,817 bioactivity endpoints and 43,255 unique compounds. The pChEMBL filter restricts data to those that are equal to a specific value. Values that are reported to be ‘greater than’ or ‘less than’ will therefore be missing in the...
Fig. 3. Use case B workflow. Open PHACTS v 1.3 API calls are shown in orange boxes along with the results obtained. Bioactivity filters and other data processing operations are shown in yellow boxes with results obtained in light grey boxes. Blue colored boxes show results included in the manuscript. Compound pharmacology at the pathway level was retrieved by consecutive execution of the API calls ‘Pathway Information: Get targets’ and ‘Target Pharmacology: List’ - the latter includes a filtering for desired activity endpoints and units - and other filtering, transformation, and normalization steps: transformation into \(-\log{\text{Activity}}\) values [molar], setting a threshold for binary representation, and subsequent filtering by keeping only the max. activity value for each compound/target pair. Retrieving GO annotations for a list of targets, and ChEBI annotations for compounds that have been tested against those targets was achieved by using the API calls ‘Target Classifications’ and ‘Compound Classifications’ and subsequent restriction to terms of the type ‘biological process’ and ‘has role’, respectively.

doi:10.1371/journal.pone.0115460.g003
final data set. Consequently, \(-\log \text{Activity}\) values appear to be a valid approach to generate data sets of bioactivity measures that span a larger range of values.

To compare the pharmacological data across different targets, each compound/target pair was represented by only one activity point, keeping the most active value in cases where several measurements were reported, and a cutoff was set for separating active from inactive compounds. A heat map representation of the compound/target space was retrieved for these binary representations (S2 Fig.). Protein targets with a greater number of measurements (having a larger portion of red/blue bars) can be distinguished from those with a lower number of activity data points (having a large portion of grey bars). For instance, targets: Cellular tumor antigen p53 (CHEMBL4096, P04637), MAP kinase ERK2 (CHEMBL4040, P28482), Epidermal growth factor receptor ErB1 (CHEMBL203, P00533), and FK506 binding protein 12 (CHEMBL2842, P42345), have the highest numbers of unique measurements (sum of unique active and inactive compounds), 36,075, 14,572, 5,028, and 4,572, respectively. In addition, one can identify targets with a higher number of unique active compounds (setting the cutoff at 6), i.e. 3,670 for p53, and 2,268 for ErB1 (see Table 2). By reducing the target/compound space to representative activity points and choosing a binary representation, easier visualization of large data collections is enabled. However, additional information on the concrete bioactivity might be desirable in cases where compounds possess activity values close to the chosen cutoff.

Apart from necessary filtering and normalization steps that limit the full illustration of the target space, we also recognized a lack of reliable compound bioactivity data specifically targeting oligomeric proteins in the pathway. For example, in ChEMBL_v17, the target ‘Epidermal growth factor receptor and ErB2 (HER1 and HER2)’ is classified as being a ‘protein family’ (CHEMBL2111431, P00533 and P04626) with 115 IC50 bioactivity endpoints. Inspecting the underlying assay descriptions however reveals the inclusion of compounds targeting either ErB1, ErB2, both proteins, or in some cases even upstream targets. For the sake of data completeness, we retained all target types in the query, but we advise to always go back to the original primary literature source and study the bioassay setup in order to make sure which effect was actually measured and if the data is reliable in cases where data is assigned to other target types than ‘single protein’.

**Studying targets related to certain diseases**

Determining the targets related to cancer or neurodegenerative diseases was accomplished by evaluating the GO [22], [23] annotations. The ‘biological process’ terms were extracted for the 23 protein targets (possessing at least 100 active compounds): 525 different (unique) annotations, with Glycogen synthase kinase-3 (CHEMBL2095188, P49840 and P49841; 93 annotations), and p53 (CHEMBL4096, P04637; 86 annotations) having the highest number of different annotation terms. The GO term most frequently associated with the 23 targets was ‘innate immune response’ (GO_0045087; annotated to 16 targets). Interestingly, brain immune cells (microglia) seem to play a major role in the development and
progress of neurodegenerative diseases such as Alzheimer’s disease [38], [39]. Other frequent terms, which appear interesting in the context of cancer include: ‘negative regulation of apoptotic process’ (GO_0043066; annotated to 9 targets), ‘positive regulation of cell proliferation’ (GO_0008284; 7 targets), ‘cell division’ (GO_0051301; 6 targets), ‘apoptotic process’ (GO_0006915; 5 targets), and ‘positive regulation of apoptotic process’ (GO_0043065; 5 targets). The information gained by such analyses can guide the selection of targets to be studied more thoroughly, in the search for novel therapeutic treatment opportunities, especially if multi-targeted therapies are in the focus of research. (A list of all GO ‘biological process’ terms that have been annotated to at least 5 of the 23 prioritized targets and ChEMBL target IDs of those targets can be found in the S3 Table.)

**Studying compounds related to certain diseases**

In parallel to the identification of GO terms for the targets, we enriched the compounds with the addition of ChEBI terms [17], [18], [19]. In total, 294
different ChEBI ‘roles’ (including the three sub-categories: ‘chemical role’, ‘biological role’, and ‘application’) have been annotated to 1036 different compounds targeting the 23 prioritized targets. Unfortunately, only a minor proportion of compounds (approximately 1.6% in this use case) possess ChEBI annotations although they are of very high quality as each entry in the database is manually annotated by experts [17]. 49 of the 294 different (unique) ChEBI terms have been annotated to at least 6 different compounds (see Suppl. Section, S4 Table). The ChEBI term ‘antineoplastic agent (ChEBI_35610)’ appears the most frequently, with annotations to 79 different compounds. We assessed these active compounds using a binary heatmap representation (see S3 Fig.) and found the targets: Tyrosine-protein kinase ABL (CHEMBL1862, P00519; 18 active compounds), Epidermal growth factor receptor ErbB1 (CHEMBL203, P00533; 15 active compounds), and Tyrosine-protein kinase SRC (CHEMBL267, P12931; 10 active compounds) with the highest numbers of active measurements.

Compounds comprising a pharmacological pattern corresponding to that (activity on CHEMBL1862, CHEMBL203, and CHEMBL267) and possessing the ChEBI annotation term ‘antineoplastic agent’ include: Erlotinib, Lapatinib, Bosutinib, Vandetanib, Sunitinib, Masitinib, Canertinib, and Sprycel. It appears interesting to experimentally test other compounds with the same ChEBI term against those three targets, especially if they possess a similar chemical structure like the compounds/drugs mentioned before. S2 File gives the names of the 79 compounds, their CHEMBL compound IDs, and the previously determined active/inactive result according to our cut-off for active molecules.

However - like all hand-curated resources - ChEBI is biased towards its annotation criteria, which in that case are already approved drugs. Thus, to date it serves best for filtering out drugs related to a certain disease. As the ChEBI database and ontology is instantly growing, it will become a more comprehensive and increasingly reliable and useful resource.

Using our Open PHACTS workflow, we could answer research questions related to complex regulatory pathways with a large number of druggable targets and requiring data from multiple sources. With an expansion of the data sources available in the next release of the Open PHACTS API (version 1.4), which will include more information on the distribution of targets in tissues and changes in relation to disease, more refinement of the antineoplastic agents found in our analyses will be possible.

Use case C: Broadening the therapeutic opportunities from the Vitamin D pathway

1,25(OH)₂D₃ or calcitriol, the biologically active form of vitamin D [40], is an important hormone that is critically required for the maintenance of mineral homeostasis and structural integrity of bones by facilitating calcium absorption from the gut and by direct action on osteoblasts, the bone forming cells [41]. Apart from its classical actions on the gut and bone, calcitriol and its synthetic analogues also possess potent anti-proliferative, differentiative and immunomo-
dulatory activities (reviewed by [46]). These pleiotropic effects are mediated through vitamin D receptor (VDR), a ligand-dependent transcription factor that belongs to the superfamily of steroid/thyroid hormone/retinoid nuclear receptors [42]. This has set the stage for therapeutic exploitation of synthetic VDR ligands for the treatment of various inflammatory indications and cancer [43], [44], [45], [46], [47], [48]. However, the use of VDR ligands for these indications in the clinic is limited by their major dose-related side effect, viz., hypercalcemia/hypercalciuria. Therefore there has been tremendous interest in generating newer vitamin D analogues that retain the desired therapeutic activity but with less toxic (calcemic) side effects.

Prior to reaching the nuclear VDR, calcitriol interacts with several key proteins, the serum vitamin D binding protein (DBP), the vitamin D-activating enzyme (CYP27B1), and the catabolic enzyme 24-hydroxylase (CYP24A1). The latter two enzymes are expressed and differentially regulated in VDR-expressing target tissues, providing a means for tissue-specific actions of VDR ligands. Affinity for the DBP is another means to control circulating calcitriol levels. The unique actions of calcitriol and its analogues thus result from their combined interactions with several key proteins in the Vitamin D pathway (http://www.wikipathways.org/index.php/Pathway:WP1531). Better understanding of these interactions and a pathway-focused approach will facilitate the design of a new generation of vitamin D analogues with a desired interaction profile against pathway components, resulting in improved therapeutic indices. Knowledge of the appropriate compound evaluation methodologies is also important to ensure that the desired bioactivity profile is being retained during chemical optimization stages. Finally, information about how the pathway is regulated, identifying novel points for therapeutic intervention, and estimating the impact of modulating these targets could allow alternative therapeutic strategies. Accordingly, our Open PHACTS workflows were designed to collect the above information and identify drug discovery opportunities in the Vitamin D metabolism pathway.

Pathway targets and pharmacology
The pathway data obtained (from workflows 1 and 2, represented in Fig. 4) afforded several insights into the Vitamin D metabolism pathway; names of targets, number of compounds tested, their specificity for these targets and approved drugs in the pathway are shown in Table 3 and S5 Table. Other pathways where these targets are present are shown in S6 Table. From these data we see that out of the 10 targets in the pathway, 4139 unique compounds are reported to have activity against the target VDR and 545 for RXR-alpha, compared to 323 compounds for all the remaining targets combined (S3 File). This provides a quick overview on which targets in the pathway have been the focus of small molecule modulatory approaches and the ‘undruggable’ targets are identified - parathyroid hormone and CYP2R1/Vit D- 25 hydroxylase. Existing approved drugs in DrugBank for single protein targets are obtained via the ‘Target Information’ API. To complement this information, we obtained pharmacology data from ChEMBL for protein complexes consisting of pathway components
| No. | UniProt Accession | Name | Active compounds (bioactivities) | Approved drugs obtained via 2 methods | Comment |
|-----|-------------------|------|----------------------------------|--------------------------------------|---------|
| 1   | Q07973            | CYP24A1 cytochrome P450 | 26 (30) | 0 | |
| 2   | Q548T3            | CYP27B1 cytochrome P450 | 1 (1) | Ergocalciferol | |
|     |                   |      |                                  | Calcidiol | |
|     |                   |      |                                  | Calcitriol | |
| 3   | Q02318            | Sterol 26-hydroxylase, mitochondrial | 5 (5) | Cholecalciferol | |
| 4   | P02774            | Vitamin D-binding protein | 39 (112) | 0 | |
| 5   | Q9UBM7            | DHCR7 7-dehydrocholesterol reductase | 4 (5) | NADH | |
|     | Q15125, Q9UBM7    | AEBS | 66 (122) | 0 | Tamoxifen, Doxorubicin |
|     |                   |      |                                  | Target is protein complex AEBS (made of DHCR7 and D8- D7 sterol isomerase). Not listed in DrugBank 3.0 | |
| 7   | Q6VVX0            | CYP2R1 cytochrome P450 | 0 (0) | Cholecalciferol, Ergocalciferol | |
| 8   | P01270            | PTH parathyroid hormone | 0 (0) | 0 | |
| 9   | P19793            | RXRA retinoid X receptor, alpha | 545 (1274) | 0 | Tazarotene, Tretinoin |
|     |                   |      |                                  |              | Acitretin, Adapalene |
|     | P37231, P19793    | RXR alpha/PPAR gamma | 33 (65) | 0 | 0 |
| 10  | P28702            | RXRB retinoid X receptor, beta | 149 (261) | 0 | Tazarotene, Tretinoin |
|     |                   |      |                                  |              | Bexarotene, Acitretin, Adapalene |
| 11  | P11473            | VDR vitamin D (1,25- dihydroxyvitamin D3) receptor | 4139 (5918) | Calcipotriol | |

doi:10.1371/journal.pone.0115460.t003
using the ‘Target Pharmacology’ API. Indeed, no approved drugs are listed in DrugBank 3.0 for DHCR7; however our workflow retrieves Tamoxifen and Doxorubicin as they target the anti-estrogen binding site (AEBS), a protein complex comprising DHCR7 and D8-D7 sterol isomerase [49]. The integration of two disparate pharmacology databases (DrugBank and ChEMBL) provides a more complete listing of all approved drugs that have potent activity against any target in the pathway, whether it is a single protein or part of a complex. Thus, in one
Table 4. Compounds active against CYP24A1 obtained from Workflow 2.

| No. | Compound name                                                                 | Assay Description                                                                                       | Activity Type | Value | Unit | pChembl | Active against other targets in pathway? | Other targets in general? |
|-----|-------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------|---------------|-------|------|---------|------------------------------------------|----------------------------|
| 1   | 4'-Chloro-N-[2R]-2-(1H-imidazol-1-yl)-2-phenylethyl-4-biphenylcarboxamide      | Inhibition of CYP24A1                                                                                   | IC50          | 15    | nM   | 7.82    | No                                       | No                         |
| 2   | 1,3-cyclohexanediol, 4-methylen-5-{[2E]-2-[[1R,3aS,7aR]-octahydro-7a-methyl-1-[1(R)-1-methyl-3-(phenylsulfonyl)propyl]-4H-inden-4-ylidene]ethylidene], (1R,3S,5Z)- | Inhibition of human CYP24 hydroxylase expressed in V79 cells                                           | IC50          | 28    | nM   | 7.55    | No                                       | No                         |
| 3   | N-(2-imidazol-1-yl-2-phenyl-ethyl)-4-[(E)-styryl]benzamide                    | Inhibition of human recombinant CYP24A1 expressed in Chinese hamster V79 cells using [3H-1-beta]calcitriol after 60 mins by scintillation counting | IC50          | 300   | nM   | 6.52    | No                                       | No                         |
| 4   | 6-methoxy-2-(2-methylbenzyl)-3,4-dihydronaphthalen-1(2H)-one                  | Inhibition of CYP24A1 expressed in CHO cells                                                          | IC50          | 900   | nM   | 6.05    | No                                       | Liver microsomes, ADMET (CYP26A1) |
| 5   | N-[2-(4-fluorophenyl)-2-imidazol-1-yl-ethyl]benzofuran-2-carboxamide          | Inhibition of human recombinant CYP24A1 expressed in Chinese hamster V79 cells using [3H-1-beta]calcitriol after 60 mins by scintillation counting | IC50          | 2800  | nM   | 5.55    | No                                       | No                         |
| 6   | N-(2-imidazol-1-yl-2-phenyl-ethyl)benzofuran-2-carboxamide                   | Inhibition of human recombinant CYP24A1 expressed in Chinese hamster V79 cells using [3H-1-beta]calcitriol after 60 mins by scintillation counting | IC50          | 4000  | nM   | 5.40    | No                                       | No                         |
| 7   | N-(2-imidazol-1-yl-2-phenyl-ethyl)-5-nitro-benzofuran-2-carboxamide          | Inhibition of human recombinant CYP24A1 expressed in Chinese hamster V79 cells using [3H-1-beta]calcitriol after 60 mins by scintillation counting | IC50          | 6400  | nM   | 5.19    | No                                       | No                         |
| 8   | 4'-chloro-N-[2-(1H-imidazol-1-yl)-2-phenylethyl]biphenyl-4-carboxamide       | Inhibition of CYP24 in human keratinocytes                                                            | IC50          | 15    | nM   | 7.82    | 25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial | No                         |
| 9   | 1-[4-4-[[2S,4R]-2-(2,4-Dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxol-4-ylmethoxy]phenyl]-1-piperazinyl]jethanone | Inhibition of CYP24A1 in human epidermal keratinocytes                                                   | IC50          | 126   | nM   | 6.90    | RXRA, VDR                               | 340 different targets (ketoconazole) |
workflow, we could quickly assess the previously published chemical space of a pathway of interest.

**CYP24A1 as a therapeutic target**

The pathway pharmacology data clearly show that the majority of efforts have been focused on targeting the VDR directly ([Table 3](#tab3)). Targets for novel therapeutic strategies to enhance VDR activation could lie upstream of ligand-receptor binding, at the level of calcitriol catabolism by CYP24A1 ([50] or transport by Vitamin D- binding protein or DBP ([51]). CYP24A1 is the major catabolic enzyme of calcitriol converting it to less active calcitroic acid ([52]), so selectively inhibiting this enzyme can be expected to raise the circulating levels of the hormone or its analogues. Therefore, using Workflow 2 (represented in Fig. 4) we looked for compounds with inhibitory activity against CYP24A1 and found 25 unique compounds, of which 12 have IC$_{50}$, 10 uM ([Table 4](#tab4)).

Five of these compounds have potent activity against two other critical targets in the pathway, CYP27A1 and CYP27B1, the key activating enzymes producing calcitriol. One of these is ketoconazole, an approved drug for fungal infections that has been extensively tested against a variety of other targets in primary HTS and ADMET assays. The remaining seven compounds (five azoles and two non-azoles) could serve as starting points for selective CYP24A1 inhibition strategies given the lack of polypharmacology data and potential for off-target effects ([Table 4](#tab4)). In addition, our data show that CYP24A1 does not have a known role in pathways other than Vitamin D metabolism ([S6 Table](#tabS6)), so inhibiting this enzyme should not affect substrates other than calcitriol (or its analogues), resulting in the desired prolongation of VDR activation. Therefore, a drug combination strategy of inhibiting CYP24A1 with one of the above compounds, while activating VDR with the natural ligand or an analogue may be considered as a valid approach to enhance VDR signaling ([53]). Alternatively, evaluating a compound’s sensitivity to CYP24A1, in parallel to VDR activation would optimize

| No. | Compound name                                                                 | Assay Description                                      | Activity Type | Value | Unit | pChembl | Active against other targets in pathway? | Other targets in general? |
|-----|--------------------------------------------------------------------------------|--------------------------------------------------------|---------------|-------|------|---------|------------------------------------------|--------------------------|
| 10  | 2-(2-Ethylbenzyl)-6-methoxy-3,4-dihydro-1(2H)-naphthalenone                    | Inhibition of CYP24A1 expressed in CHO cells           | IC$_{50}$     | 1920  | nM   | 5.72    | Sterol 26-hydroxylase, mitochondrial     | Yes                      |
| 11  | 6-Methoxy-2-(2-(trifluoromethyl)-benzyl)-3,4-dihydro-1(2H)-naphthalenone      | Inhibition of CYP24A1 expressed in CHO cells           | IC$_{50}$     | 2080  | nM   | 5.68    | Sterol 26-hydroxylase, mitochondrial     | Yes                      |
| 12  | (2E)-6-Methoxy-2-{2-[(E)-2-phenylvinyl]benzylidene}-3,4-dihydro-1(2H)-naphthalenone | Inhibition of CYP24A1 expressed in CHO cells           | IC$_{50}$     | 5080  | nM   | 5.29    | Sterol 26-hydroxylase, mitochondrial     | Yes                      |

Compounds 1–7 ranked according to potency (in bold) have no activity against additional targets based on polypharmacology records, whereas compounds 8–12 inhibit calcitriol activating enzymes, VDR and RXRA.

doi:10.1371/journal.pone.0115460.t004
medicinal chemistry efforts to synthesize improved VDR ligands with better metabolic stability. Our polypharmacology (S3 File) data retrieved a vitamin D analogue (CHEMBL564855) with considerably less sensitivity to CYP24A1 catabolism (binding affinity to human CYP24A1 relative to calcitriol = 2%) compared to the natural hormone while having high binding affinity to VDR (binding affinity to bovine thymus VDR relative to calcitriol = 180%), that could serve as a starting point for this approach [54].
Evaluating compound affinity for VDR and DBP orthologues

There is considerable Structure Activity Relationship (SAR) data on the VDR as compared to the DBP, although the latter is a critical determinant of Vitamin D analogue availability in vivo. However, of the 669 human VDR-activating compounds retrieved, only two have been tested for human DBP binding (S3 File). The amino acid sequence of the VDR ligand-binding domain (residues 192–427) is highly conserved, with the bovine and porcine orthologues sharing 96% and 97% similarity, respectively, with that of the human VDR, allowing comparisons to be made for binding assays. We therefore expanded our search to orthologues of these two targets (S7 Table) to retrieve compounds with binding affinity data for VDR from three species (workflow 3 represented in Fig. 5). We identified 35 such compounds that also had binding affinity data for human DBP; a more reasonable number for SAR analysis (S4 File). Preliminary observations show that most compounds involve modifications of side chain or A-ring structures but a more limited set of four compounds are non-steroidal structures. Interestingly, these newer analogues have no affinity for DBP compared to the classical steroidal analogues but are capable of binding VDR with moderate affinity and moreover show lower calcemic activity [55]. It is reasonable to speculate that designing analogues with lower DBP binding will enable higher target tissue concentration and lower their lower calcemic effects in vivo. Indeed several reports describing other non-steroidal Vitamin D analogues can be found in the literature [56], [57], [58], [59], [60]. However, as they have been explicitly

### Table 5. Regulators of Vitamin D signaling obtained from Workflow 3.

| GO terms associated with Vitamin D regulation | Associated Genes (Human) | In pathway? |
|-----------------------------------------------|--------------------------|-------------|
| Parents                                      | Children                 | UniProt Accession | TargetName/Gen Name |          |
| GO:0010979 regulation of vitamin D 24-hydroxylase activity | GO:0010980 positive regulation of vitamin D 24-hydroxylase activity | O15528 | 25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial | YES |
| P11473 Vitamin D3 receptor | P935590 | YES |
| GO:0060556 regulation of vitamin D biosynthetic process | GO:0060557 positive regulation of vitamin D biosynthetic process | P01579 | Interferon gamma | NO |
| GO:0070562 regulation of vitamin D receptor signaling pathway | GO:0070564 positive regulation of vitamin D receptor signaling pathway | O15528 | 25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial | YES |
| Q13573 SNW domain-containing protein 1 | Q9GZV9 | NO |
| GO:0060556 regulation of vitamin D biosynthetic process | GO:0010957 negative regulation of vitamin D biosynthetic process | O43623 | Zinc finger protein SNAI2 | NO |
| O95863 Zinc finger protein SNAI1 | Q13573 | NO |
| GO:0070562 regulation of vitamin D receptor signaling pathway | GO:0070563 negative regulation of vitamin D receptor signaling pathway | O43623 | Zinc finger protein SNAI2 | NO |

Terms in bold are discussed in the text.

To view this table as it appears in the HTML version of this paper, please click on the table link.
tested for DBP binding, they could not be included in the SAR analysis set for non-secosteroidal analogues.

**Regulation of the pathway**

We used Gene Ontology (GO) annotations [22] for a preliminary assessment of factors that regulate Vitamin D signaling in general, and those that specifically regulate key enzymes in the pathway (Workflow 4 represented in Fig. 5). In addition to external factors, we identified pathway components that regulate Vitamin D signaling via inherent feedback loops. For example, CYP24A1, the main catabolic enzyme of 1,25(OH)_{2}D_{3} is upregulated by the VDR, providing an efficient negative feedback loop to terminate calcitriol actions in normal conditions (Table 5). Conversely, abnormally elevated CYP24A1 in certain disease states, such as hypophosphatemia [61], [62] and certain types of cancer [63] associates with decreased vitamin D status and with vitamin D resistance. CYP24A1 may thus be a predictive marker of 1,25(OH)_{2}D_{3} efficacy as an adjunctive therapy in patients with cancer. Next, we see that the transcription factors SNAIL1 and SNAIL2 repress Vitamin D signaling by inhibiting VDR expression (Table 5). Interestingly, these factors have been shown to be elevated in several types of cancers and thought to be the mechanism by which these cancers are resistant to tumor suppressor action by endogenous 1,25(OH)_{2}D_{3} [64], [65], [66]. Patients with high levels of SNAIL1 and SNAIL2 can be expected to have lower VDR expression and, therefore, will be poor responders to anti-cancer therapy with 1,25(OH)_{2}D_{3} or its analogs. Thus, tumor expression of SNAIL1 and SNAIL2 could also be used as biomarkers of adequacy for this type of therapy [67]. The GO annotations extended our knowledge of the interactions between pathway components to gain valuable insights into the mechanisms for feedback regulation, as well as identify potential biomarkers for selecting tumors most likely to respond to Vitamin D analogue therapy. In conclusion, knowledge of the Vitamin D metabolism pathway obtained through these workflows supports and informs on a multi-pronged drug discovery approach, wherein properties like DBP binding and sensitivity to CYP24A1 catabolism are evaluated in parallel using the appropriate bioassays, rather than focusing on VDR activation alone. An effective analogue should potently activate VDR, be resistant to catabolism by CYP24A1 and have low affinity for DBP. Alternatively, co-administration with a selective CYP24A1 inhibitor could also extend analogue lifetime. Most tissues express VDR, so tissue-specific actions of VDR ligands are instead governed by differential expression and regulation of CYP27B1, which permits localized synthesis of additional calcitriol, and CYP24A1, which inactivates the hormone. Tissue expression profiles as well as interacting proteins for a given target can be obtained in future versions of the Open PHACTS Discovery Platform with the incorporation of neXtProt data and tissue ontologies, thereby enabling a better prediction of 1,25(OH)_{2}D_{3} analogue efficiency in different cellular contexts.
Conclusions and Future Directions

The Open PHACTS Discovery Platform makes available the data needed to answer a wide range of questions applicable to pharmaceutical research by broadly covering critical aspects of chemistry and biology. A multitude of potential use cases of the Open PHACTS Discovery Platform can be envisaged: target identification and validation, discovery of interaction profiles of compounds and targets, detection of potential toxic interactions, repositioning of existing drugs to new therapeutic areas, and many other drug discovery questions [6]. We present three challenging example use cases to demonstrate the requirement for comprehensive integration from multiple data sources to address real world questions. Workflows systems (e.g. KNIME nodes and Pipeline Pilot components) using the Open PHACTS Discovery Platform enable the seamless integration between pathway, target, and compound, permitting retrieval of diverse and complex data from one interface. Additionally, working via the Open PHACTS API solves many unrealized data integration problems for the individual scientist by tackling in the background, data licensing, formatting, and querying issues. Moreover, some of these issues have been further assessed by an empirical evaluation to benchmark improvements across a number of Semantic Web technologies [68]. Most importantly, the platform retains and gives full transparency on data provenance. The Open PHACTS Discovery Platform not only creates connections between heterogeneous data sets but also provides the tools that can help scientist exploit the data available from the API.

The three exemplar use cases demonstrate how the application of Open PHACTS API services can support drug-discovery research. One workflow emphasizes a search strategy across proprietary and public pharmacology databases for a comprehensive identification of chemical compounds targeting the dopamine receptor D2. Using a proprietary dictionary generated for in-house data, the different target and compound nomenclatures were reconciled with the public domain data for a comprehensive and meaningful ranking of existing chemical compounds active against the target of interest. The other use case examples leverage the semantically integrated knowledge in the Open PHACTS Discovery Platform on pathways to derive testable hypotheses concerning therapeutic targets. The two pathways, ErbB signaling and Vitamin D metabolism, are representative of a) complex regulatory processes involving a large number of druggable targets and corresponding chemical compounds, and b) comparatively simple and well-defined metabolic processes with few druggable targets. The differences between the two pathways serve to highlight divergent analyses possible via differently combined queries. In one case, pharmacological bioactivity data and its enrichment by integrated annotation terms originating from GO and the ChEBI ontology was turned into a reasonable number of data points, and visualized as heat map representations. While in the other case, key pathway targets (VDR, CYP24A1 and DBP) were explicitly evaluated to identify strategies for designing improved Vitamin D analogues with the desired bioactivity profile.
The workflows developed for the present use cases can be broadly used by drug discovery scientists to exploit the wealth of publicly available information for other targets and pathways of interest. As all the accessed data sets reside in the public domain, the results from the present use cases could, in principle, be derived without the use of the Open PHACTS Discovery Platform. However, it has been previously demonstrated that manual access methods require considerable time and resource investment due to the complexity of data access and licensing for multiple databases, the use of different data formats and identifiers, need for bio- and chemo-informatics expertise and post-processing of data retrieved [1], [6]. Such an exercise is non-trivial for scientists unskilled in programming languages or database management. By providing these example workflows, we hope to encourage the use of the technology to a wide research audience to increases the productivity of both academic and industrial drug discovery projects. Features of the Open PHACTS Discovery Platform useful for our research questions are summarized in Table 6.

Together, these examples serve to demonstrate some of the operations made possible via a semantically integrated pharmacology platform. A plethora of other queries requiring the linkage of target-compound-pathway concepts can be envisioned and answered by combining an appropriate sequence of API calls with workflow tools; and, the possibilities for new use cases continue to grow as more data sources are added to the platform. In future releases of the platform, gene-disease association data, protein sequence features, and tissue expression data are scheduled for integration. Additionally, many opportunities exist for the inclusion of new data sets such as text mining data from scientific publications and patents as well as proprietary or commercial data sources [11]. Going forward, the continuation of the infrastructure development and data integration will be carried out in the context of the Open PHACTS Foundation (http://www.openphactsfoundation.org). The Open PHACTS Foundation is the not-for-profit

| Benefits of using the Open PHACTS platform for drug discovery research |
|-------------------------------------------------------------|
| Mapping identifiers to external databases not required       |
| Avoids different interfaces to online knowledge and the need to go back and forth between protein, pathway and bioactivity databases |
| All integrated data available under “Creative Commons” type licenses |
| Bioactivity values normalized via QUDT ontology              |
| Getting approved drug status for a list of compounds, including those responsible for off-target or non-approved indications |
| Getting a list of homologues for a given target              |
| Integration of ontology tags, i.e. from GO and ChEBI and hierarchies with other datasets |
| Data provenance facilitates enrichment of knowledge from primary literature |
| Possibility to create specialized API-compatible KNIME nodes to enable other user-defined queries |
| Existing pipelining workflows can be re-used entirely or in modules to answer other research questions |
| Results can be easily updated to benefit from future upgrades to the Open PHACTS platform |

doi:10.1371/journal.pone.0115460.0006
successor organization set up to sustain and continue the growth of the achievements of the Open PHACTS project. Specifically the mission is to maintain a sustainable, open, vibrant and interoperable information infrastructure for applied life science research and development.

**Supporting Information**

S1 Fig. Pipeline Pilot workflows for retrieving data for Use Case A; lines 1, 2, and 3 show the components used for retrieving data from Open PHACTS discovery platform; lines 4 and 5 show the components used for retrieving data from Thomson Reuters; and, lines 6, 7, and 8 show the components used for retrieving data from GVKBio GOSTAR.

doi:10.1371/journal.pone.0115460.s001 (TIF)

S2 Fig. Binary heatmap representation of the pharmacological space in the human ErbB signalling pathway (considering ‘-logActivity values [molar]’ and a cutoff of 6); abscissae: targets with ChEMBL target ID’s; ordinate: compounds; red bars indicate ‘actives’, blue bars ‘inactives’, grey areas indicate that no activity value was reported.

doi:10.1371/journal.pone.0115460.s002 (TIF)

S3 Fig. Binary heatmap representation for compounds annotated with ‘antineoplastic agent’ in ChEBI (considering ‘-logActivity values [molar]’ and a cutoff of 6); abscissae: targets with ChEMBL target ID’s; ordinate: compounds; red bars indicate ‘actives’, blue bars ‘inactives’, grey areas indicate that no activity value was reported.

doi:10.1371/journal.pone.0115460.s003 (TIF)

S1 Table. List of current resources available through the Open PHACTS Discovery Platform.

doi:10.1371/journal.pone.0115460.s004 (XLSX)

S2 Table. Examples of free text and URI inputs used in the API calls.

doi:10.1371/journal.pone.0115460.s005 (XLSX)

S3 Table. List of all GO ‘biological process’ terms that have been annotated to at least 5 of the 23 prioritized targets (plus ChEMBL target IDs of those targets).

doi:10.1371/journal.pone.0115460.s006 (XLSX)

S4 Table. List of all ChEBI classification terms for the 23 prioritized targets that have been annotated to at least 6 compounds.

doi:10.1371/journal.pone.0115460.s007 (XLSX)

S5 Table. Specificity of compounds targeting proteins in the Vitamin D pathway.

doi:10.1371/journal.pone.0115460.s008 (XLSX)

S6 Table. Additional pathways for targets in the Vitamin D pathway.

doi:10.1371/journal.pone.0115460.s009 (XLSX)
Acknowledgments

The authors would like to acknowledge the contribution of the many Open PHACTS Consortium members for their various critical inputs to scientific discussions, manuscript preparation and general insight. A list of Consortium members can be found here: https://www.openphacts.org/partners/consortium. The authors also wish to acknowledge the input of Prof. Roman Perez-Fernandez (University of Santiago de Compostela) in the form of helpful discussions regarding the Vitamin D pathway and its relevance to public health.

Author Contributions

Conceived and designed the experiments: JR BZ DD JMN HT AW GB CHC LR CTE EJ SS MIL GFE CC. Performed the experiments: BZ DD ECR JMN AW CC. Analyzed the data: JR BZ JMN DD ECR CC HT LR. Contributed reagents/materials/analysis tools: BZ RS CHC DD JMN ECR LR AW. Wrote the paper: JR BZ DD HT CC. Aided in manuscript preparation: GB CTE JB. Supervised the study: EJ SS MIL GFE CC.

References

1. Lanfear J (2002) Dealing with the data deluge. Nat Rev Drug Discov 1: 479.
2. Samwald M, Jentzsch A, Bouton C, Kallesoe CS, Willighagen E, et al. (2011) Linked open drug data for pharmaceutical research and development. J Cheminform 3: 19-2946-3-19.
3. Keiser MJ, Setola V, Irwin JJ, Laggner C, Abbas Al, et al. (2009) Predicting new molecular targets for known drugs. Nature 462: 175–181.
4. Bender A, Young DW, Jenkins JL, Serrano M, Mikhailov D, et al. (2007) Chemogenomic data analysis: Prediction of small-molecule targets and the advent of biological fingerprint. Comb Chem High Throughput Screen 10: 719–731.

5. Gregori-Puigjane E, Setola V, Hert J, Crews BA, Irwin JJ, et al. (2012) Identifying mechanism-of-action targets for drugs and probes. Proc Natl Acad Sci U S A 109: 11178–11183.

6. Williams AJ, Harland L, Groth P, Pettifer S, Chichester C, et al. (2012) Open PHACTS: Semantic interoperability for drug discovery. Drug Discov Today 17: 1188–1198.

7. Gray AJ, Groth P, Loizou A, Askjaer S, Brenninkmeijer C, et al. (2014) Applying Linked Data Approaches to Pharmacology: Architectural Decisions and Implementation. Semantic Web Journal 10.3233/SW-2012-0088

8. Belleau F, Nolin MA, Tourigny N, Rigault P, Morissette J (2008) Bio2RDF: Towards a mashup to build bioinformatics knowledge systems. J Biomed Inform 41: 706–716.

9. Chen B, Dong X, Jiao D, Wang H, Zhu Q, et al. (2010) Chen2Bio2RDF: A semantic framework for linking and data mining chemogenomic and systems chemical biology data. BMC Bioinformatics 11: 255-2105-11-255.

10. Hardy B, Douglas N, Helma C, Rautenberg M, Jeliakova N, et al. (2010) Collaborative development of predictive toxicology applications. J Cheminform 2: 7-2946-2-7.

11. Azzouzi K, Jacoby E, Senger S, Rodriguez EC, Loza M, et al. (2013) Scientific competency questions as the basis for semantically enriched open pharmacological space development. Drug Discov Today 18: 843–852.

12. Kelder T, van Iersel MP, Hanspers K, Kutmon M, Conklin BR, et al. (2012) WikiPathways: Building research communities on biological pathways. Nucleic Acids Res 40: D1301–7.

13. Berthold M, Cebron N, Dill F, Gabriel T, Kotter T, et al. (2008) KNIME: The konstanz information miner. In: Preischach C, Burkhardt H, Schmidt-Thieme L, Decker R, editors.: Springer Berlin Heidelberg. pp.319–326.

14. Accelrys (2010) Pipeline pilot. Available: http://accelrys.com/products/pipeline-pilot/. Accessed 2014 Nov 30.

15. Gaulton A, Bellis LJ, Bento AP, Chambers J, Davies M, et al. (2012) ChEMBL: A large-scale bioactivity database for drug discovery. Nucleic Acids Res 40: D1100–7.

16. Bento AP, Gaulton A, Hersey A, Bellis LJ, Chambers J, et al. (2014) The ChEMBL bioactivity database: An update. Nucleic Acids Res 42: D1083–90.

17. Degtyarenko K, de Matos P, Ennis M, Hastings J, Zbinden M, et al. (2008) ChEBI: A database and ontology for chemical entities of biological interest. Nucleic Acids Res 36: D344–50.

18. de Matos P, Alcantara R, Dekker A, Ennis M, Hastings J, et al. (2010) Chemical entities of biological interest: An update. Nucleic Acids Res 38: D249–54.

19. Hastings J, de Matos P, Dekker A, Ennis M, Harsha B, et al. (2013) The ChEBI reference database and ontology for biologically relevant chemistry: Enhancements for 2013. Nucleic Acids Res 41: D456–63.

20. Knox C, Law V, Jewison T, Liu P, Ly S, et al. (2011) DrugBank 3.0: A comprehensive resource for ‘omics’ research on drugs. Nucleic Acids Res 39: D1035–41.

21. Williams AJ, Tkachenko V, Golotvin S, Kidd R, McCann G (2010) ChemSpider - building a foundation for the semantic web by hosting a crowd sourced databasing platform for chemistry. Journal of Cheminformatics 2: O16–O16. doi: 10.1186/1758-2946-2-s1-o16

22. Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, et al. (2000) Gene ontology: Tool for the unification of biology, the gene ontology consortium. Nat Genet 25: 25–29.

23. Hill DP, Smith B, McC Andrews-Hill MS, Blake JA (2008) Gene ontology annotations: What they mean and where they come from. BMC Bioinformatics 9 Suppl 5:: S2-2105-9-S5-S2.

24. Apweiler R, Bairoch A, Wu CH, Barker WC, Boeckmann B, et al. (2004) UniProt: The universal protein knowledgebase. Nucleic Acids Res 32: D115–9.

25. Bairoch A (2000) The ENZYME database in 2000. Nucleic Acids Res 28: 304–305.
26. Chichester C, Mons B (2011) Collaboration and the Semantic Web. In: Collaborative Computational Technologies for Biomedical Research. Ekins S, Hupcey MAZ, Williams AJ editors. John Wiley & Sons, Inc. New Jersey. pp453–465

27. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, et al. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25: 3389–3402.

28. Berridge KC (2007) The debate over dopamine’s role in reward: The case for incentive salience. Psychopharmacology (Berl) 191: 391–431.

29. Beaulieu JM, Gainetdinov RR (2011) The physiology, signaling, and pharmacology of dopamine receptors. Pharmacol Rev 63: 182–217.

30. Sibley DR, Monsma FJ Jr (1992) Molecular biology of dopamine receptors. Trends Pharmacol Sci 13: 61–69.

31. Civelli O, Bunzow JR, Grandy DK (1993) Molecular diversity of the dopamine receptors. Annu Rev Pharmacol Toxicol 33: 281–307.

32. Seeman P (2010) Dopamine D2 receptors as treatment targets in schizophrenia. Clin Schizophr Relat Psychoses 4: 56–73.

33. Hynes NE, Lane HA (2005) ERBB receptors and cancer: the complexity of targeted inhibitors. Nat Rev Cancer 5: 341–354

34. Gondi CS, Dinh DH, Klopfenstein JD, Gujrati M, Rao JS (2009) MMP-2 downregulation mediates differential regulation of cell death via ErbB-2 in glioma xenografts. Int J Oncol 35: 257–263.

35. Yarden Y, Sliwkowski MX (2001) Untangling the ErbB signaling network. Nat Rev Mol Cell Biol 2: 127–137.

36. Yarden Y, Pines G (2012) The ERBB network: at last, cancer therapy meets systems biology. Nat Rev Cancer 12: 553–563.

37. Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, et al. (2006) Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell 127: 635–648.

38. Ridolfi E, Barone C, Scarpini E, Galimberti D (2013) The role of the innate immune system in Alzheimer’s disease and frontotemporal lobar degeneration: an eye on microglia. Clin Dev Immunol 2013: 939786.

39. Blach-Olszewska Z, Leszek J (2007) Mechanisms of over-activated innate immune system regulation in autoimmune and neurodegenerative disorders. Neuropsychiatr Dis Treat 3: 365–372.

40. Holick MF, Schnoes HK, DeLuca HF (1971) Identification of 1,25-dihydroxycholecalciferol, a form of vitamin D3 metabolically active in the intestine. Proc Natl Acad Sci U S A 68: 803–804.

41. Fleet JC (2006) Molecular regulation of calcium and bone metabolism through the vitamin D receptor. J Musculoskelet Neuronal Interact 6: 336–337.

42. Haussler MR, Whitfield GK, Haussler CA, Hsieh JC, Thompson PD, et al. (1998) The nuclear vitamin D receptor: Biological and molecular regulatory properties revealed. J Bone Miner Res 13: 325–349.

43. Deluca HF, Cantorna MT (2001) Vitamin D: Its role and uses in immunology. FASEB J 15: 2579–2585.

44. Mathieu C, Adorini L (2002) The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. Trends Mol Med 8: 174–179.

45. van Etten E, Mathieu C (2005) Immunoregulation by 1,25-dihydroxyvitamin D3: Basic concepts. J Steroid Biochem Mol Biol 97: 93–101.

46. Baeke F, Etten EV, Overbergh L, Mathieu C (2007) Vitamin D3 and the immune system: Maintaining the balance in health and disease. Nutr Res Rev 20: 106–118.

47. Brown AJ, Slatopolsky E (2008) Vitamin D analogs: Therapeutic applications and mechanisms for selectivity. Mol Aspects Med 29: 433–452.

48. Fleet JC (2008) Molecular actions of vitamin D contributing to cancer prevention. Mol Aspects Med 29: 388–396.

49. Kedjouar B, de Medina P, Oulad-Abdelghani M, Payre B, Silvente-Poirot S, et al. (2004) Molecular characterization of the microsomal tamoxifen binding site. J Biol Chem 279: 34048–34061.
50. Jones G, Prosser DE, Kaufmann M (2012) 25-hydroxyvitamin D-24-hydroxylase (CYP24A1): Its important role in the degradation of vitamin D. Arch Biochem Biophys 523: 9–18.

51. Gomme PT, Bertolini J (2004) Therapeutic potential of vitamin D-binding protein. Trends Biotechnol 22: 340–345.

52. Prosser DE, Jones G (2004) Enzymes involved in the activation and inactivation of vitamin D. Trends Biochem Sci 29: 664–673.

53. Luo W, Hershberger PA, Trump DL, Johnson CS (2013) 24-hydroxylase in cancer: Impact on vitamin D-based anticancer therapeutics. J Steroid Biochem Mol Biol 136: 252–257.

54. Saito N, Suhara Y, Abe D, Kusudo T, Ohta M, et al. (2009) Synthesis of 2alpha-propoxy-1alpha,25-dihydroxyvitamin D3 and comparison of its metabolism by human CYP24A1 and rat CYP24A1. Bioorg Med Chem 17: 4296–4301.

55. Zhou X, Zhu GD, Van Haver D, Vandewalle M, De Clercq PJ, et al. (1999) Synthesis, biological activity, and conformational analysis of four seco-D-15,19-bisnor-1alpha,25-dihydroxyvitamin D analogues, diastereomeric at C17 and C20. J Med Chem 42: 3539–3556.

56. Boehm MF, Fitzgerald P, Zou A, Elgort MG, Bischoff ED, et al. (1999) Novel nonsecosteroidal vitamin D mimics exert VDR-modulating activities with less calcium mobilization than 1,25-dihydroxyvitamin D3. Chem Biol 6: 265–275.

57. Swann SL, Bergh J, Farach-Carson MC, Ocasio CA, Koh JT (2002) Structure-based design of selective agonists for a rickets-associated mutant of the vitamin d receptor. J Am Chem Soc 124: 13795–13805.

58. Perakyla M, Malinen M, Herzig KH, Carlberg C (2005) Gene regulatory potential of nonsteroidal vitamin D receptor ligands. Mol Endocrinol 19: 2060–2073.

59. Ma Y, Khalifa B, Yee YK, Lu J, Memezawa A, et al. (2006) Identification and characterization of noncalcemic, tissue-selective, nonsecosteroidal vitamin D receptor modulators. J Clin Invest 116: 892–904.

60. Asano L, Ito I, Kuwabara N, Waku T, Yanagisawa J, et al. (2013) Structural basis for vitamin D receptor agonism by novel non-secosteroidal ligands. FEBS Lett 587: 957–963.

61. Bai X, Miao D, Goltzman D, Karaplis AC (2007) Early lethality in Hyp mice with targeted deletion of Pth gene Endocrinology, 148 (10). pp.4974–4983.

62. Roy S, Martel J, Ma S, Tenenhouse HS (1994) Increased renal 25-hydroxyvitamin D3-24-hydroxylase messenger ribonucleic acid and immunoreactive protein in phosphate-deprived hyp mice: A mechanism for accelerated 1,25-dihydroxyvitamin D3 catabolism in X-linked hypophosphatemic rickets. Endocrinology 134: 1761–1767.

63. Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. (2006) Expression of VDR and CYP24A1 mRNA in human tumors. Cancer Chemother Pharmacol 57: 234–240.

64. Larriba MJ, Martin-Villar E, Garcia JM, Pereira F, Pena C, et al. (2009) SNAIL2 cooperates with SNAIL1 in the repression of vitamin D receptor in colon cancer. Carcinogenesis 30: 1459–1468.

65. Larriba MJ, Casado-Vela J, Pendas-Franco N, Pena R, Garcia de Herreros A, et al. (2010) Novel SNAIL1 target proteins in human colon cancer identified by proteomic analysis. PLoS One 5: e10221.

66. Palmer HG, Larriba MJ, Garcia JM, Ordonez-Moran P, Pena C, et al. (2004) The transcription factor SNAIL represses vitamin D receptor expression and responsiveness in human colon cancer. Nat Med 10: 917–919.

67. Larriba MJ, Bonilla F, Munoz A (2010) The transcription factors Snail1 and Snail2 repress vitamin D receptor during colon cancer progression. J Steroid Biochem Mol Biol 121: 106–109.

68. Loizou A, Angles R, Groth P (2014) On the Formation of Performant SPARQL Queries. Submitted: Journal of Web Semantics, June 2014