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Database issue

INTEDE 2.0: the metabolic roadmap of drugs

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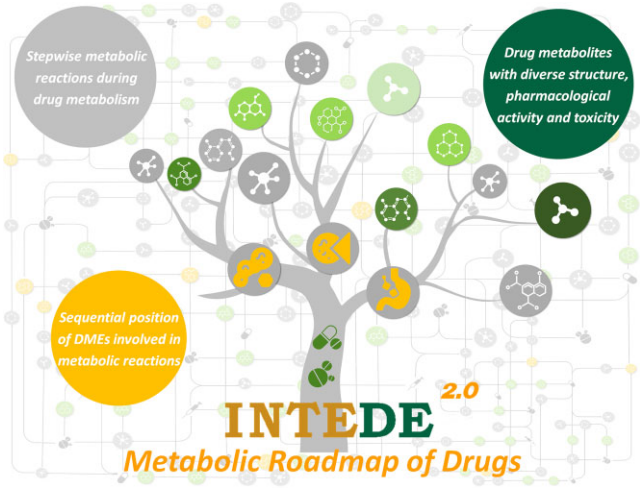
Abstract

The *metabolic roadmap of drugs* (MRD) is a comprehensive atlas for understanding the stepwise and sequential metabolism of certain drug in living organisms. It plays a vital role in lead optimization, personalized medication, and ADMET research. The MRD consists of three main components: **(i)** the sequential catalyses of drug and its metabolites by different drug-metabolizing enzymes (DMEs), **(ii)** a comprehensive collection of metabolic reactions along the entire MRD and **(iii)** a systematic description on efficacy & toxicity for all metabolites of a studied drug. However, there is no database available for describing the comprehensive *metabolic roadmaps of drugs*. Therefore, in this study, a major update of INTEDE was conducted, which provided the stepwise & sequential metabolic roadmaps for a total of 4701 drugs, and a total of 22 165 metabolic reactions containing 1088 DMEs and 18 882 drug metabolites. Additionally, the INTEDE 2.0 labeled the pharmacological properties (pharmacological activity or toxicity) of metabolites and provided their structural information. Furthermore, 3717 drug metabolism relationships were supplemented (from 7338 to 11 055). All in all, INTEDE 2.0 is highly expected to attract broad interests from related research community and serve as an essential supplement to existing pharmaceutical/biological/chemical databases. INTEDE 2.0 can now be accessible freely without any login requirement at: http://idrblab.org/intede/

Graphical abstract



Introduction

The *metabolic roadmap of drugs* (MRD) is a comprehensive atlas that illustrates the stepwise and sequential metabolism of certain drug from administration to elimination in living organisms (1). It includes diverse metabolic reactions (MRs), drug metabolites (DMs) & drug-metabolizing enzymes (DMEs) (2,3), and is critical for lead optimization, personalized medication, ADMET research, etc. (4–6).

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Particularly, an MRD consists of three main components (i) *the sequential catalyses of drug and its metabolites by different DMEs*, which describes the dynamic and sequential influence of multiple DMEs on drug metabolism (7); (ii) *a comprehensive collection of MRs along the entire MRD*, which illustrates the catalytic sites on both drug & its corresponding DME and the variations in DMs' physicochemical properties and structural conformations (8) and (iii) *a systematic description on efficacy & toxicity for all metabolites of a studied drug*, which provides valuable information to the studies of structure-activity relationship and adverse drug reaction (9). In other words, these components jointly determine the chemical fate of drugs, which are essential for clarifying the mechanism underlying drug metabolism, resistance, toxicity, and so on (10–13). Therefore, it is highly demanded to have a database that offers systematic and valuable information of MRD for diverse drugs of clinical importance.

So far, a variety of popular databases related to drug metabolism has been constructed. Some of them provide the general data of enzymatic classification, chemical structure or chromatographic analysis for various metabolites as part of a broad collection of biological/chemical information, such as: HMDB (14), VMH (15), BRENDA (16), MetaCyc (17) and PubChem (18); some others describe diverse metabolic pathways covering very limited amount (less than 70) of drugs, such as: KEGG (19), Reactome (20), WikiPathways (21) and SMPDB (22); the remaining include two popular databases: DrugBank (23) and PhamGKB (24), which illustrate the metabolic processes of about 500 FDA approved drugs by largely ignoring clinical trial drugs and investigative agents. The majority of the above databases are published on NAR, and have attracted extensive research interests and citations from related research communities. However, none of them systematically provides the MRD information for either clinical trial drugs or investigative agents, and the MRD information for FDA approved drugs is far from comprehensive in terms of the numbers of drugs, MRs, and DMEs. In other words, there is no database available for describing the comprehensive *metabolic roadmap of drugs*. Therefore, it is urgently needed to construct database that provides systematic data of MRD for diverse drugs of clinical importance (not only for FDA approved ones, but also for those in clinical trial and experimental investigation).

Herein, a major update of INTEDE was therefore performed to provide the stepwise & sequential metabolic roadmap for a total of 4701 drugs (1698 approved, 1957 clinical trials, 360 preclinical & investigative drugs, 686 discontinued drugs), including 127 prodrugs activated by DMEs or acidic environments. The constructed metabolic roadmaps contain 22 165 MRs, composing of 1088 DMEs and 18 882 DMs, which are clearly illustrated and explicitly described in the MRD of INTEDE 2.0 (illustrated in Figure 1). For those 18 882 DMs, their pharmacological activities against drugs' original targets, experimentally/clinically-validated toxicities, physicochemical characteristics and structural information were manually collected, which resulted in 1212 annotations of major DMs, 1277 annotations of active DMs, 332 annotations of toxicity or side effects of DMs and 16 274 chemical structures of DMs.

All in all, the comprehensive and sequential drug metabolic roadmaps provided in INTEDE 2.0 are key for the researchers working in the whole process of modern drug discovery (from target identification, to drug design, to lead optimization, to ADME assessment, to toxicity evaluation, to preclinical test, to clinical trials) (25,26). As such, INTEDE 2.0 is highly expected to attract broad interests from related research community and serve as an essential supplement to existing pharmaceutical/biological/chemical databases. The latest version of INTEDE 2.0 can be freely assessed by all users at: http://idrblab.org/intede/.

Factual content and data retrieval

Systematic collection of metabolic roadmap data for drugs

The metabolic profiles of drugs *in vivo* are largely dependent on the sequential metabolic reactions of drugs and the exogenous or endogenous factors influencing the expression or biological activities of DMEs, which directly determines the therapeutic effects or drives the clinical adverse reactions (27–30). Thus, INTEDE 1.0 (31) was updated with the detailed and sequential metabolic process of drugs in the organism as the main update element. The MRDs mapped by the new version would provide valuable information for drug development and clinical medication guidance (6,32). To ensure the quality, comprehensiveness, and timeliness of the data, multiple sources of drug molecular data were integrated, including ∼2600 approved drugs (the US Food and Drug Administration (US FDA) website (33), ∼6300 drugs that were previously in clinical trials (the ClinicalTrial.gov website) (34), and ∼16 000 preclinical and investigational drugs (the existing databases, such as TTD (35) and DrugMAP (36). Based on the drug molecular information collected above, a comprehensive and in-depth literature search was conducted on PubMed (37) using 'drug names and synonyms' + 'keyword' combinations, including: 'Drug Name' + metabolism, 'Drug Name' + metabolite, 'Drug Name' + biotransformation, 'Drug Name' + pharmacokinetics, 'Drug Name' + metabolic pathway, 'Drug Name' + reaction and so on. The identified literature was evaluated manually to extract any information about the *metabolic roadmap of drugs*. The collected data included: DMEs involved in the sequential metabolism of drugs; the full name and species origin of the DMEs; the information on the metabolic reactions involved (such as *N*-dehydroxylation, esterase hydrolysis, monohydroxylation, *etc.*); the detailed information of DM, such as DM names (IUPAC name or ID code), chemical structures, markers of 'major' & 'pharmacologically active' and so on.

The construction of MRDs depended on reliable experimental data. Therefore, the information related to the drug metabolism obtained from *in vitro* and *in vivo* samples (e.g. co-incubation solution, plasma, excreta, or tissue extracts, etc.) detected by HPLC, MS and NMR should be focused during the literature search process. Additionally, the well-known metabolism prediction tool BioTransformer 3.0 (38) was used to predict drugs without metabolic pathway data, thereby providing certain reference value for drug metabolism research and new drug development. Then, combined with comprehensive literature organization and tool prediction, the drug metabolism process was manually sorted out and the sequential metabolic pathways were mapped out as shown in Figure 1.

All in all, a total of 4701 drugs with stepwise and orderly drug metabolism information were sorted into MRDs, including 1698 FDA-approved drugs, 1957 clinical stage drugs, 360

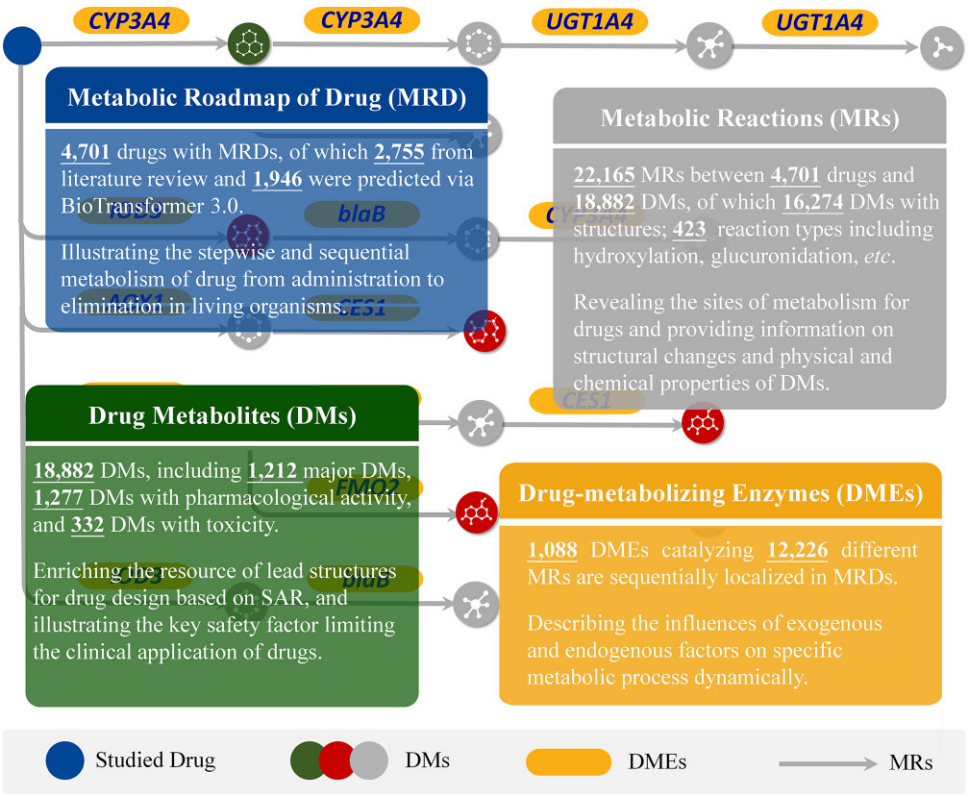

Figure 1. The *metabolic roadmap of drugs* (MRD) and corresponding statistics updated in INTEDE 2.0. (i) MRD and their corresponding drugs (blue), (ii) comprehensive collection of metabolic reactions along the entire MRD (gray); (iii) DM: the systematic description on efficacy and toxicity for all the drug metabolites in the MRD (green); (iv) DME data involved in the sequential catalyses of drugs (orange).

preclinical and research drugs, and 686 discontinued drugs. The summarized MRDs were composed of 22 165 metabolic reactions and 18 882 DMs and 1088 DMEs were localized to specific reaction nodes. A schematic diagram of the drug metabolic roadmap and detailed data information was shown in Figure 1.

Annotation of drug metabolites

In order to trace the biological impacts of the DMs produced during the sequential metabolism of drugs on the organism, the pharmacological information of DMs was also collected during the construction of MRDs and marked as 'major metabolite' and 'pharmacologically active metabolite' (39). In addition, data on the characteristics of toxicity or adverse reactions that could be caused by DMs (40) were also collated through searching the PubMed using the combinations of 'DMs names or synonyms' and 'keyword', including: 'DMs name' + toxic, 'DMs name' + side effects, 'DMs name' + toxicity, 'DMs name' + adverse, 'DMs name' + reactive, *etc*. The defined literature was assessed manually to extract any information about the drug metabolites. The collected data included: the general toxicological information of DMs (such as cardiovascular toxicity, carcinogenicity, genotoxicity, reproductive/developmental toxic, hepatotoxicity); the physical and chemical properties of DMs; and structural characteristics of DMs.

In summary, INTEDE 2.0 collected 1212 annotations of major DMs, 1277 annotations of active DMs, and 332 annotations of toxicity or side effects of DMs (summarized in Table 1). For DMs without biological activity data, the well-known molecular pharmacokinetic prediction tool ADMET Lab 2.0 (41) was used to evaluate the toxic properties of DMs. The above integrated information could further profile the details of drug metabolism, which was conducive to elaborating the molecular mechanisms underlying therapeutic significance or adverse reactions due to metabolism.

Metabolic roadmap data for drugs provided in INTEDE 2.0

Sequential catalyses of drug and its metabolites by different DMEs

The sequential catalyses of drug and its metabolites by different DMEs in MRDs are crucial for elucidating the metabolic profile of drug metabolism in the body (42). Just like dams can regulate the flow rate of rivers and the amount of water stored upstream and downstream, DMEs can directly determine the metabolism rate of drugs in the body, changes in plasma concentration, and the metabolic fate of drugs in the body. The catalytic activities of DMEs are easily affected by endogenous and exogenous factors, and the metabolic results of drugs in different bodies become complex and unpredictable, resulting in individual differences in drug metabolism (43,44).

Taking Adenosine's metabolic roadmap as an example, there were four metabolic pathways in the body for this drug, and the enzymes catalyzing each biological process were located at the corresponding reaction nodes (as shown in Figure 2). According to Figure 2, the metabolic profiles of

Table 1. Statistics of new features added to the 2024 update of INTEDE 2.0

a. Metabolism roadmaps of drugs			
No. of DMEs in metabolism roadmaps	No. of drugs with metabolism roadmap (approved/in clinical/other)	No. of metabolic reactions	No. of drug metabolites
1088	4701 (1698 / 1957 / 1046)	22 165	18 882
b. Annotation of drug metabolites			
No. of toxic drug metabolites	No. of drug metabolites with structures	No. of major drug metabolites	No. of active drug metabolites
332	16 274	1212	1277
c. Data supplementation compared to INTEDE 1.0			
	No. of metabolic interactions between drugs and DMEs	No. of drugs with interactions	No. of DMEs
INTEDE 2.0	11 055	2466	1897
(INTEDE 1.0)	(7338)	(1890)	(1046)

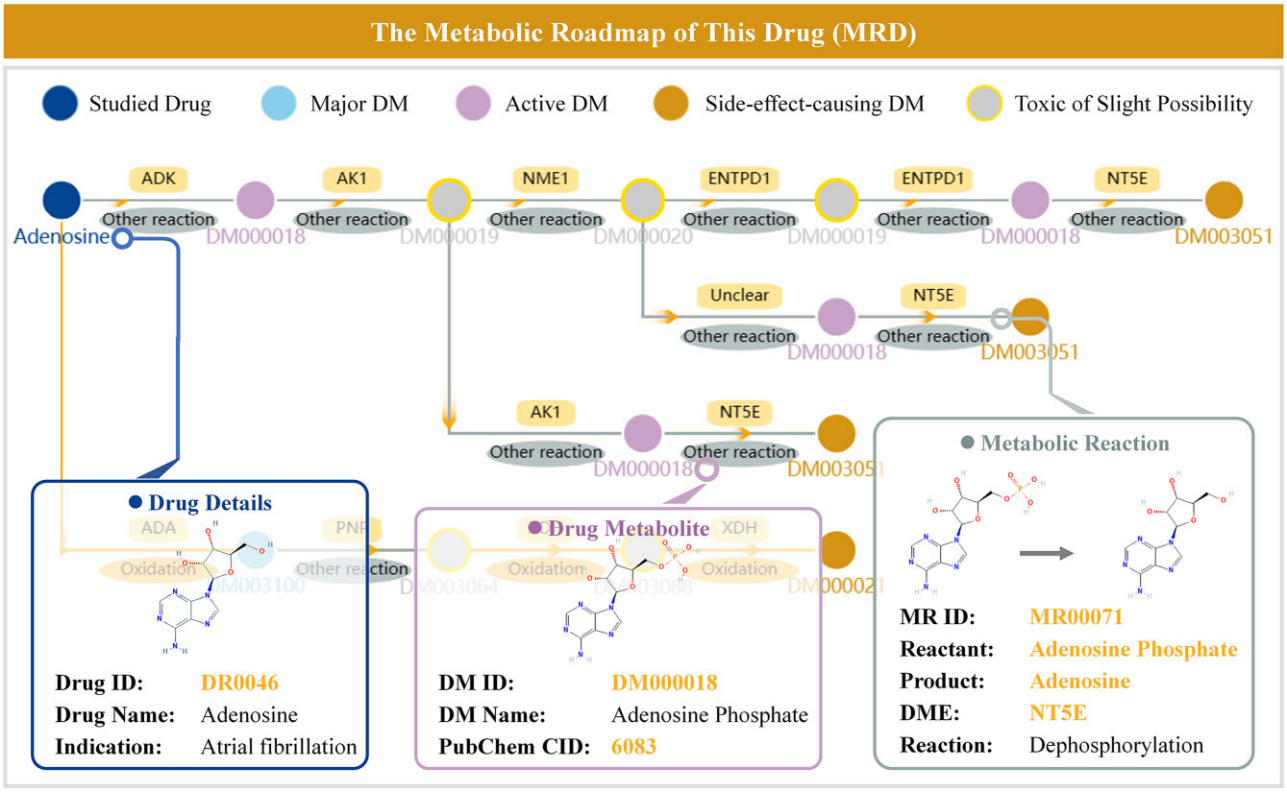

Figure 2. A typical page in INTEDE 2.0 offers the sequential position of DMEs involved in MRD. Taking the metabolic profiles of Adenosine as an example, there were four metabolic pathways in the body for this drug, and the enzymes catalyzing each biological process were located at the corresponding reaction nodes.

Adenosine in Pathway I was mainly determined by the drug metabolizing enzyme (ADK, AK1, NME1, ENTPD1 and NT5E) (45). When the expression of certain enzyme was affected by endogenous or exogenous factors (for example, miR-30a-5p inhibiting the expression of NT5E, Azacitidine upregulating the expression of NT5E) (46), the metabolism of Adenosine on this pathway would also be affected accordingly, that was, the drug metabolite adenosine phosphate (its DM ID in INTEDE 2.0 is DM000018) would accumulate to a certain extent (47). The information of sequential catalyses of drug and its metabolites by different DMEs could be found in the page of 'Drug Metabolic Roadmap' in the website of INTEDE 2.0. Collectively, combining the sequential positioning information of DMEs in MRD with the influencing factors of DMEs could help to further understand the mechanisms of drug metabolism, explore the mechanisms of drug metabolism differences, drug resistance, drug–drug interactions, and adverse reactions among individuals, and more accurately guide clinical medication (48–53).

Stepwise metabolic reactions during drug metabolism

INTEDE 2.0 collates drug MRs information in the 'Metabolic Reaction of Drug/Drug Metabolite' interface, providing the enzyme catalyzed reaction diagram of a drug or DM, showing the chemical structural changes (as illustrated in Figure 3). The stepwise and orderly MRs in MRDs could help to understand the structural changes during drug metabolism at the molecular level, reveal the metabolic sites of drugs, illustrate the metabolic characteristics of the drug molecular

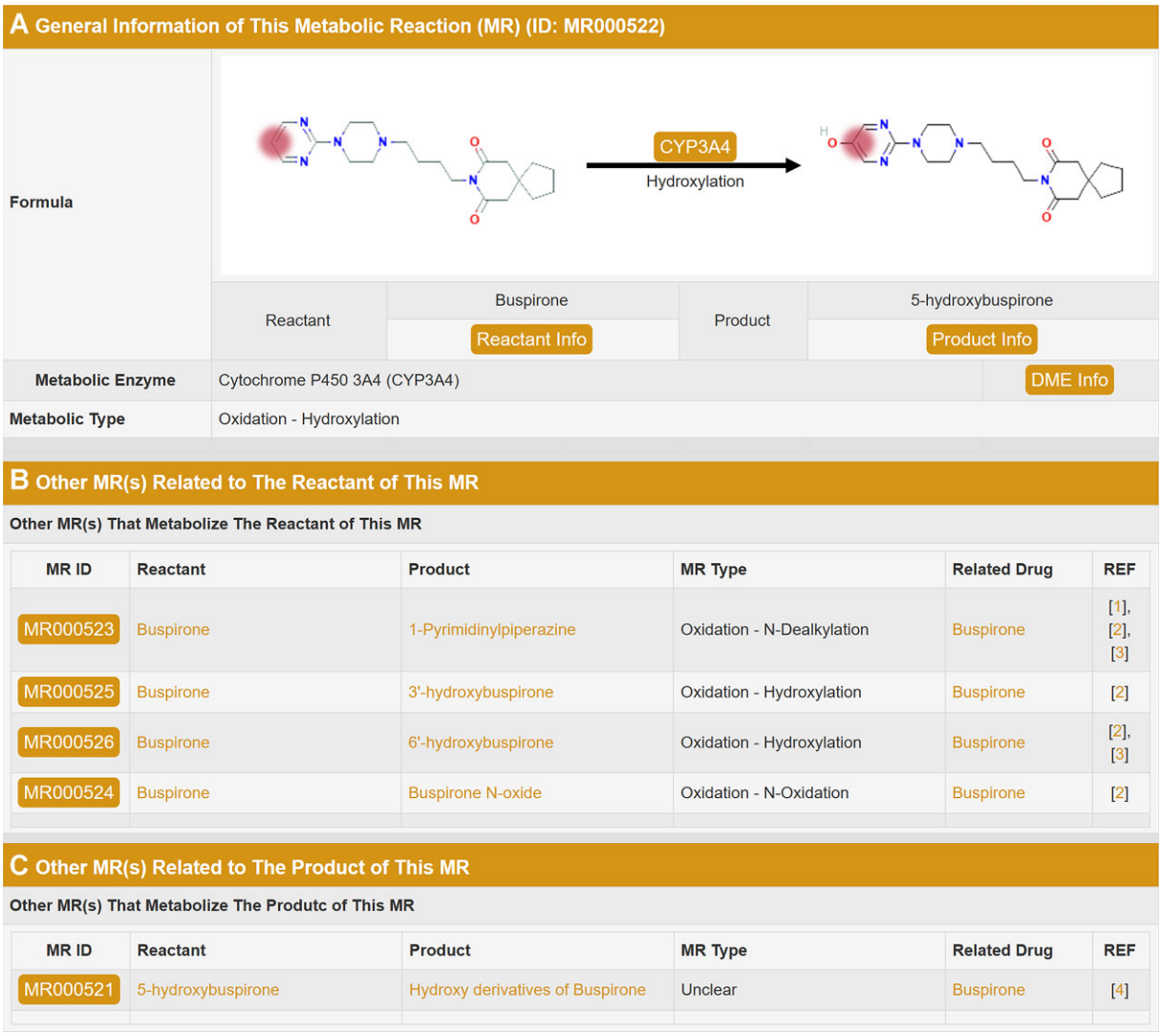

Figure 3. A typical page in INTEDE 2.0 presents the details of the metabolic reaction. Taking the 5-HT1A receptor agonist buspirone as an example: (**A**) general information of metabolic reaction of this drug, including its reactant, product, metabolic enzyme and metabolic type; (**B**) other metabolic reactions related to the reactant of this MR, including all reactions with the specific reactant as a metabolic substrate in INTEDE 2.0 and (**C**) other metabolic reactions related to the product of this MR, including all reactions with the specific product as a metabolic substrate in INTEDE 2.0.

structure, which guiding the design of drugs with desired metabolic properties (54,55).

Taking the 5-HT1A receptor agonist buspirone as an example (56), this drug exerted anti-anxiety effects, but had poor metabolic stability (short $t_{1/2}$), resulting in increased frequency of medication. By integrating the metabolic reaction information of buspirone, INTEDE 2.0 revealed the metabolic site information (5-position of the pyrimidine ring), further revealed the pharmacokinetic properties of the drug, which also provided guidance for the structural optimization of the drug. Additionally, this interface also provided information on other metabolic reactions in which the drug participated as a reactant (as illustrated in Figure 3). The stepwise metabolic reactions data could be retrieved through different types of search strategies in the 'Home' page or the menu of 'Metabolic Reaction' (Figure 3). Overall, stepwise metabolic reactions during drug metabolism in INTEDE 2.0 may provide a data base and inspirational guidance for drug development and optimization.

DMs with diverse structure, pharmacological activity and toxicity

The drug metabolism process can produce metabolites with diverse structures, some of which have ideal biological activity or bioavailability, or even better than the original drug (40,57). For example, nortriptyline (6), the active metabolite of amitriptyline, was applied to treat mental disorders, including depression and anxiety. INTEDE 2.0 marked the activity characteristics of the included DMs and provided valuable resources of lead structures for drug research and development. Certainly, the reactive metabolites, which covalently adducting to DNA, CYP family enzymes, or susceptible proteins in target tissue and so on, would lead to mutagenicity, liver injury, and drug-drug adverse interactions, which was a negative feature in drug development (58–62). For example, troglitazone could be metabolized by CYP2C8 and CYP3A4 enzymes to produce electrophilic quinone metabolite, which in turn produced liver toxicity and limited clinical application (63,64).

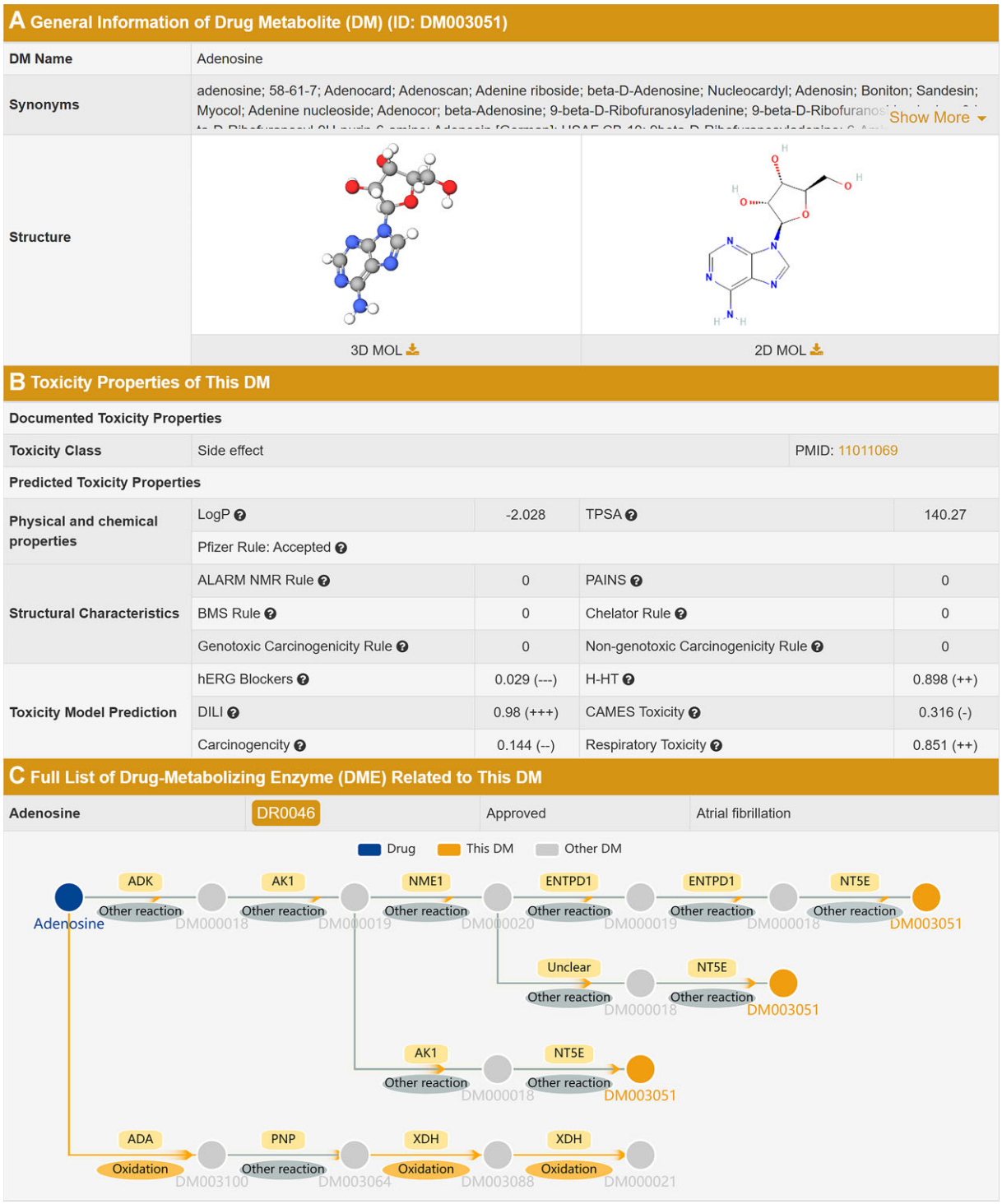

Figure 4. A typical page in INTEDE 2.0 offers the detailed information about the corresponding metabolite in the database. (**A**) general information of drug metabolite, including its synonyms, structure, and pharmaceutical properties; (**B**) the toxicity properties of drug metabolite, including its physical and chemical properties, structural characteristics and toxicity model prediction; (**C**) full list of drug(s) that produce this DM by metabolism, including all MRDs that produce this DM.

Through the comprehensive literature search, INTEDE 2.0 sorted out the toxicity information of DMs, which facilitated the identification of new structural alerts. In addition, combining the structural variations of metabolites and the toxicity information of the corresponding metabolites in the metabolic process could help improve the efficiency and reduce the cost of drug research and development (65). In order to enhance the user's ability to discover and utilize the diverse structure, pharmacological activities and toxicity information of DMs, INTEDE 2.0 delivered a wide range of available search strategies on the 'Home' page or in the 'Drug Metabolite' menu. These available search strategies enable clinicians and researchers to obtain the diverse information about the corresponding metabolite in the database, such as general information (DM name, synonyms, structure), toxicity properties, and metabolism-related details (as illustrated in Figure 4).

Data supplementation compared to INTEDE 1.0

Drug metabolism data was mainly addressed in the industrial setting rather than in academia, with the consequence that the detailed metabolic information wasn't available to the scientific community at large (2). Thus, in the process of constructing the MRDs, the drug metabolism-related data also collected the metabolic relationship between the drug and DMEs. In order to make the data included in this updated version more comprehensive and provide more valuable information for drug metabolism research, the comprehensive literature search on PubMed for the metabolic relationship was conducted by using keyword combinations, such as 'Drug Name + drug metabolizing enzyme', 'Drug Name + metabolism', 'Drug Name + enzyme', 'Drug Name + drug metabolism' and 'Drug Name + metabolized'. Finally, 3717 drug metabolism relationships were supplemented (increased from 7338 to 11 055), of which 2031 metabolic relationships came from the newly added 576 drugs and different DMEs, and 1686 metabolic relationships were derived from updating the originally included drug metabolism relationships (as shown in Table 1).

Statistics, data standardization, access and retrieval

To facilitate the users to access and use INTEDE 2.0 data, all collected data were systematically cleaned and then standardized, including: (i) the latest version of International Classification of Diseases (ICD-11) (66) released by World Health Organization was applied to standardized the diseases included in the INTEDE 2.0; (ii) DME enzymatic families were standardized according to the Enzyme Commission (EC) (16) nomenclature; (iii) the structures of drugs and metabolites were standardized into SDF format (2D and 3D); the extended information of drugs, the corresponding DMs and involved DMEs could be obtained via the crosslink to CAS Registry Number (67), PubChem (18), UniProt (68), BRENDA (16), TTD (35), DrugMAP (36), VARIDT (69) and Drugs@FDA (70), etc. The latest version of INTEDE can now be accessed free by all users at: http://idrblab.org/intede/

Conclusion and perspectives

The *metabolic roadmap of drugs* (MRD) plays a critical role in understanding the stepwise and sequential metabolism of drugs in living organisms (42). INTEDE 2.0, a major update of the INTEDE database, provided a comprehensive and organized resource for drug metabolism research. The update mainly focuses on three modules within INTEDE 2.0: the 'Drug Metabolism Roadmap', 'Drug Metabolites' and 'Metabolic Reactions of Drugs/Drug Metabolites'. These modules offer comprehensive and valuable information for drug metabolism research. The 'Drug Metabolism Roadmap' module described the sequential catalyses of drugs and their metabolites by different DMEs, highlighting the dynamic and sequential influence of multiple DMEs on drug metabolism. The 'Drug Metabolites' module offered detailed data on the pharmacological activities, validated toxicities, structural conformations, and physicochemical characteristics of drug metabolites. The 'Metabolic Reactions of Drugs/Drug Metabolites' module encompassed a comprehensive collection of metabolic reactions, including the catalytic sites on both drugs and their corresponding DMEs, as well as variations in the physicochemical properties and structural conformations of drug metabolites.

The comprehensive and organized drug metabolism roadmap provided by INTEDE 2.0 is crucial for researchers involved in the process of modern drug discovery (71–73). From target identification to drug design (74,75), lead optimization to ADME evaluation (6,76), toxicity assessment to preclinical and clinical trials (77), the MRD information offered by INTEDE 2.0 guides researchers at multiple stages. By serving as a supplement to existing drug databases, INTEDE 2.0 fills the gap by providing reliable data for constructing drug metabolism-related models, facilitating further research in this field (78). In conclusion, INTEDE 2.0 is a valuable resource that enhances our understanding of drug metabolism. Its comprehensive and organized approach to presenting MRD information supports researchers in the development of novel drugs and the improvement of drug safety and efficacy. With its contributions to the field of drug metabolism research, INTEDE 2.0 stands as an essential tool for the scientific community and serves as a foundation for future advancements in drug discovery and development.

Data availability

INTEDE 2.0 is accessible freely without any login requirement at: http://idrblab.org/intede/.

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Conflict of interest statement

None declared.

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