Database for drug metabolism and comparisons, NICEdrug.ch, aids discovery and design

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Abstract

The discovery of a new drug takes around 14 years and enormous research efforts and financial investments, with a high risk of failure. To reduce this burden, we developed the NICEdrug.ch database, which incorporates 250,000 bioactive molecules, and studies their metabolic targets, fate, and toxicity. NICEdrug.ch includes a new reactive site centric fingerprint to identify reactive similarity between drug/drug and drug/metabolite pairs. We use NICEdrug.ch to identify all potential inhibited enzymes by the anticancer drug 5fluorouracil, explain new reasons for its toxicity, and suggest avenues to alleviate its side effects. We validate clustering based on reactive

site centric fingerprint in statins and identify drugs for repurposing. Last, we propose shikimate 3phosphate to target liver-stage malaria parasites with minimal impact on the human host cell. The NICEdrug.ch database is accessible online for researchers and clinicians to systematically identify reactivity of new small molecules and druggable enzymes with practical applications in lead discovery and drug repurposing.

Keywords

Ligand-based drug discovery, drug clustering, drug repurposing, drug metabolic fate, prodrug, drug similarity, drug toxicity, drug side effects, reactive site similarity, enzyme inhibition.

Introduction

New small molecules are always needed to assure effective therapies for previously untreated illness, emerging diseases, and personalized medicine, but the process to develop new drugs is complex, costly, and time consuming. This is especially problematic considering about 90% of drug candidates in clinical trials are discarded due to unexpected toxicity or other secondary effects. This inefficiency threatens our health care system and economy (Wong et al., 2019). Improving how we discover and design new drugs could reduce the time and costs involved in the developmental pipeline and hence is of primary importance to define efficient medical therapies.

Current drug discovery techniques often involve high-throughput screens with candidates and a set of target enzymes presumably involved in a disease, which leads to the selection for those candidates with the preferred activity. However, the biochemical space of small molecules and possible targets in the cell is huge, which limits the possible experimental testing. Computational methods for drug pre-screening and discovery are therefore promising. *In silico,* one can systematically search the maximum biochemical space for targets and molecules with desired structures and functions to narrow down the molecules to test experimentally.

There are two main *in silico* strategies for drug discovery: a data-driven approach based on machine learning, or a mechanistic approach based on the available biochemical knowledge. Machine learning (ML) has been successfully used in all stages of drug discovery, from the

prediction of targets to the toxicity and side effects of drug candidates, as shown in some recent studies (Shilo et al., 2020; Stokes et al., 2020; Vamathevan et al., 2019). However, ML approaches require big, high-quality data sets of drug activity and associated physiology (Vamathevan et al., 2019), which might be challenging to obtain when studying drug action mechanisms and side effects in humans. ML also uses trained neural networks, which can lack interpretability and repeatability. This can make it difficult to explain why the neural networks has chosen a specific result, why it unexpectedly failed for an unseen dataset, and the final results may vary (Vamathevan et al., 2019).

Mechanistic-based approaches can also rationally identify small molecules in a desired system and do not require such large amounts of data. Such methods commonly screen based on structural similarity to a native enzyme substrate (antimetabolite) or to a known drug (for drug repurposing), considering the complete structure of a molecule to extract information about protein-ligand fitness (Jarvis and Ouvry, 2019; Verlinde and Hol, 1994). However, respecting enzymatic catalysis, the reactive sites and neighboring atoms play a more important role than the rest of the molecule when assessing molecular reactivity (Hadadi et al., 2019), and this is normally not accounted for. Furthermore, neither ML nor mechanistic-based approaches consider the metabolism of the patient, even though the metabolic fate of the drug and the existence of additional targets in the cell might give rise to toxicity. To our knowledge, no available method accounts for the human biochemistry to refine the search of drugs.

In this study, we present the development of the NICEdrug.ch database using a more holistic and updated approach to a traditional mechanistic-based screen by (1) adding a more detailed analysis of drug molecular structures and target enzymes based on structural aspects of enzymatic catalysis and (2) accounting for drug metabolism in the context of human biochemistry. NICEdrug.ch assesses the similarity of the reactivity between a drug candidate and a native substrate of an enzyme based on their common reactive sites and neighboring atoms (i.e., the NICEdrug score) in an analogous fashion as the computational tool BridgIT (Hadadi et al., 2019). It also identifies all biochemical transformations in the cellular metabolism that can modify and degrade a drug candidate using the reaction prediction tool BNICE.ch (Finley et al., 2009; Hadadi and Hatzimanikatis, 2015; Hadadi et al., 2016; Hafner et al., 2020; Hatzimanikatis

et al., 2005; Henry et al., 2010; Soh and Hatzimanikatis, 2010; Tokic et al., 2018). With NICEdrug.ch, we automatically analyzed the functional, reactive, and physicochemical properties of around 250,000 small molecules to suggest the action mechanism, metabolic fate, toxicity, and possibility of drug repurposing for each compound.

To validate NICEdrug.ch, we (1) show how it provides a comprehensive understanding of the enzymatic inhibition caused by the anticancer drug 5-fluorouracil or the downstream products of its metabolic fate; (2) use a case study of 5-fluorouracil to demonstrate how NICEdrug.ch can predict the toxic effects of downstream metabolic products, also identifying new possible mechanisms for this drug's toxicity; (3) demonstrate how NICEdrug.ch benefits drug repurposing in a case study of statins; and (4) prove the application of NICEdrug.ch to identify drugs that target essential enzymes for liver-stage development of a malaria parasite and have minimal impact on the human host cell. Strikingly, the analysis of drug candidates to target liver-stage malaria is in high agreement with previously proven drugs (Antonova-Koch et al., 2018), and NICEdrug.ch further reveals their inhibitory mechanism. We propose to repurpose shikimate 3-phosphate to target liver-stage malaria parasites and their essential shikimate metabolic pathway (Stanway et al., 2019), with non-observed toxicity in human cell (Díaz-Quiroz et al., 2018). Finally, we provide NICEdeug.ch as an online resource (https://lcsb-databases.epfl.ch/Nicedrug). Overall, NICEdrug.ch combines knowledge of molecular structures, enzymatic reaction mechanisms (as included in BNICE.ch (Finley et al., 2009; Hadadi and Hatzimanikatis, 2015; Hadadi et al., 2016; Hafner et al., 2020; Hatzimanikatis et al., 2005; Henry et al., 2010; Soh and Hatzimanikatis, 2010; Tokic et al., 2018)), and cellular biochemistry (currently human, *Plasmodium*, and *Escherichia coli* metabolism) to provide a promising and innovative approach to accelerate the discovery and design of novel drugs.

Results

Discovery of 200,000 molecules one reaction step away from known drugs.

To have a collection of all small molecules presumably suitable for treating human disease, we gathered over 70,000 existing molecules from three source databases: KEGG, ChEMBL, and DrugBank (Figure S1, Materials and Methods). We eliminated duplicate molecules, curated

available information, computed thermodynamic properties, and applied the Lipinski rules (Lipinski et al., 2001) to keep in NICEdrug.ch only molecules that have drug-like properties (Figure 1, Materials and Methods). NICEdrug.ch currently includes 48,544 unique small molecules from the source databases.

To evaluate the reactivity of the 48,544 drugs, we searched for all possible reactive sites on each drug with BNICE.ch (Hatzimanikatis et al., 2005) (Figure 1, Materials and Methods). All of the 48,544 drugs contain at least one reactive site and hence might be reactive in a cell. In total, we identified more than 5 million potential reactive sites (183k unique) on the 48,544 molecules and matched them to a corresponding enzyme by assigning them to an Enzyme Commission (E.C.) number. All of these enzymes belong to the human metabolic network (Materials and Methods). Interestingly, 10.4% of identified reactive sites correspond to the p450 class of enzymes, which are responsible for breaking down compounds in the human body by introducing reactive groups on those compounds, also known as phase I of drug metabolism (Figure S2A). The sites that were identified varied greatly from simple and small (i.e., comprising a minimum number of one atom) to more complex sites that covered large parts of the molecule (Figure S2B).

Given the important role of metabolism in the biochemical transformations and toxicity of drugs, we investigated the metabolism of the 48,544 input molecules in human cells. We predicted the downstream metabolites of all NICEdrug.ch small molecules in a human cell (i.e., reacting with known human metabolites and cofactors) using a retro-biosynthetic analysis with BNICE.ch (Figure 1, Materials and Methods). With this approach, we discovered 197,246 unique compounds connected to the input drugs via one step or reaction (products of the first generation), and the associated hypothetical biochemical neighborhood consists of 630,449 reactions (Figure S2). The 197,246 unique compounds are part of a new set of bioactive molecules in NICEdrug.ch that might act as drugs or prodrugs (i.e., compounds that are metabolized into active drugs) in a human cell. We stored the total number of 245,790 small molecules (i.e., the curated set of 48,544 drugs and the new set of 197,246 bioactive compounds), their calculated

properties, and biochemistry in our open-access database of drug metabolism, NICEdrug.ch (Materials and Methods).

To boost the usage of NICEdrug.ch for the identification of pairs of drug-drug or drug-metabolite sharing reactivity and target enzymes, we developed a new metric called the *NICEdrug score* (Figure S3). The NICEdrug score is calculated based on the information of the structure of the reactive site and its neighborhood (as computed using the BridgIT methodology (Hadadi et al., 2019)) that is stored in the form of a fingerprint (Materials and Methods). The fingerprint of a molecule's reactive site and neighborhood, or *reactive site-centric fingerprint,* serves to compare this site-specific similarity with other molecules. We recently showed that the reactive site-centric fingerprint of a reaction provides a better predictive measure of similar reactivity than the overall molecular structure (Hadadi et al., 2019). Here, we generated reactive site-centric fingerprints for all 20 million reactive sites identified in the 48,544 drugs and 197,246 one-step away molecules included in NICEdrug.ch. The 20 million reactive site-centric fingerprints for the total 245,790 small molecules are available in NICEdrug.ch to be used in similarity comparisons and classification of molecules (Materials and Methods).

NICEdrug.ch enables us to generate reports that define the hypothetical reactivity of a molecule, the molecule's reactive sites as identified by target enzymes, and the NICEdrug score between drug-drug and drug-metabolite pairs. The NICEdrug.ch reports can be used for three main applications: (1) to identify the network of metabolism for small molecules; (2) to suggest drug repurposing; and (3) to evaluate the druggability of an enzyme in a desired cell or organism (Figure 1), as we show in the next result sections. Currently, NICEdrug.ch includes metabolic information for human cells, a malaria parasite, and *Escherichia coli*. However, it is easily extendible to other organisms in future.

NICEdrug.ch suggests inhibitory mechanisms of the anticancer drug 5-FU and avenues to alleviate its toxicity.

As a case study, we used NICEdrug.ch to investigate the mode of action and metabolic fate of one of the most commonly used drug to treat cancer, 5-fluorouracil (5-FU), by exploring its

reactivity and the downstream products, or intermediates, that are formed during the cascade of biochemical transformations. Intermediates in the metabolism of 5-FU interferes with DNA synthesis as an antimetabolite (Longley et al., 2003), meaning that its various intermediates like 5-fluorodeoxyuridine monophosphate (FdUMP) are similar enough to naturally occurring substrates and they can act as competitive inhibitors in the cell.

To study the potential transformation products of 5-FU, we generated all possible intermediates up to four reaction steps away from the drug (Table S1), which is a reasonable range to occur in the body after 5-FU treatment (Testa, 2010). This analysis identified 407 compounds (90 biochemical and 317 chemical molecules) that have the biochemical potential to inhibit certain enzymes in human cell. We assessed the NICEdrug score of the intermediates compared to the native human metabolites of these enzymes, which ranged from no similarity (NICEdrug score of 0) to the equivalent substructure on compound (NICEdrug score of 1). More importantly, some of the 407 metabolites were known inhibitors that have been investigated for their implication in 5-FU toxicity. The remaining majority of the identified compounds do not have any known effects on human metabolism and could therefore serve as avenues for future research into alleviating the side effects of this drug.

We investigated these 407 compounds in more detail, looking first at the set of already validated metabolite inhibitors. 5-Fluorouridine (two steps or reactions away from 5-FU) and UDP-L-arabinofuranose (four steps away from 5-FU) are very similar to uridine, with NICEdrug scores of 0.95 and 1, respectively. Uridine is recognized as a substrate by two human enzymes, cytidine deaminase (EC: 3.5.4.5) and 5'-nucleotidase (EC: 3.1.3.5) (Figure 2). Therefore, NICEdrug.ch predictions show that the degradation metabolism of 5-FU generates downstream molecules similar to uridine, which likely leads to the inhibition of these two enzymes. This effect has already been investigated as a potential method for reducing the toxicity of 5-FU, wherein it was proposed that high concentrations of uridine could compete with the toxic 5-FU metabolites (Ma et al., 2017).

NICEdrug.ch also identified a few potential metabolites that have not been previously studied for their effects. These metabolites share a reactive site with native human metabolites and differ slightly in the reactive site neighborhood, and we refer to them as *para-metabolites (Sartorelli*

and Johns, 2013). 6-Methyl-2'-deoxyadenosine, purine-deoxyribonucleoside, and 2'-deoxyisoguanosine structurally resemble the reactive site neighborhood of deoxyadenosine, with respective NICEdrug scores of 1, 1, and 0.91. Similarly, 2-aminoadenosine, 2-chloroadenosine, and 2-methylaminoadenosine (four steps from 5-FU) have the same reactive site neighborhood as adenosine, with NICEdrug scores of 1, 1, and 0.96, respectively. Adenosine and deoxyadenosine are both native substrates of the adenosine kinase (EC: 2.7.1.20) and 5'-nucleotidase (EC: 3.1.3.5) (Figure 2). Therefore, we suggest that the 5-FU derivatives 2-aminoadenosine and 2-chloroadenosine are competitive inhibitors for the two enzymes adenosine kinase and 5'-nucleotidase. With these new insights from NICEdrug.ch, we hypothesize that co-administering adenosine or deoxyadenosine and uridine (Figure 2) with 5-FU might be required to reduce its toxic effects and hopefully alleviate the side effects of the 5-FU cancer treatment.

In the previous case study, we have shown inhibitors that contain the identical active site to the native enzyme. However, a slightly different reactive site might still be able to bind to an enzyme and compete with a native substrate, also defined as *anti-metabolite* (Matsuda et al., 2014). We explored the possibility of competitive substrate binding for each 5-FU intermediate in two steps. We first identified all atoms around a reactive site to compare the binding characteristics between the native molecule and putative inhibitor. Next, we compared the reactive sites of the native molecule and the putative inhibitor, and we scored the latter based on the similarity (Materials and Methods). Following these two steps, we assessed the similarity between the intermediates in the 5-FU metabolic neighborhood and human metabolites. Among all 407 compounds in the 5-FU metabolism (Table S1), we found 8 that show a close similarity to human metabolites (NICEdrug score above 0.9, Figure 3), and might act as competitive inhibitors or anti-metabolites. Inside the reactive site, the original hydrogen atom is bioisosterically replaced by fluorine. F-C bonds are extremely stable and therefore block the active site by forming a stable complex with the enzyme. NICEdrug.ch predicts the 5-FU downstream metabolites tegafur, 5-fluorodeoxyuridine, F-dUMP, 5flurim, 5-fluorodeoxyuridine triphosphate, 5-fluorodeoxyuridine triphosphate, 5-fluorouridine diphosphate, and 5-fluorouridine triphosphate to act as

antimetabolites (Figure 3). Among the predicted antimetabolites, the inhibitory effect of the intermediates tegafur, 5-fluorodeoxyuridine, and F-dUMP (one to two reaction steps away) has been confirmed in studies by Kobayakawa et.al (Kobayakawa and Kojima, 2011) and Bielas et.al (Bielas et al., 2009). Based on the insights from NICEdrug.ch about down, we suggest the inhibitory and side effect of 5-FU treatment might be more complex than previously thought. 5-FU downstream products are structurally close to human metabolites and might form stable complexes with native enzymes. This knowledge could serve to further refine the pharmacokinetic and pharmacodynamic models of 5-FU and ultimately the dosage administered during treatment.

NICEdrug.ch identifies toxic alerts in the anticancer drug 5-FU and its products from metabolic degradation.

The concept of drug toxicity refers to toxic effects at medical doses (Guengerich, 2011), which often occur due to the degradation products generated through drug metabolism. Extensive efforts have been expended to identify toxic molecules or, more generally, to extract the substructures that are responsible for toxicity (called structural alerts). The Liver Toxicity Knowledge Base (LTKB) and the super toxic database include 1,036 and about 60k toxic molecules, respectively (Schmidt et al., 2009; Thakkar et al., 2018). ToxAlert provides around 1,200 alerts related to different forms of toxicity (Sushko et al., 2012). However, the number of molecules that are analyzed and labeled as toxic in databases is disproportionally low compared to the space of compounds. Additionally, structural alerts are indicated for many compounds, and current alerts might identify redundant and over-specific substructures, which questions their reliability (Yang et al., 2017).

To quantify the toxicity of downstream products of drugs in NICEdrug.ch, we collected all of the molecules cataloged as toxic in the LTKB and super toxic databases (approved toxic molecules) along with their lethal dose (LC_{50}), as well as the existing structural alerts provided by ToxAlert. We measure the similarity of an input molecule with all approved toxic molecules using the reactive site centric fingerprints implemented in BridgIT and the NICEdrug score (Materials and Methods). Next, we scan both the toxic reference molecule and the input molecule for structural

hints of toxicity here referred to as *NICEdrug toxic alerts*. We keep common NICEdrug toxic alerts between the reference, which is a confirmed toxic compound, and input molecule. With this procedure in place, NICEdrug.ch finds for each input molecule the most similar toxic molecules along with their common toxic alerts, and serves to assess the toxicity of a new molecule based on the mapped toxic alerts. Additionally, the NICEdrug toxic alerts and toxicity level of drug intermediates can be traced with NICEdrug.ch through the whole degradation pathway to reveal the origin of the toxicity.

As a case study, we herein tested the ability of NICEdrug.ch to identify the toxicity in 5-FU metabolism. First, we queried the toxicity profile of all intermediates in the 5-FU metabolic neighborhood, integrating both known and hypothetical human reactions (Materials and Methods). In this analysis, we generated all compounds within four reactions or steps away from 5-FU. Based on the toxicity report of each potential degradation product, we calculated a relative toxicity metric that adds the LC_{50} value, NICEdrug score, and number of common NICEdrug toxic alerts with all approved toxic drugs (Materials and Methods). We calculated this toxicity metric for each compound in the metabolic neighborhood of 5-FU (Table S1). Remarkably, we show that the top most toxic intermediates match the list of known toxic intermediates in 5-FU metabolism (Figure 4) (Krauß and Bracher, 2018). Based on the toxicity analysis in NICEdrug.ch for 5-FU, we hypothesize that there are highly toxic molecules in the vicinity of 5-FU drug metabolism that have not yet been identified as toxic, neither experimentally nor computationally. These new molecules are predicted to have similar score of toxicity as the known compounds, which are in the top most toxic intermediates in our ranking, it might be necessary to evaluate experimentally their toxicity to recalibrate the dosage of the 5-FU treatment.

NICEdrug.ch accurately clusters statins of type I and II and guides drug repurposing.

Repurposing approved drugs for other diseases can reduce the medical risks as well as the development expenses, because potential side effects of an approved drug are already documented and the drug has passed the approval process. As an example, the antitussive noscapine has been repurposed to treat some cancers (Mahmoudian and Rahimi-Moghaddam, 2009; Rajesh, A. and International, 2011). Because NICEdrug.ch can search for functional (i.e.,

reactivity) and structural (i.e., structural pattern) similarities between molecules while accounting for human biochemistry, we wanted to determine if NICEdrug.ch could therefore suggest drug repurposing strategies.

As a case study, we investigated the possibility of drug repurposing to replace statins, which are a class of drugs often prescribed to lower blood cholesterol levels and to treat cardiovascular disease. Indeed, data from the National Health and Nutrition Examination Survey indicate that nearly half of adults 75 years and older in the United States use prescription cholesterol-lowering statins (US Preventive Services Task Force, 2016). Since some patients do not tolerate these drugs and many still do not reach a safe blood cholesterol level (Kong et al., 2004), there is a need for alternatives. Being competitive inhibitors of the cholesterol biosynthesis enzyme 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMG-CoA reductase) (Jiang et al., 2018; Mulhaupt et al., 2003), all statins share the same reactive site. BNICE.ch labeled this reactive site, in a linear or circular form, as corresponding to the EC class 4.2.1.- (Istvan, 2001). NICEdrug.ch includes 254 molecules with the same reactive site that are recognized by enzymes of the E.C. class 4.2.1.-, ten of which are known statins. We used the NICEdrug score to cluster the 254 molecules into different classes (Table S2, Figure 5). Two of the classes correspond to all currently known statins, which are classified based on their activity into type 1 and 2, wherein statins of type 2 are less active and their reactive site is more stable compared to type 1. This property is well distinguished in the clustering based on the NICEdrug score (Figure 5A).

In addition to properly classifying the ten known statins (Figure 5B and 5C, molecules non-marked), we identified seven other NICEdrug.ch molecules that clustered tightly with these statins (Figure 5B and 5C, molecules marked with *). These new molecules share the same reactive site and physicochemical properties, and have the highest similarity with known statins in atoms neighboring the reactive site. In a study by Endo *et al.*, these seven NICEdrug.ch molecules were introduced as Mevastatin analogues for inhibiting cholesterol biosynthesis (Endo and Hasumi, 1993). Therefore, they were already suggested as possible candidates for treating high blood cholesterol and could be a good option for repurposing. Furthermore, we found eight known drugs not from the statin family among the 254 scanned molecules (Table S3). One of them, acetyl-L-carnitine (Figure 5C, molecule marked with **) is mainly used for treatment of

neuropathic pain (Li et al., 2015), though Tanaka *et al.* have already confirmed that it also has a cholesterol-reducing effect (Tanaka et al., 2004).

Overall, NICEdrug.ch was able to characterize all known enzymatic reactions that metabolize statins, including proposed alternatives and new hypothetical reactions that could be involved in their metabolism within human cells (Figure 5A, Figure S4). The identification of seven drugs that clustered around the statins and were already designed as alternatives to statins verifies the ability of NICEdrug.ch and the NICEdrug score to search broad databases for similar compounds in structure and function. Furthermore, the discovery of the eight compounds unrelated to known statins offer alternative candidates for the inhibition of the HMG-CoA reductase and, eventually, the treatment of high cholesterol, though further *in vitro* characterization and preclinical experiments would be required to validate their clinical benefits.

NICEdrug.ch suggests over 500 drugs to target liver-stage malaria and simultaneously minimize side effects in human cells, with shikimate 3-phosphate as a top candidate.

Efficiently targeting malaria remains a global health challenge. Malaria parasites (*Plasmodium*) are developing resistance to all currently known drugs. Moreover, antimalarials cause undesired side effects such as hemolytic toxicity. (Marcsisin et al., 2016; World Health Organization, 2018). We applied NICEdrug.ch to identify drug candidates that target liver-stage developing malaria parasites and lessen or avoid side effects in human cells.

We previously identified 178 essential genes and enzymes for liver-stage development in the malaria parasite *Plasmodium berghei* (Stanway et al., 2019) (Table S4, Materials and Methods). Out of 178 essential *Plasmodium* enzymes, 33 enzymes are not essential in human cells (Wang et al., 2015) (Table S4, Materials and Methods). We extracted all human substrates of these 33 enzymes uniquely essential in *Plasmodium*, which resulted in 68 metabolites and 157 unique metabolite-enzyme pairs (Table S4, Materials and Methods). We used NICEdrug.ch to examine the druggability of the 33 essential *Plasmodium* enzymes with the curated 48,544 drugs (Figure 1) and the possibility to repurpose them to target malaria. To identify potential prodrugs, we evaluated potential derivatives up to three reactions steps away from all 48,544 drugs in

NICEdrug.ch. Suitable candidates to target liver-stage malaria are those molecules that have a good NICEdrug score (NICEdrug score above 0.5) with any of the 157 *Plasmodium* metabolite-enzyme pairs. We identified 538 such drug candidates, targeting 14 essential *Plasmodium* enzymes (Table S5, Materials and Methods). Furthermore, 1,018 other drugs appear in the metabolic neighborhood of 538 identified drugs (between one and three reaction steps away). Interestingly, out of the 538 identified drug candidates, two (Estradiol-17beta and digoxigenin) have been previously validated as antimalarials (Antonova-Koch et al., 2018), and 93 are in the metabolic neighborhood of already approved antimalarial drugs (Antonova-Koch et al., 2018), which suggests these antimalarials might be prodrugs.

Being an intracellular parasite, antimalarial treatments should not only be efficient to target *Plasmodium* but also assure the integrity of the host cell (Figure 6sA). To tackle this challenge, we identified 1,497 metabolites participating in metabolic reactions catalyzed with essential human enzymes (Table S5, Materials and Methods), and excluded the antimalarial drug candidates that shared reactive site-centric similarity with the extracted human metabolite set (to satisfy NICEdrug score below 0.5). Out of all 538 drug candidates that might target liver-stage *Plasmodium*, a reduced set of 69 molecules minimize inhibition of essential human enzymes (Table S5, Materials and Methods) and are hence optimal antimalarial candidates.

Among our set of 69 optimal antimalarial candidates, a set of 10 drugs targeting *Plasmodium* shikimate metabolism arose as the top candidate. Shikimate biosynthesis is absent in human cells but essential for liver-stage malaria development, as we recently showed (Stanway et al., 2019). The set of drugs targeting shikimate metabolism include 27 prodrugs (between one and three reaction steps away) that have been shown to have antimalarial activity (Antonova-Koch et al., 2018). NICEdrug.ch identifies molecules among the prodrugs with high number of toxic alerts like Nitrofen and four molecules with similar scaffolds as 1-(4-chlorobenzoyl)pyrazolidin-3-one in the metabolic neighborhood (two or three steps away) of shikimate and derivatives. This result suggests that downstream compounds of the 27 prodrugs might target the *Plasmodium* shikimate pathway but also cause side effects on human cells (Table S5).

To this end, NICEdrug.ch identified shikimate 3-phosphate as a top candidate antimalarial drug. We propose shikimate 3-phosphate inhibits the essential *Plasmodium* enzyme 3-Dehydroquinate hydro-lyase (aroC, E.C.: 4.2.1.10, PBANKA_0304000) without side effects in the host cell (Figure 6, Table S5). Excitingly, shikimate 3-phosphate has been used to treat *E. coli* and *Streptococcus* infections without appreciable toxicity in patient (Díaz-Quiroz et al., 2018). Furthermore, recent studies have shown that inhibition of the shikimate pathway by 7-deoxy-sedoheptulose is an attractive antimicrobial and herbicidal strategy with no cytotoxic effects on mammalian cells (Brilisauer et al., 2019). Experimental studies should now validate the capability of shikimate 3-phosphate to efficiently and safely target liver malaria, and could further test other NICEdrug.ch antimalarial candidates (Table S5).

Discussion

Discovering new drugs with reduced toxicity/side effects is required to cure arising diseases and infections, target drug resistance, and develop personalized treatments (Weaver and Valentin, 2019). However, the process of identifying, testing, and approving a single small molecule can take over twenty years and billions of dollars. In addition, there is a high risk that the proposed drug candidate fails at any step due to toxicity and side effects. Indeed, the need to find and rapidly develop new, safe, and effective drugs remains one of the biggest public health challenges of our time. There is an urgent need to define strategies that accelerate drug discovery and computational screening of *all* possible targets and molecules can help toward this aim. Computational approaches to date have focused on capturing molecular structures without considering the reactivity of the molecules in a cell (Lin et al., 2020). However, reactive site information and drug metabolism determine which enzymes the drugs will target as competitive inhibitors (Ramsay and Tipton, 2017), the drug's metabolic fate or degradation (Li et al., 2019; Ramsay and Tipton, 2017), and the potential source of its toxicity and side effects (Attia, 2010). Understanding drug effects in the context of cellular metabolism also offers great promise to evaluate the reactivity of a new potential drug, the druggability of an enzyme, and the possibility of drug repurposing. Yet, *in silico* mechanistic analysis of drug biochemistry is relatively

unexplored, and no computational large-scale studies of drug metabolism in human cells have ever been performed.

To systematically illuminate the metabolism and *all* enzymatic targets (competitively inhibited) of known drugs and hypothetical prodrugs, we have used the Biochemical Network Integrated Computational Explorer (BNICE.ch) (Hatzimanikatis et al., 2005) in combination with the analysis of neighboring atoms of reactive sites as developed in BridgIT (Hadadi et al., 2019) to perform the first large-scale computational analysis of drug biochemistry and toxicity in the context of human metabolism. We collected and curated over 250,000 small molecules, computed their bio- and physicochemical drug properties, and we made them available to researchers, clinicians, and industry partners in the open-source drug database and analysis platform NICEdrug.ch. Excitingly, NICEdrug.ch revealed 20 million potential reactive sites at the 250,000 small molecules of the database, and there exist over 3,000 enzymes in the human metabolism that can be subject to inhibition with the 250,000 molecules. This is because NICEdrug.ch can identify *all* potential metabolic intermediates of a drug and scans these molecules for substructures that can interact with catalytic sites across all enzymes in a desired cell.

NICEdrug.ch adapts the metric previously developed for reactions in BridgIT (Hadadi et al., 2019) to precisely compare drug-drug and drug-metabolite pairs based on similarity of reactive site and neighborhood, which we have recently shown to outperform other molecular comparison metrics (Hadadi et al., 2019). Since NICEdrug.ch shows high specificity in the identification of such reactive sites and neighborhood, it provides a better mechanistic understanding than currently available methods (Robertson, 2005). Despite these advances, it remains challenging to systematically identify non-competitive inhibition or targeting of non-enzymatic biological processes. We suggest coupling NICEdrug.ch drug metabolic reports with other *in silico* and experimental analyses accounting for signaling induction of small molecules and other non-enzymatic biological processes like transport of metabolites in a cell. The combined analysis of drug effects on different possible biological targets (not uniquely enzymes) will ultimately increase the coverage of molecules for which a mechanistic understanding of their mode of action is assigned.

A better understanding of the mechanisms of interactions and the specific nodes where the compounds act can help re-evaluate pharmacokinetic and pharmacodynamic models, dosage, and treatment. Such understanding can be used in the future to build models that correlate the pharmacodynamic information with specific compounds and chemical substructures in a manner similar to the one used for correlating compound structures with transcriptomic responses. We have shown for one of the commonly used anticancer drugs 5-FU that NICEdrug.ch identifies and ranks alternative sources of toxicity and hence can guide the design of updated models and treatments to alleviate the drug's side-effects.

The mechanistic understanding will also further promote the development of drugs for repurposing. While current efforts in repurposing capitalize on the accepted status of known drugs, some of the issues with side effects and unknown interactions are limiting their development as drugs for new diseases. Given that repurposing of these drugs will require new dosage and administration protocols, the understanding of their interactions with the human metabolism will be very important to identify, develop, and interpret unanticipated side effects and physiological responses. We evaluated the possibility of drug repurposing with NICEdrug.ch to substitute the statins, which are broadly used to reduce cholesterol but show side effects. NICEdrug.ch and its reactive site-centric comparison accurately cluster both family types of statins although they are in their overall molecular structure similar and show different reactivity. In addition, NICEdrug.ch suggests a set of new molecules with potentially less side effects (Endo and Hasumi, 1993; Tanaka et al., 2004) that share reactive sites with statins.

Last, a better mechanistic understanding of drug targets can guide the design of treatments against infectious diseases, for which we need effective drugs targeting pathogens and avoiding side effects in the host cell. This is arguably the most challenging type of problems in drug design, and indeed machine learning has continuously has, as of today, not fulfilled its promise to guide drug design, which might be due to the inherent difficulty to acquire large, consistent, and high quality data sets in human patients. To demonstrate the power of NICEdrug.ch to tackle this problem, we sought to identify drugs that target liver-stage malaria parasites and minimize

impact on the human host cell. We identified over 500 drug candidates that might inhibit essential *Plasmodium* enzymes in the liver stages and minimize the impact on the human host cell. Our top drug candidate satisfying these conditions is shikimate 3-phosphate targeting the parasite's shikimate metabolism, which we recently identified as essential in a high-throughput gene knockout screening in *Plasmodium* (Stanway et al., 2019). Excitingly, our suggested antimalarial candidate shikimate 3-phosphate has already been used for *Escherichia* and *Streptococcus* infections without considerable side effects (Díaz-Quiroz et al., 2018).

Overall, we believe that a systems level or metabolic network analysis coupled with an investigation of reactive sites will likely accelerate the discovery of new drugs and provide additional understanding regarding metabolic fate, action mechanisms, and side effects. We propose the generation of metabolic reports with NICEdrug.ch to understand the reactivity of new small molecules, possibility of drug repurposing, and druggability of enzymes. Our results using NICEdrug.ch suggest that this database can be a novel avenue towards the systematic pre-screening and identification of drugs and antimicrobials. In addition to human metabolic information, NICEdrugh.ch currently includes information for *P. berghei* and *E. coli*'s metabolism. Because we are making it publicly available (https://lcsb-databases.epfl.ch/Nicedrug), our hope is that scientists and medical practitioners alike can make use of this unique database to better inform their research and clinical decisions, saving time, money, and ultimately lives.

Acknowledgements

H.M. was funded by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 72228. A.C-P. and V.H. were funded by grant 2013/155 (MalarX), N.H. and J.H. were funded by grant 2013/158 (MicroScapesX); both are SystemsX.ch grants awarded by the SNSF. K.H. ,H.M and J.H were supported by the Ecole Polytechnique Fédérale de Lausanne (EPFL). Biorender was used to make figure 6 and the graphical abstract.

Author Contributions

Conceptualization, V.H., H.M., N.H., A.C-P.; Methodology, V.H., H.M., K.H., N.H., A.C-P.; Software, H.M., K.H., J.H.; Formal analysis, H.M., K.H.; Investigation, H.M., K.H., A.C-P; Writing Draft, H.M., A.C-P.; Writing- Review & Editing, V.H., H.M., A.C-P., J.H., K.H., N.H.; Visualization, H.M., A.C-P.; Supervision, V.H.; Project Administration, V.H., H.M.; Funding Acquisition, V.H.

Declaration of interests

The authors declare no competing interests.

Main figure title and legends

Figure 1. Pipeline to construct and use the NICEdrug.ch database.

NICEdrug.ch (I) curates available information and calculates the properties of an input compound, (II) identifies the reactive sites of that compound, (III) explores the hypothetical metabolism of the compound in a cell, (IV) stores all functional, reactive, bio-, and physico-chemical properties in open-source database, and (V) allows generation of reports to evaluate (V.i) reactivity of a small molecule, (V.ii) drug repurposing, and (V.iii) druggability of an enzymatic target.

Figure 2. Similarity in reactive site and neighborhood defines para-metabolites in 5-FU metabolism and inhibited human metabolic enzymes.

Eight para-metabolites that are two and four reaction steps away in the 5-FU metabolic neighborhood. We show the most similar native human metabolites to each para-metabolite based on the NICEdrug score (value under para-metabolite name). Reactive sites common to para-metabolites and native human metabolites are shaded with colors (violet, gold, turquoise) matching the color of the enzymes (packmen) that are inhibited. Neighborhood (seven atoms away, as considered in NICEdrug score) of reactive sites are surrounded in para-metabolites and native human metabolites with the same color as the reactive sites and enzymes. We also present the reaction that is inhibited and its native product.

Figure 3. Difference in reactive site but similarity in neighborhood defines top anti-metabolites in 5-FU metabolism and inhibited human metabolic enzyme.

Eight anti-metabolites of dUMP that are one and two reactions or steps away in the 5-FU metabolic neighborhood in NICEdrug.ch. dUMP is the most similar native human metabolite to all anti-metabolites based on the NICEdrug score (value under anti-metabolite name). These anti-metabolites are the most similar to any native human substrates (NICEdrug score above 0.9) in the 5-FU metabolic neighborhood. Reactive sites in anti-metabolites (orange) and the native human metabolite (dUMP, blue) are highlighted. Note that the reactive site of the anti-metabolites (orange) is different than the one of the native human metabolite (blue), but the neighborhood is highly similar (blue surrounding line), which determines the high NICEdrug score. We present the human enzyme (dTMP synthase, blue packmen) that is predicted to be inhibited based on the NICEdrug score, the inhibited reaction, and its native product. Compounds marked with * are confirmed inhibitors (see references in the main text).

Figure 4. Comparing downstream products to known toxic molecules and analyzing their common structural toxic alerts explains metabolic toxicity of 5-FU.

Example of six suggested toxic molecules that are one, two, three, and four reactions or steps away in the 5-FU metabolic neighborhood. We show toxic compounds from the supertoxic and hepatotoxic databases that lead to the highest NICEdrug toxicity score (number under toxic intermediate name, calculated using equation 1 in Materials and Methods) for each of the six example toxic molecules. The NICEdrug toxicity score is calculated between each molecule in the 5-FU metabolic neighborhood and two approved toxic compounds; one from the supertoxic database and one from the hepatotoxic database. The NICEdrug toxicity score considers six values (three numbers under each approved toxic compound name): twice the NICEdrug score, twice the number of common structural alerts, once the severity degree of the hepatotoxic compound, and once the log(LC_{50}) of the supertoxic compound. The highlighted substructures are corresponding to structural alerts in common between intermediate and most similar toxic molecule. Five examples of NICEdrug toxic alerts are visualized: genotoxic carcinogenicity and mutagenicity (blue), non-biodegradability (red), acute aquatic toxicity (green), pyridinium-

related toxicity (orange), and skin sensitization (lila). From the six potential toxic molecules in the 5-FU metabolic neighborhood that we identified with NICEdrug toxicity analyses, there are three (5-Fluorouridine-3P, F-dUMP, and 5-Fluorodeoxyuridin-3P; marked with *) that are confirmed as toxic in the literature (see references in the main text).

Figure 5. Clustering of molecules with statin reactive sites based on NICEdrug score suggests drugs for repurposing.

(A) Pairwise NICEdrug score between all molecules with statin reactive sites (heat map) and number of metabolic reactions in which they participate (left). We highlight clusters of statins of type 1 (cluster a) and type 2 (cluster b), and clusters of most similar molecules to type 1 statins (cluster c) and type 2 statins (cluster d). Within the metabolic reactions, we indicate the total number of reactions (green) and the number of reactions that involve statins' reactive site (blue). **(B)** Examples of statins and Mevastatin analogues of type 1 from cluster c (blue) and of type 2 from cluster d (gold). We left unmarked the known statins, which are appropriately clustered together based on the NICEdrug score, and we mark with * new molecules that cluster with statins that NICEdrug.ch suggests could be repurposed to act as statins. Reactive sites in type 1 statins and type 2 statins are colored in blue and orange, respectively. The reactive site neighborhood as considered in the NICEdrug score is also marked.

Figure 6. NICEdrug.ch suggests shikimate 3-phosphate as a top candidate to target liver-stage malaria and minimize side effects in host human cells.

(A) Schema of ideal scenario to target malaria in which a drug is efficient inhibiting an essential enzyme for survival of the malaria parasite and does not cause side effect inhibition of essential enzymes in the host human cell. **(B)** Shikimate 3-phosphate inhibits the enzyme *aroC* in the *Plasmodium* shikimate metabolism, which is essential for liver-stage development of the parasite. Shikimate 3-phosphate does not inhibit any enzyme in the human host cell since it is not a native human metabolite, and it does not show similarity to any native human metabolite.

Supplementary figure title and legends

Supplementary figure 1. Overview of number of molecules in NICEdrug.ch and their structural curation, related to Figure 1.

(A) Venn diagram showing the number of compounds in NICEdrugh.ch and their source database: KEGG, DrugBank, ChEMBLE NTD, and ChEMBLE. **(B)** Representation on how different kekulé forms affect the identification of reactive sites and prediction of biological activity for an example molecule.

Supplementary figure 2. Distribution of reactive sites and metabolic reactions as of E.C. numbers linked to all molecules in NICEdrug.ch, related to Figure 1.

(A) Distribution of reactive sites identified in all molecules of NICEdrug.ch among classes of E.C. numbers. **(B)** Specificity of reactive sites identified in drugs based on length and types of participating atoms. **(C)** Distribution of drug metabolic reactions based on class of E.C. number. **(D)** Distribution of Gibbs free energy for the drug metabolic reactions, which are the reactions linked to all molecules of NICEdrug.ch.

Supplementary figure 3. Description of NICEdrug score, related to Figures 1, 2, 3, 4, 5, and 6.

Example of NICEdrug score calculation. The NICEdrug score takes into account the structure of a molecule's reactive site and its seven-atom-away neighborhood for similarity evaluation, analogous to BridgIT.

Supplementary figure 4. Clustering based on NICEdrug score, molecular weight, and reactivity of statin like molecules, related to Figure 5.

Hierarchical clustering based on the NICEdrug score of all molecules in NICEdrug.ch that contain statin reactive site (left). We report the molecules' molecular weight (middle left) and number of drug metabolic reactions or reactions in which these drugs participate (middle). The molecular weight seems to be inversely correlated with the number of drug metabolic reactions. We highlight six clusters of drugs (a-f, middle right) and an example representative molecule (left). Interestingly, these clusters also group molecules based on bio- or physico-chemical properties: cluster a involve a range of silicon-containing chemical molecules, cluster b are drug like

molecules of type 2 statins, cluster c includes chemical molecules with a long chain connected to the reactive site, cluster d involves molecules with 1-indanone fused with a tetrahydropyran ring, cluster e drug like molecules of type 1 statins, and cluster f are 16-membered ring macrolide antibiotics.

MATERIALS AND METHODS

Curation of input molecules used in the construction of NICEdrug.ch

We constructed the NICEdrug.ch database to gather small molecules suitable for treatment of human diseases. We collected the SMILES structure, synonyms, and any available bio- and physico-chemical property included from three source databases: KEGG, ChEMBL, and DrugBank, which added up to 70,976 molecules by January 2018 (Figure S1A). Only molecules that were fully structured were imported to our database. We further curated the imported molecules by removing duplicate structures and merging annotations from different databases into one molecule entry in the database. For removing duplicate structures we used canonical SMIELS (Weininger, 1988) generated by openbabel (O'Boyle et al., 2011) version 2.4.0. This unification method is based on atoms and their connectivity in a molecule in terms of a molecular graph that is captured by the canonical SMILES. Therefore, different resonance forms, stereoisomers, as well as dissociated and charged states of the same compound are mapped to one entry in database. Furthermore, we filtered molecules based on Lipinski rules (Lipinski et al., 2001): (1) the molecular weight should be less than 500 Dalton, (2) the number of hydrogen bond donors should be less than five, (3) the number of hydrogen bond acceptors should be less than ten, and (4) an octanol-water partition coefficient (log P) should be less than five. According to Lipinski rules an active orally drug does not violate more than one of the above criteria. We calculated criteria one, two and three based on the structural information from SMILES of molecules. To assess criterion four, we relied on reported data in the source database. We kept in the NICEdrug.ch database those compounds for which the partition coefficient was not available.

We performed a separate analysis to account for non-unique graph representations of aromatic rings, also called *kekulé structures*. The existence of aromatic rings and the fact that bond-electrons are shared within the ring make several single-double bond assignments possible,

which results in multiple kekulé representations for a single molecule (Figure S1B). We included all such kekulé structures to account for alternative atom-bond connectivity and associated reactivity. We call "effective forms" to the kekulé representations that show different reactive sites than their canonical structures. For example, there can be two effective forms plus the canonical structure (Figure S1B). In total, we found 42,092 effective forms for 29,994 aromatic compounds in NICEdrug.ch database and we kept them for further analysis.

We also computed the thermodynamic properties of all drugs in NICEdruch.ch. Specifically, we computed the Gibbs free energy of formation ($\Delta_f G'^{\circ}$) using the group contribution method of Mavrovouniotis (Jankowski et al., 2008).

The NICEdrug.ch database includes a total number of 48,544 unique and curated small molecules (Figure S1A).

Identification of reactive sites in drugs

The 3D structures of enzyme pockets are complex and mostly unknown. Therefore, evaluating and comparing docking of two small molecules in the pocket of a specific target is impossible most of the times. Using BNICE.ch, we focused on the complementary structure of active sites on substrates, also called *reactive site*. To recognize the potential reactive sites on molecules, we scanned molecules using expert-curated generalized reaction rules of BNCIE.ch (Hadadi et al., 2016), which mimic the identification of substrates by the enzyme pocket and account for the promiscuous activity of enzymes. Theses reaction rules incorporate the information of biochemical reactions and have third-level Enzyme Commission (EC) identifiers. Each BNICE.ch reaction rule accounts for three levels of information: (1) atoms in reactive sites of compounds, (2) connectivity and configuration of atom bonds in the reactive site, and (3) mechanism of bond breakage and formation during the reaction. As of May 2020, BNICE.ch contains 450 bidirectional generalized reaction rules that can reconstruct 8118 KEGG reactions (Hadadi et al., 2016). Here, we include all BNICE.ch rules to identify all possible reactive sites on a given drug in two steps. First, a BNICE.ch rule identifies all atoms in a compound that belong to the rule's reactive site. Second, the rule evaluates the connectivity of the atoms previously identified. The candidate

compounds for which a BNICE.ch rule identified a reactive site were validated as metabolically reactive and considered for analysis in NICEdrug.ch.

It is important to note that thanks to the generalized reaction rules, which abstract the knowledge of thousands of biochemical reactions, BNICE.ch is able to reconstruct known biotransformations and also propose novel metabolic reactions. This was demonstrated in the reconstruction of the ATLAS of Biochemistry (Hadadi et al., 2016), which involves up to 130,000 reactions between known compounds.

Analysis of drug metabolism in human cells.

To mimic biochemistry of human cells and simulate human drug metabolism, we collected all available information (metabolites and metabolic activities or EC numbers of enzymes) on human metabolism from three available databases: the human metabolic models Recon3D (Brunk et al., 2018) and HMR (Pornputtapong et al., 2015), and the Reactome database (Croft et al., 2011). These three databases include a total of 2,266 unique human metabolites and 2,066 unique EC numbers of enzymes (Table S5).

To explore the biochemical space beyond the known human metabolic reactions and compounds, we used (1) the generalized enzymatic reaction rules of BNICE.ch that match up to the third EC level the collected human enzymes (Table S5.1), and (2) all of the collected human metabolome (Table S5.2). We evaluated the reactivity of each drug in a human cell using the retro-biosynthesis algorithm of BNICE.ch, which predicts hypothetical biochemical transformations or *metabolic neighborhood* around the drug of study. We generated with BNICE.ch metabolic reactions in which each drug and all known human metabolites (Table S5) could participate as substrate or products. We also allowed a set of 53 known cofactors to react with the human metabolites (Table S5.3).

We define the boundaries of the metabolic neighborhood of a molecule with a maximum number of reactions or *steps away* that separate the input molecule (drug of study) from the furthest compound. In BNICE.ch, a generation *n* of compounds involves all metabolites that appear for the first time in the metabolic neighborhood of a drug after *n* reactions or steps happened. For example, in the case study of 5-FU we find the compound 5-Fluorouridine in generation 2 or 2

steps away, which means there are two metabolic reactions that separate 5-FU and 5-Fluorouridine (Figure 2).

In NICEdrug.ch, there exist 197,246 compounds in generation 1 (1 step away) from all input drugs. The 197,246 compounds are part of the potential drug metabolic neighborhood in human cells. Out of all generation 1 molecules, 13,408 metabolites can be found in human metabolic models and HMDB database (Wishart et al., 2018), 16,563 metabolites exist in other biological databases, and the remaining 167,245 metabolites are catalogued as known compounds in chemical databases (i.e., PubChem). Note that HMDB includes native human metabolites and non-native human compounds, like food ingredients.

The 197,246 products that are one-step away of all NICEdrug.ch molecules are part of a hypothetical biochemical neighborhood of 630,449 drug metabolic reactions. Of all drug metabolic reactions, 5,306 reactions are cataloged in biological databases, and the remaining 625,143 reactions are novel. A majority of the reactions involved oxidoreductases (42.54%), broken down into 27.45% of lyases, 7.15% of hydrolases, 6.28% of transferases, 1% of isomerases, and 15.58% of ligases. Interestingly based on the previously identified reactive sites, out of the 265,935 (42.54% of 625,143) oxidoreductase reactions, 49.92% are catalyzed by the p450 family of enzymes, which are known to be responsible for the metabolism of drug (Figure S2C).

Using NICEdrug.ch database for analysis of the metabolic neighborhood of a drug

In NICEdrug.ch webserver, users can look up for a drug using the drugs' name and other identifiers like ChEMBL, DrugBank and KEGG. NICEdrug.ch will report a unique identifier for the compound that will be input for upcoming analysis modules. The *predict metabolism* module allows to study the metabolic network around an input molecule. The input to this module is: (1) the unique identifier of the drug of interest, (2) a maximum number of reactions or *steps away* that shall separate the input drug to the furthest compound in the metabolic neighborhood.

The output of this analysis is a report in the form of a csv file that includes all compounds and metabolic reactions in the metabolic neighborhood of the input drug. One can also export the

neighborhood in the form of a visual graph, in which nodes are molecules and edges are reactions.

Definition of the NICEdrug score

Based on the theory of lock and key, two metabolites that can be catalyzed by the same enzyme may have similar reactive sites and also neighboring atoms. In order to quantify the similarity inside and around reactive sites of two molecules, we developed a metric called *NICEdrug score* (Figure S3), which is inspired on BridgIT (Hadadi et al., 2019). BridgIT assesses the similarity of two reactions, considering the reactive site of the participating substrates and their surrounding structure until the seventh atom out of the reactive site.

The NICEdrug score is an average of two similarity evaluations: (1) the atom-bond configuration inside reactive site (α parameter), and (2) the 7 atom-bond chain molecular structure around the reactive site (β parameter). The NICEdrug score, and its parameters α and β, range between 0 and 1 when they indicate no similarity and identical structure, respectively. Different constraints on the α and β parameters determine the identification of different types of inhibition like para-metabolites and anti-metabolites (see other sections in this Materials and Methods).

We show the evaluation of NICEdrug scores for three example compounds (Figure S3). In this example, Digoxin, Labriformidin and Lanatoside C all share the reactive site corresponding to EC number 5.3.3.- (α=1). Starting from the atoms of the identified reactive site, eight description layers of the molecule were formed, where each layer contains a set of connected atom-bond chains. Layer zero includes types of atoms of reactive site and their count. Layer 1 expands one bond away from all of the atoms of reactive site and accounts for atom-bond-atom connections. This procedure is continued until layer 7, which includes the sequence of 8 atoms connected by 7 bonds. Then, we compare the fingerprint of each molecule to the other participants of the class based on the Tanimoto similarity scores. A Tanimoto score near 0 designates no or low similarity, whereas a score near 1 designates high similarity in and around reactive site. Lanatoside C and Digoxin share the same substructure till 8 layers out of reactive site which is presented in the NICEdrug score by preserving score 1 in all layers, so the overall Tanimoto score for these two compounds in the context of EC number 5.3.3.- is 1 (α=1 and β=1). However, the structure of two

compounds are not exactly the same and actually Lanatoside C has 8 more carbon atoms and 6 more oxygen atoms, shaped as an extra benzenehexol ring and an ester group. Although this part is far from the reactive site, based on the NICEdrug score they both can perfectly fit inside the binding pocket of a common protein related to this reactive site. This hypothesis is proved by experiments reported in KEGG and DrugBank. According to DrugBank and KEGG, Lanatoside C has actions similar to Dioxin and both of them have the same target pathways: Cardiac muscle contraction and Adrenergic signaling in cardiomyocytes. Furthermore, target protein for both of them is ATP1A.

Also, the NICEdrug score effectively captures and quantifies differences around the reactive site. The substructure around the reactive site in Labriformidin is slightly different (α=1 and β <1). The difference is calculated trough different layers of the NICEdrug score.

In the case study of 5-FU, in order to predict competitive inhibition, we analyzed all the metabolites that share reactive site with 5-FU or its downstream products (α=1) and then we ranked the most similar metabolites based on their similarity in neighborhood of reactive site to 5-FU or its downstream products (β). To assess the structural differences in the reactive sites themselves (α), we implemented the Levenshtein edit distance algorithm (Levenshtein, 1966) to determine how many deletions, insertions, or substitutions of atom/bonds are required to transform one pattern of reactive site into the other one. Here, the edit distance explains the difference between the reactive sites of the intermediate and the human metabolite. However, even slight changes in the reactive site affect its interaction with the binding site. To ensure that the divergence retained the appropriate topology, we compared the required edit on reactive site with interchangeable groups, termed bioisosteric groups (Papadatos and Brown, 2013). These bioisosteric groups contain similar physical or chemical properties to the original group and largely maintain the biological activity of the original molecule. An example of this is the replacement of a hydrogen atom with fluorine, which is a similar size that does not affect the overall topology of the molecule. For this analysis, we used 12 bioisosteric groups adapted from the study by Papadatos et.al. (Papadatos and Brown, 2013).

To predict irreversible Inhibitors in metabolism of 5-FU, we kept only molecules with a similarity score greater than 0.9 to metabolites (β>0.9), to preserve a high similarity in the neighborhood

of the reactive sites. Then, we checked which ones contained reactive sites that differed only in the replacement of bioisosteric groups (α~1).

Classification of drugs based on the NICEdrug score

Classification of compounds with similar structure is normally used to assign unknown properties to new compounds. For instance, one can infer ligand-protein binding for a drug when its action mechanism or the structure of the target proteins are not known. In this study, we have demonstrated four strategies to classify drugs (Figure 1), which are from less to more stringent: classifying (1) molecules that participate in reactions with the same EC up to the 3th level, (2) molecules that in addition share a BNICE.ch reaction rule, (3) molecules that in addition to both previous points share reactive site, (4) molecules that show high similarity of reactive site and neighborhood based on the NICEdrug score.

The EC number guarantees that molecules are catalyzed with similar overall reaction mechanism. Generalized reaction rules from BNICE.ch further capture different submechanisms inside an EC number (Hadadi et al., 2016). A BNICE.ch reaction rule might involve more than one reactive site. Hence, information of reactive sites provide further insights into the molecule's reactivity. Furthermore, similarity of reactive sites and their neighborhoods based on the NICEdrug score increase the comparison resolution and this is the basis of the classification in NICEdrug.ch.

In NICEdrug.ch database there exist 95,342 classes that comprise all drugs and human compounds sharing EC, BNICE.ch rule, and reactive site (classification based on our strategy 3). We computed the NICEdrug score between all pairs of molecules in a class and this information is available in NICEdrug.ch.

Identification of drugs acting as para-metabolites based on NICEdrug score

Small molecules that share reactive site and are structurally similar to native human metabolites enter and bind the pocket of native enzymes and competitively inhibiting catalysis acting as para-metabolites (Ariens, 2012). In this study, we define as para-metabolite any drug or any of its metabolic neighbors that (1) shares reactive site with native metabolites (α=1), and (2) preserves a high NICEdrug score with respect to the reactive site neighborhood (β>0.9).

Identification of drugs acting as anti-metabolites based on NICEdrug score

Small molecules that do not share reactive site but are structurally similar to native human metabolites might enter the binding pocket of native enzymes and inhibiting catalysis acting as anti-metabolites (Ariens, 2012). In this study, we define as anti-metabolite any drug or any of its metabolic neighbors that (1) differs slightly in reactive site from a native metabolite (α~1), and (2) preserves high similarity in the reactive site neighborhood (β>0.9). We hypothesize that a low divergence in the reactive site, still allows a non-native compound to enter and bind the enzyme pocket since it is structurally similar enough to the native substrate.

Identification of NICEdrug toxic alerts

We obtained all NICEdrug toxic alters from ToxAlert database (Sushko et al., 2012). ToxAlert database includes about 1,200 structural toxic alerts associated with particular types of toxicity. Toxic alerts are provided in the form of SMART patterns that are searchable in SMILES structure of molecules. NICEdrug.ch uses openbable tool (O'Boyle et al., 2011) to search for these structural alerts on SMILES of compounds.

Collection of reference toxic molecules in NICEdrug.ch

Studying the adverse effects of chemicals on biological systems has led to development of databases cataloging toxic molecules. The Liver Toxicity Knowledge Base (LTKB) integrates 1,036 molecules annotated with human Drug-induced liver injury risk (severity). Super toxic DB include about 60k toxic molecules, that are annotated with their toxicity estimate, LC_{50}/LD_{50} i.e., lethal dose or concentration at which 50% of a population dies.

As a resource of approved toxic molecules, we collected all of the molecules cataloged as toxic in LTKB and super toxic databases. We used this collection as a reference to compare the similarity of drugs or and products of drug metabolism with approved toxic molecules.

Definition of a toxicity score in NICEdrug.ch

The number of molecules labeled as toxic in databases is disproportionally low compared to the space of compounds. On the other hand, toxic alerts are defined for a big number of compounds and are linked to redundant molecular structures.

We measured the similarity of drugs and their metabolic neighbors with the collection of reference toxic molecules using the NICEdrug score. We assigned toxic alerts to molecules in NICEdrug.ch if a molecule and toxic molecule shared a molecular substructure linked to the toxic alert.

Finally, NICEdrug.ch provides a toxicity report in the form of a csv file for each molecule in the metabolic neighborhood including six values linked to the most similar toxic molecules in both toxic reference databases (LTKB and supertoxic databases): (1) the NICEdrug score between the drug and those most similar toxic molecules, (2) the severity degree of the hepatotoxic compound, and log(LC_{50}) of the supertoxic compound, and (3) the number of common toxic alerts between the drug and the most similar toxic molecules. The list of toxic alerts is also provided.

We combined the six values of the toxicity report into a toxicity score defined as follows:

$$\sum_{i} \text{NICEdrug score} \times (\log(\text{LC}_{50}) \text{ or severity degree}) \times \text{number of common NICEdrug toxic alerts}$$

$$\text{i} \in \{\text{the most similar approved toxic molecules in LTKB and supertoxic databases}\}$$

The toxicity score in NICEdrugh.ch served to quantify the toxicity of each molecule in the metabolic neighborhood of a drug, recapitulate known toxic molecules, and suggest new toxic compounds (Figure 4).

Analysis of essential enzymes and linked metabolites in *Plasmodium* and human cells

We extracted information of essential genes and enzymes for liver-stage malaria development from our recent study (Stanway et al., 2019). In this study, we developed the genome-scale metabolic model of *Plasmodium berghei*, which shows high consistency (approximately 80%) with the largest gene knockout datasets in *Plasmodium* blood (Bushell et al., 2017) and liver stages (Stanway et al., 2019). There are 178 essential genes for *P. berghei*'s growth simulating

liver-stage conditions (Stanway et al., 2019). Here, we identified the substrates of those essential metabolic enzymes, which comprise a set of 328 metabolites (Table S4). To further minimize on the host cell, we filtered out those *Plasmodium* enzymes that share 4th level E.C. with human essential enzymes. We used available CRISPR gene essentiality data in various human cell lines (Wang et al., 2015) to identify essential genes and enzymes in human cells (Table S4). We further identified essential metabolites in human cells (Table S4) using the latest human genome-scale metabolic model (Robinson et al., 2020) and the metabolic information associated to the essential human genes. Subtracting essential parasite and human enzymes resulted in the analysis of 33 essential *Plasmodium* enzymes catalyzing 68 metabolites and 157 unique metabolite-enzyme pairs in the parasite (Table S5).

Identification of drugs to target malaria and minimize side effects on human cells

Those molecules that themselves and their downstream products cannot act as inhibitors of essential metabolic enzymes in the human host cell while they can target essential *Plasmodium* enzymes are attractive antimalarial candidates.

We first used NICEdrug.ch to look for small molecules that share reactive site with the 33 essential *Plasmodium* enzymes and they have good similarity score in reactive site neighborhood to native substrates of essential enzymes of parasite, i.e. NICEdrug score above 0.5 (Table S5). We also identified prodrugs that might lead to downstream products with similar reactive site and neighborhood (NICEdrug above 0.5) to any of the essential *Plasmodium* metabolites (Table S5). We suggest those drugs and downstream products act as antimetabolites and competitively inhibit the essential enzymes in the parasite. Overall, we identified 538 drugs that directly compete with essential metabolites and 1,018 prodrugs that need to be biochemically modified between one to three times in human cell to render inhibition of essential enzymes.

We next combined information of essential *Plasmodium* and human metabolites to screen further the drug search using NICEdrug.ch. Out of the hypothetical 538 antimalarial candidates, we identified 68 drugs that share reactive site with parasite metabolites (NICEdrug score above 0.5) and not with human metabolites (NICEdrug score below 0.5), making them good candidates for drug design (Table S5).

Supplemental table titles and legends

Table S1. Metabolic neighborhood of 5-FU, related to Figures 2, 3, and 4.

(1) List of compounds in the 5-FU metabolic neighborhood including up to four reactions or steps away. **(2)** Description of reactions in the 5-FU metabolic neighborhood including up to four reactions or steps away.

Table S2. NICEdrug score between all molecules with reactive site of statins in NICEdrug.ch, related to Figure 5.

We report the NICEdrug score between each pair of the whole set of 254 molecules in the NICEdrug.ch DB with reactive site of statins.

Table S3. Description of nine drugs candidates for repurposing to replace statins based on NICEdrug.ch, related to Figure 5.

These drugs can act as competitive inhibitors of HMG-CoA reductase like statins.

Table S4. Essential genes/enzymes and linked metabolites in liver-stage *Plasmodium* and a human cell, related to Figure 6.

(A) List of essential genes and associated reactions in liver-stage *Plasmodium*, as obtained from the study Stanway et al. **(B)** List of essential genes and associated reactions in a human cell, as obtained from the study Wang et al. **(C)** List of metabolites linked to essential genes in liver-stage *Plasmodium*. **(D)** List of metabolites linked to essential genes in a human cell.

Table S5. Information of human metabolism considered in this study, related to Figures 2, 3, 4, 5, and 6.

(A) List of essential metabolite-EC pairs in *Plasmodium* parasite that are not essential in human. **(B)** NICEdrug.ch information for drugs similar to metabolites of essential *Plasmodium* enzymes (NICEdrug > 0.5). **(C)** List of essential metabolite-EC pairs in human. **(D)** NICEdrug.ch information for drugs in Table S5.2 and their most similar *Plasmodium* metabolite and human metabolite. **(E)**

Description of all comparisons between drugs, *Plasmodium* metabolites, and human metabolites. **(F)** Description of drugs and prodrugs identified the malaria analysis with NICEdrug.ch and approved in the study by Antonova-Koch *et al* along with their most similar *Plasmodium* metabolite and human metabolite.

Table S6. Information of human metabolism considered in this study, related to Figures 2, 3, 4, 5, and 6.

(A) List of cofactors, **(B)** list of metabolites, and **(C)** list of E.C. numbers considered in BNICE.ch for the generation of reactions in the analysis of drug metabolism in a human cell. These cofactors are allowed to react with drugs and native metabolites in the prediction of the drug's metabolic neighborhood using the retrobiosynthesis algorithm in BNICE.ch. We report 2,266 unique metabolites and 2,066 unique E.C. numbers in the context of human biochemistry. This set integrates all human metabolites and metabolic activities (E.C. numbers) that are collected from three human metabolic databases: Recon3D, HMR, and Reactome databases.

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Figure 1

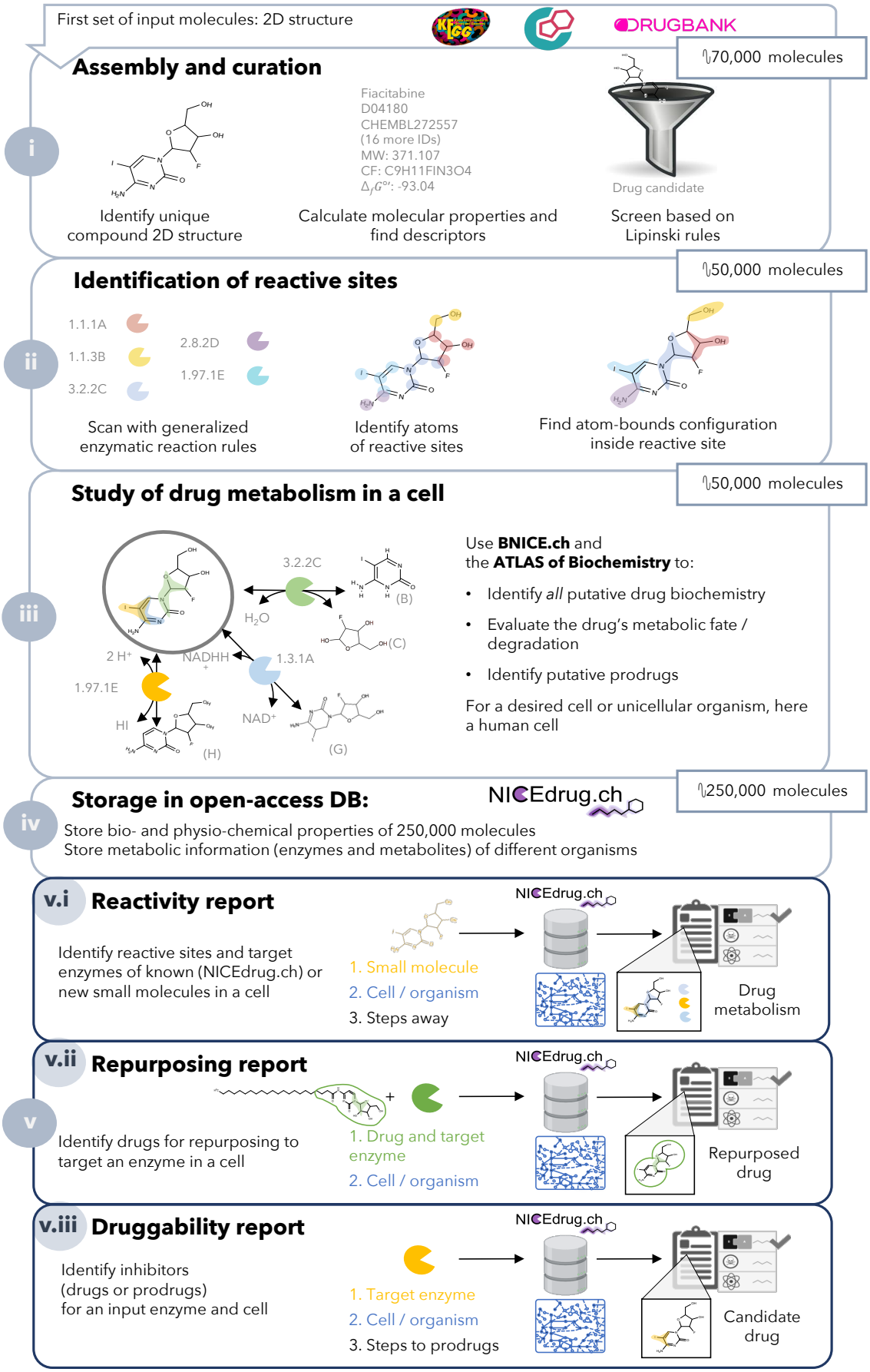

First set of input molecules: 2D structure
DRUGBANK
~70,000 molecules
i
Assembly and curation
Fiacitabine
D04180
CHEMBL272557
(16 more IDs)
MW: 371.107
CF: C9H11FIN3O4
Δ_f G°': -93.04
Drug candidate
Identify unique compound 2D structure
Calculate molecular properties and find descriptors
Screen based on Lipinski rules
~50,000 molecules
ii
Identification of reactive sites
1.1.1A
1.1.3B
3.2.2C
2.8.2D
1.97.1E
Scan with generalized enzymatic reaction rules
Identify atoms of reactive sites
Find atom-bounds configuration inside reactive site
~50,000 molecules
iii
Study of drug metabolism in a cell
3.2.2C
H2O
(B)
(C)
2 H+
NADHH+
1.3.1A
1.97.1E
HI
NAD+
(H)
(G)
Use BNICE.ch and the ATLAS of Biochemistry to:
• Identify all putative drug biochemistry
• Evaluate the drug's metabolic fate / degradation
• Identify putative prodrugs
For a desired cell or unicellular organism, here a human cell
~250,000 molecules
iv
Storage in open-access DB:
NICEdrug.ch
Store bio- and physio-chemical properties of 250,000 molecules
Store metabolic information (enzymes and metabolites) of different organisms
v
v.i Reactivity report
Identify reactive sites and target enzymes of known (NICEdrug.ch) or new small molecules in a cell
1. Small molecule
2. Cell / organism
3. Steps away
NICEdrug.ch
Drug metabolism
v.ii Repurposing report
Identify drugs for repurposing to target an enzyme in a cell
1. Drug and target enzyme
2. Cell / organism
NICEdrug.ch
Repurposed drug
v.iii Druggability report
Identify inhibitors (drugs or prodrugs) for an input enzyme and cell
1. Target enzyme
2. Cell / organism
3. Steps to prodrugs
NICEdrug.ch
Candidate drug

Figure 2

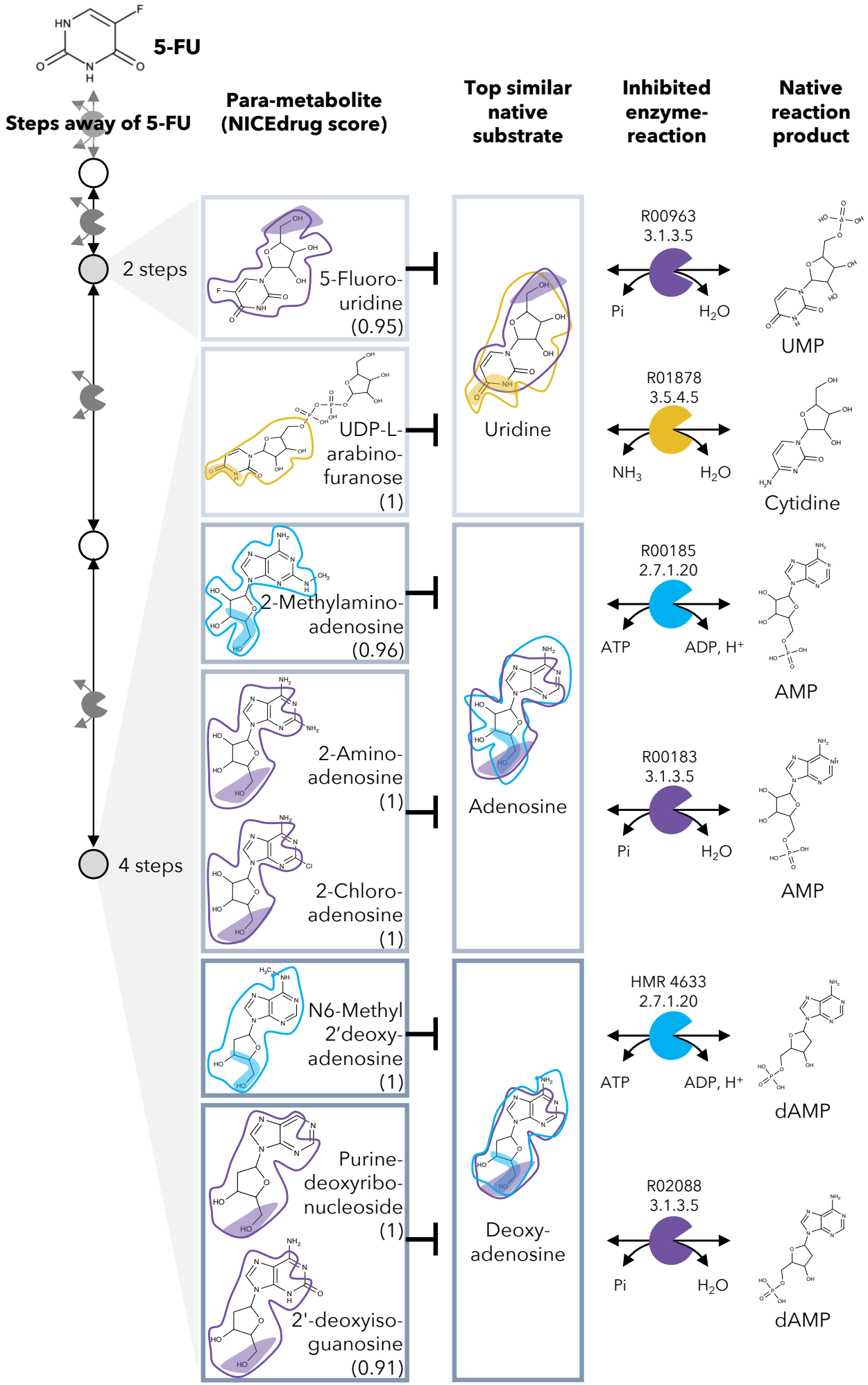

5-FU
Steps away of 5-FU
Para-metabolite (NICEdrug score)
Top similar native substrate
Inhibited enzyme-reaction
Native reaction product
2 steps
4 steps
5-Fluoro-uridine (0.95)
UDP-L-arabino-furanose (1)
2-Methylamino-adenosine (0.96)
2-Amino-adenosine (1)
2-Chloro-adenosine (1)
N6-Methyl 2'deoxy-adenosine (1)
Purine-deoxyribo-nucleoside (1)
2'-deoxyiso-guanosine (0.91)
Uridine
Adenosine
Deoxy-adenosine
R00963
3.1.3.5
Pi
H2O
UMP
R01878
3.5.4.5
NH3
H2O
Cytidine
R00185
2.7.1.20
ATP
ADP, H+
AMP
R00183
3.1.3.5
Pi
H2O
AMP
HMR 4633
2.7.1.20
ATP
ADP, H+
dAMP
R02088
3.1.3.5
Pi
H2O
dAMP

Figure 3

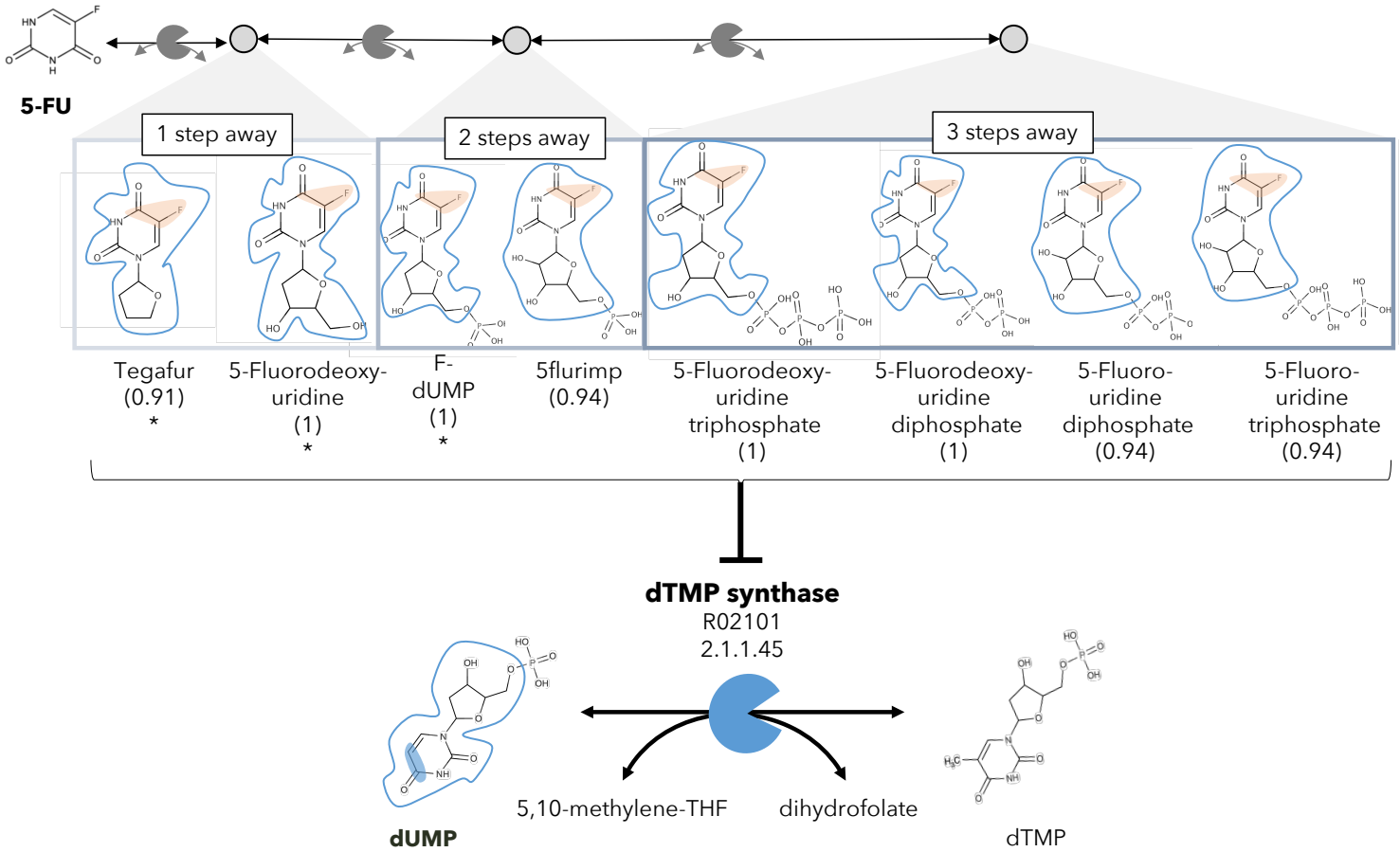
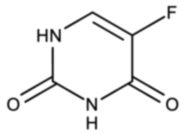

5-FU
1 step away
2 steps away
3 steps away
Tegafur (0.91) *
5-Fluorodeoxy-uridine (1) *
F-dUMP (1) *
5flurimp (0.94)
5-Fluorodeoxy-uridine triphosphate (1)
5-Fluorodeoxy-uridine diphosphate (1)
5-Fluoro-uridine diphosphate (0.94)
5-Fluoro-uridine triphosphate (0.94)
dTMP synthase
R02101
2.1.1.45
dUMP
5,10-methylene-THF
dihydrofolate
dTMP

Figure 4



5-FU

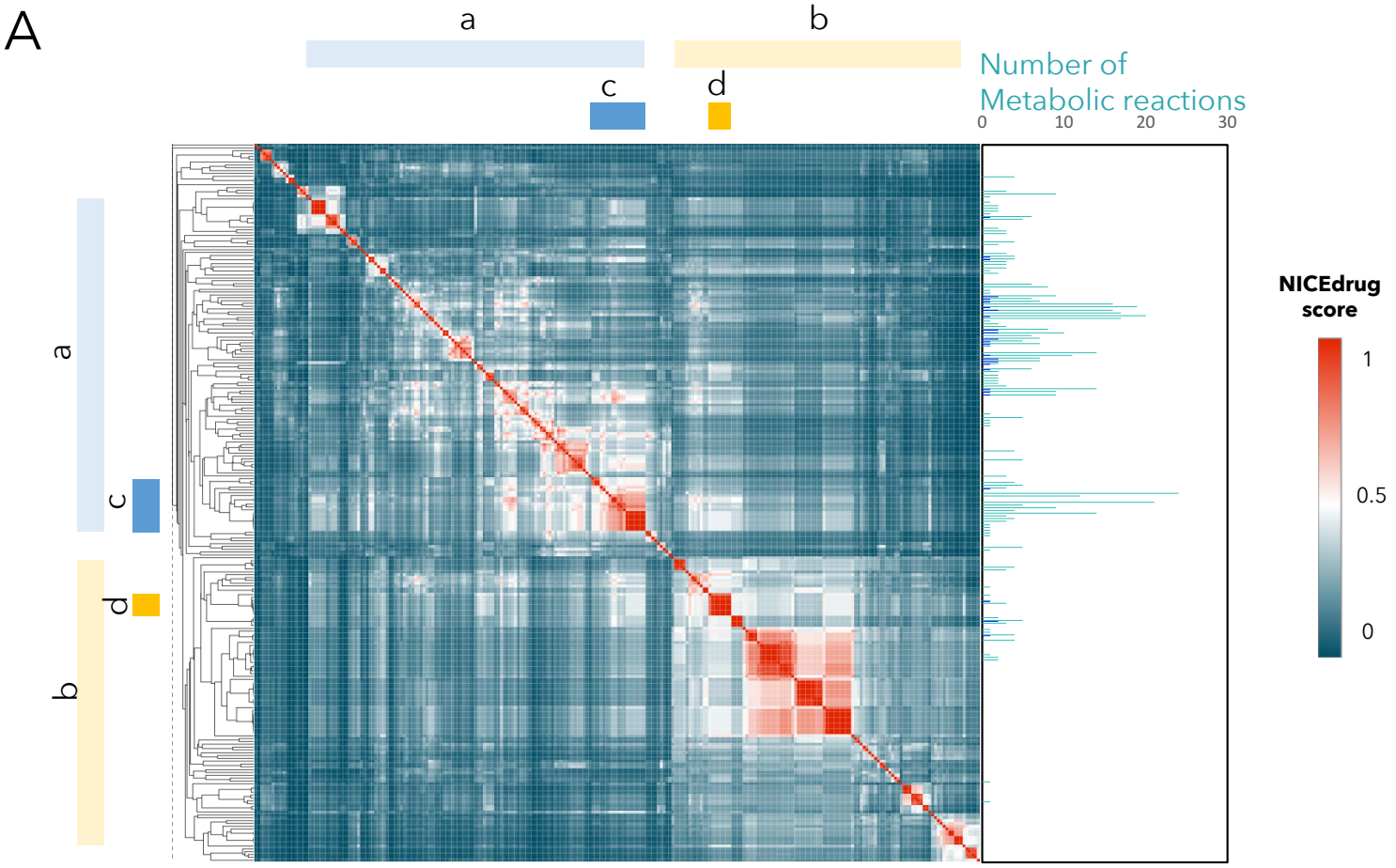
Suggested toxic metabolic neighbor (NICEdrug toxicity score)	**Supertoxic DB** (NICEdrug score, log(LC_{50}), ∩alerts**)	**Hepatotoxic DB** (NICEdrug score, severity, ∩alerts**)
1 step: Cytidine (29.4)	Enocitabine (0.98, 4, 8)	Cytidine (1, 3, 8)
2 steps: 5-Fluorouridine (31)	5-Fluorouridine (1, 7, 4)	Thymidine (0.78, 2, 3)
3 steps: Azacitidine (33)	Azacitidine (1, 4, 11)	Azacitidine (1, 8, 11)
4 steps: 5-Fluorouridine-3P (34) *	5-Fluorouridine (0.99, 7, 16)	Deoxythymidine (0.77, 2, 2)
4 steps: 5flurimp (30)	CMP(1, 4, 7)	Deoxythymidine (0.77, 2, 2)
4 steps: F-dUMP (29.6) *	CHEMBL3228489 (0.87, 4, 6)	Deoxythymidine (0.82, 2, 2)
4 steps: 5-Fluoro-deoxyuridin-3P (28.9) *	Clevudine (0.8, 4, 6)	Deoxythymidine (0.82, 2, 2)

**∩alerts: number of NICEdrug common toxic alerts
* Toxicity validated

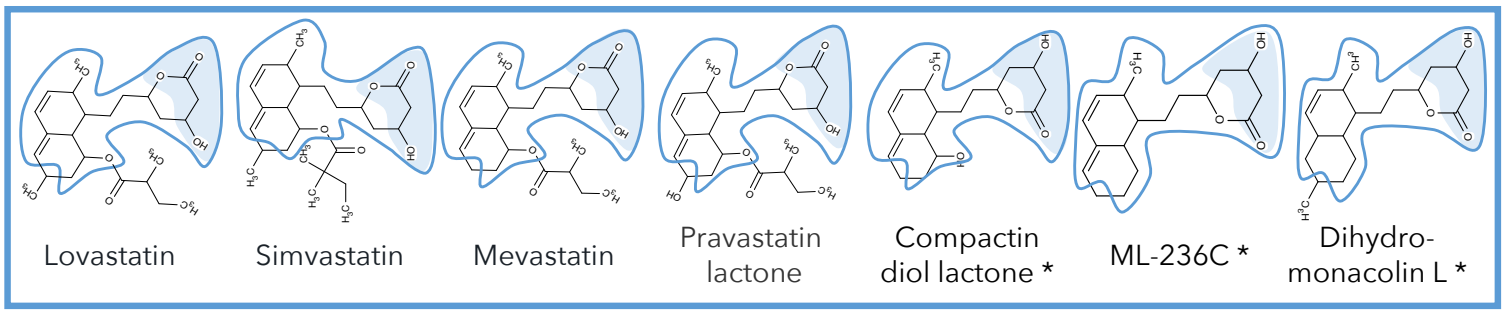
- Genotoxic carcinogenicity, mutagenicity
- Non-biodegradability
- Acute aquatic toxicity
- Pyridinium-related toxicity
- Skin sensitization

Figure 5

A


a
b
c
d
Number of Metabolic reactions
0
10
20
30
NICEdrug score
1
0.5
0

B


Lovastatin
Simvastatin
Mevastatin
Pravastatin lactone
Compactin diol lactone *
ML-236C *
Dihydro-monacolin L *

C

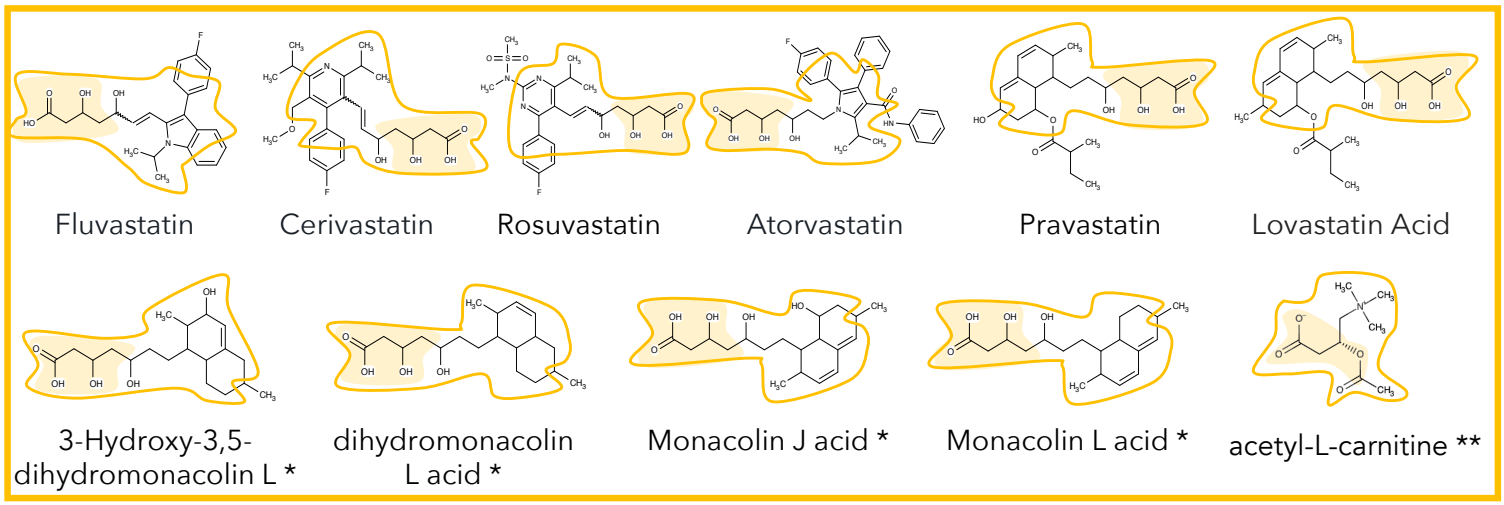
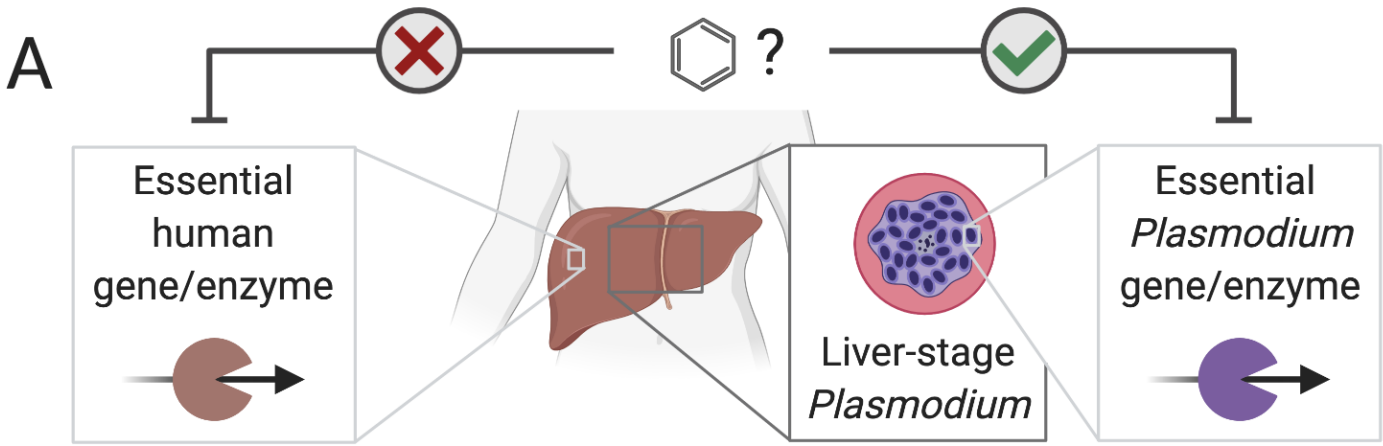
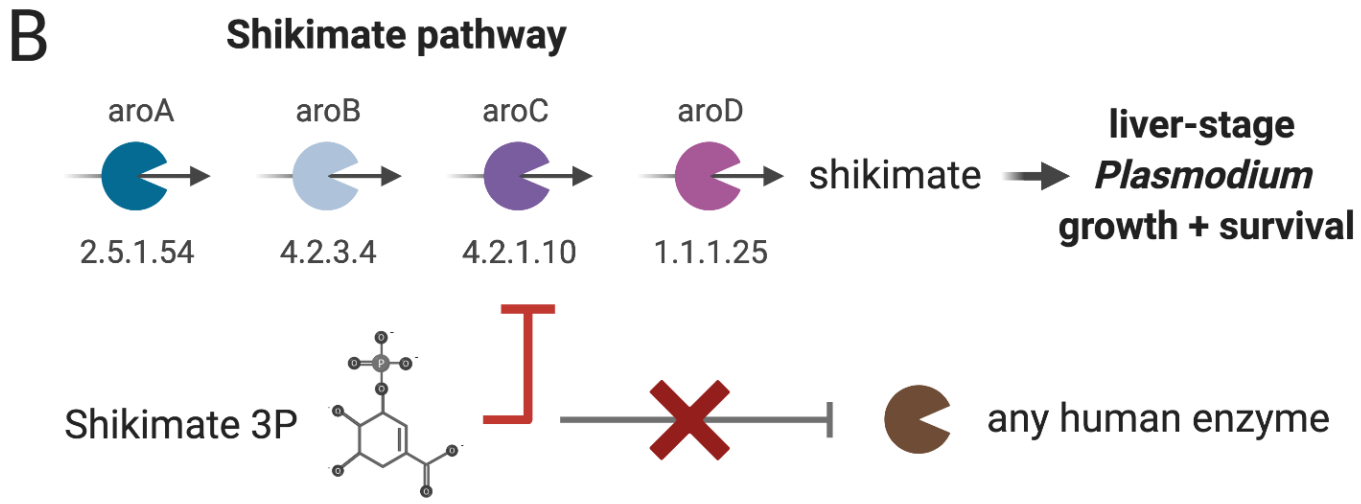

Fluvastatin
Cerivastatin
Rosuvastatin
Atorvastatin
Pravastatin
Lovastatin Acid
3-Hydroxy-3,5-dihydromonacolin L *
dihydromonacolin L acid *
Monacolin J acid *
Monacolin L acid *
acetyl-L-carnitine **

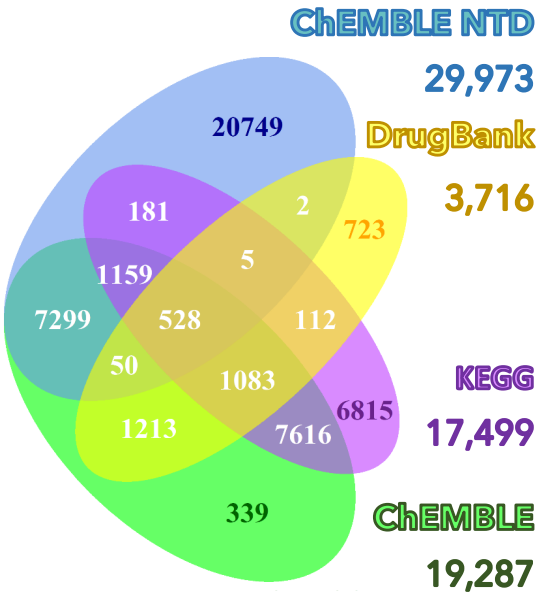
Figure 6


A
?
Essential human gene/enzyme
Liver-stage Plasmodium
Essential Plasmodium gene/enzyme

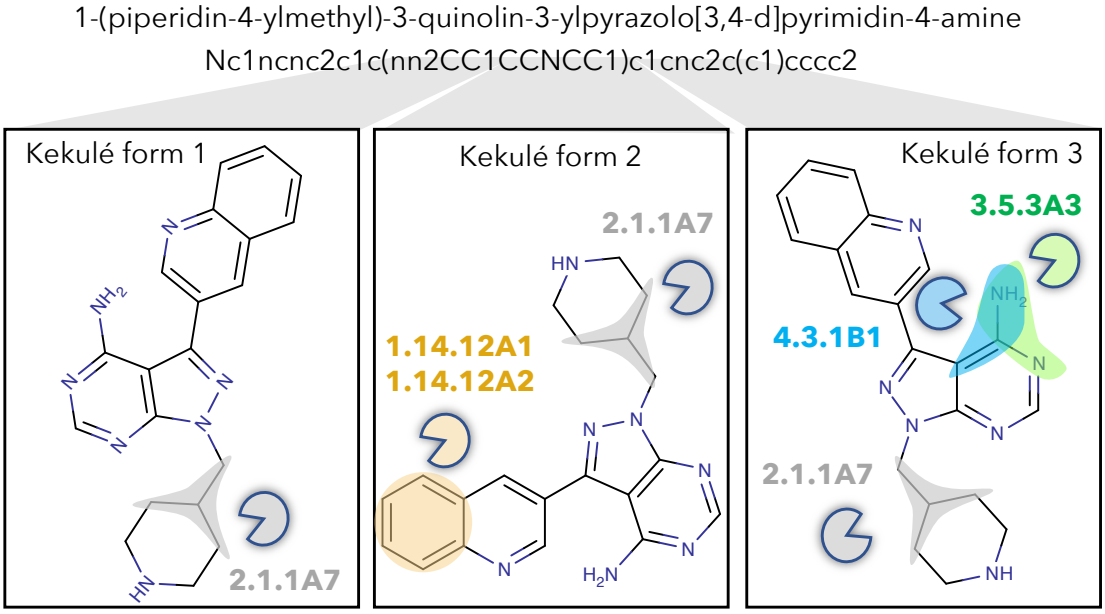

B
Shikimate pathway
aroA
aroB
aroC
aroD
2.5.1.54
4.2.3.4
4.2.1.10
1.1.1.25
shikimate
liver-stage Plasmodium growth + survival
Shikimate 3P
any human enzyme

Supplementary figure 1

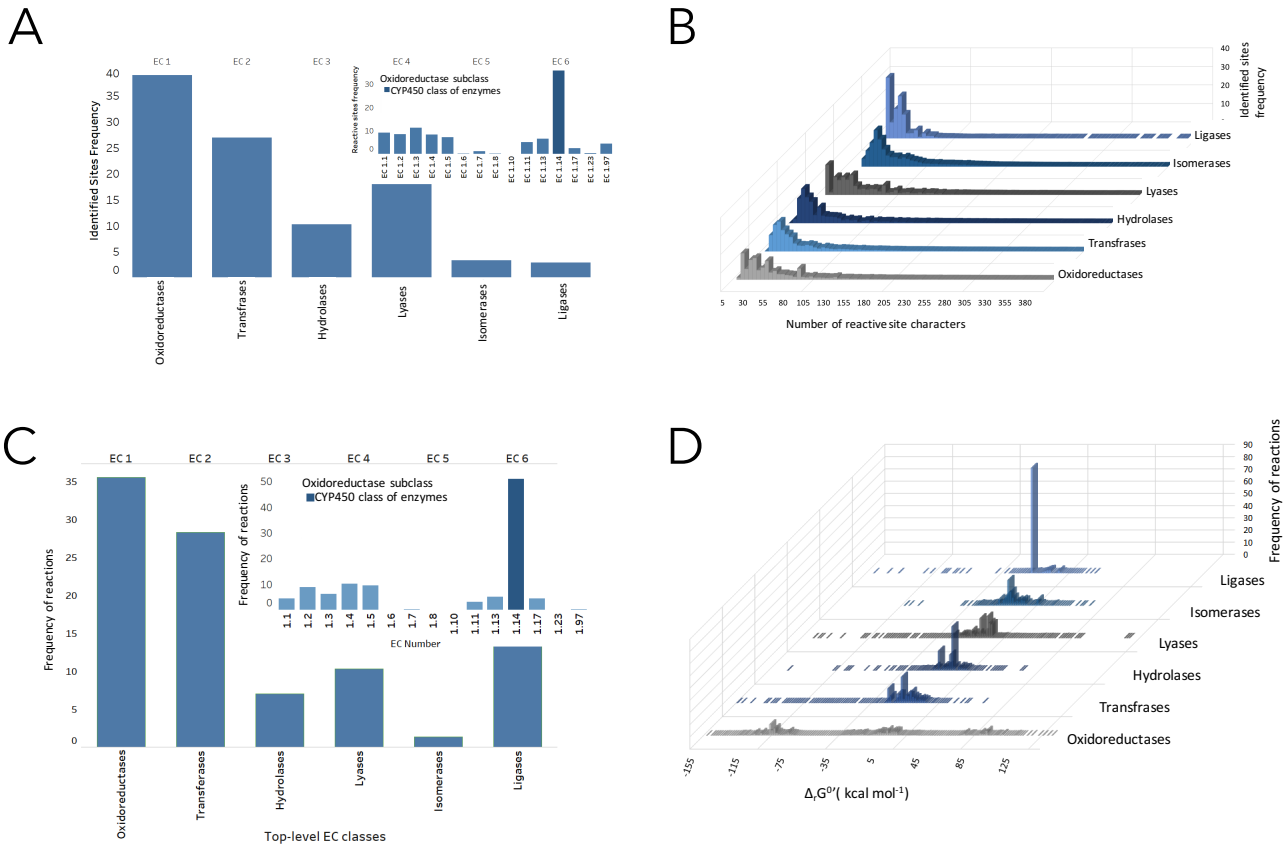
A



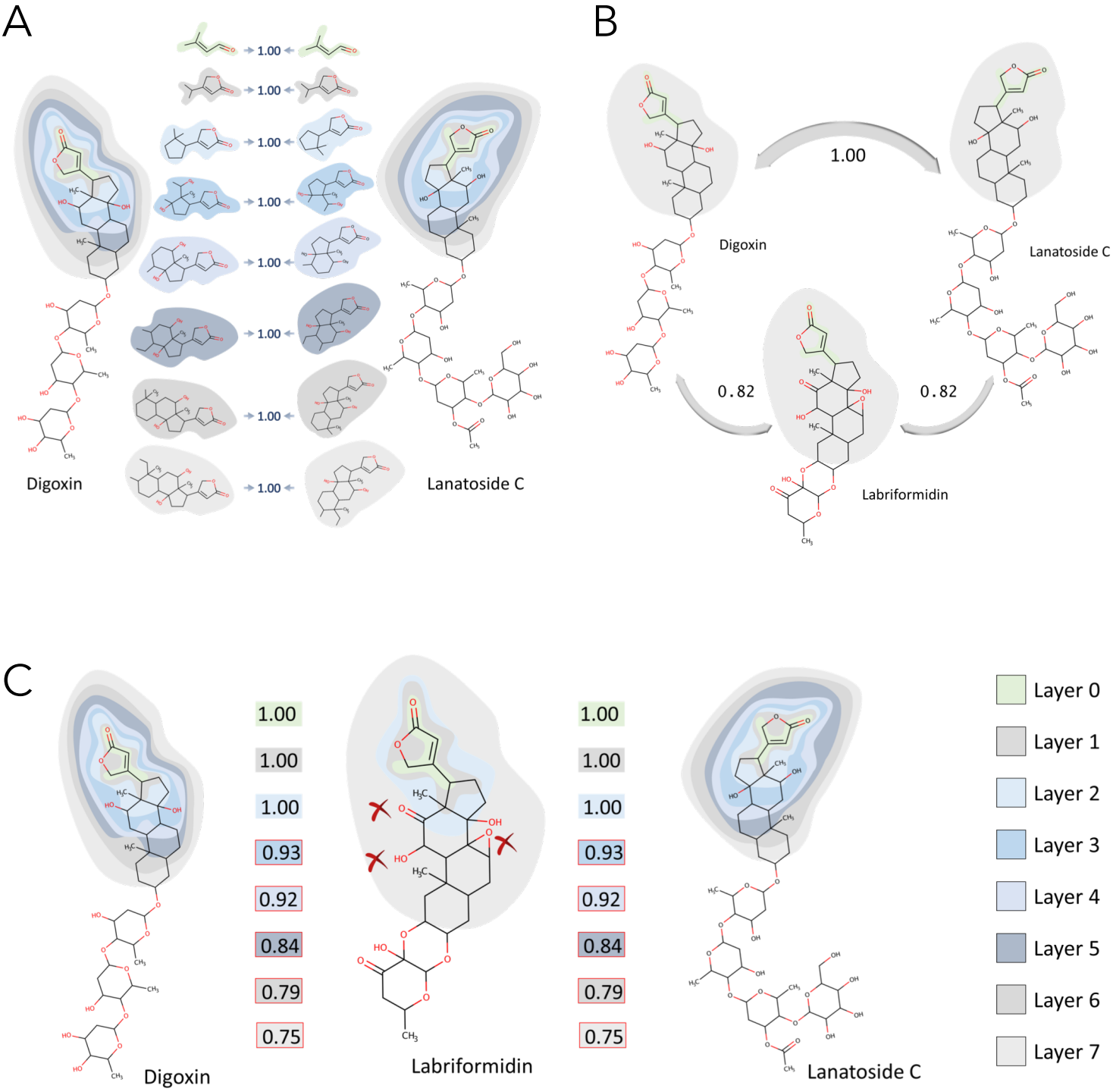
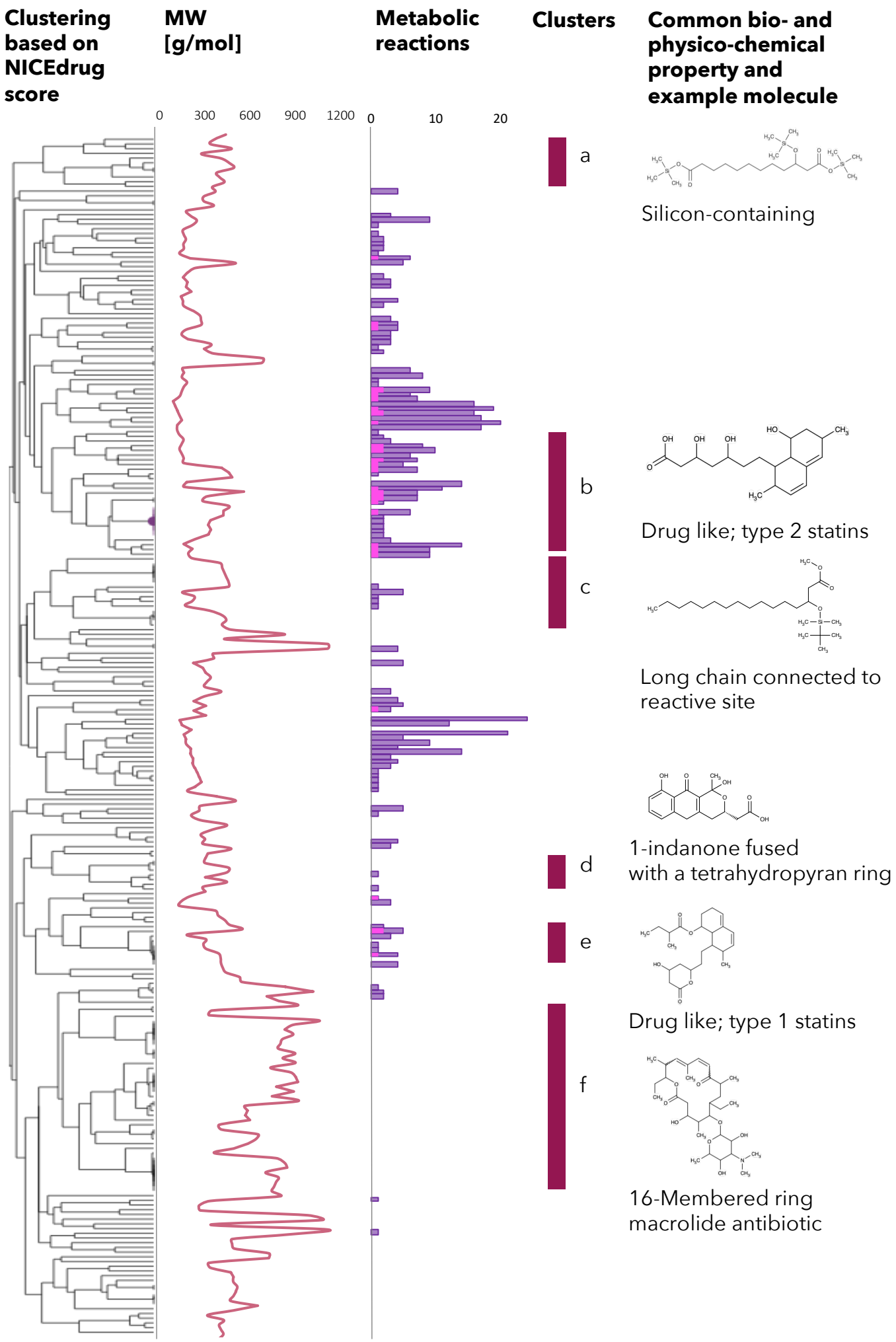
B



Supplementary figure 2



Supplementary figure 3



Supplementary figure 4