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RESEARCH ARTICLE

A novel hypothesis-generating computational workflow utilizing reverse pharmacophore mapping—A drug repurposing perspective of istradefylline towards major depressive disorder

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Background and Purpose: Drug repurposing (DR) offers a compelling alternative to traditional drug discovery's lengthy, resource-intensive process. DR is the process of identifying alternative clinical applications for pre-approved drugs as a low-risk and low-cost strategy. Computational approaches are crucial during the early hypothesis-generating stage of DR. However, 'large-scale' data retrieval remains a significant challenge. A computational workflow addressing such limitations might improve hypothesis generation, ultimately benefit patients and advance DR research.

Experimental Approach: We introduce a novel computational workflow (combining free-accessible computational platforms) to provide 'proof-of-concept' of the pre-approved drug's suitability for repurposing. Three key phases are included: target fishing (via reverse pharmacophore mapping), target identification (via disease- and drug-target pathway identification) and retrospective literature and drug-like analysis (via in silico ADMET properties determination). Istradefylline is a Parkinson's disease-approved drug with literature-attributed antidepressant properties remaining unclear. Practically applied, istradefylline's antidepressant activity was assessed in the context of major depressive disorder (MDD).

Key Results: Data mining aided by target identification resulted in istradefylline potentially representing a novel antidepressant drug class. Retrieved drug targets (KYNU, MAO-B, ALOX12 and PLCB2) associated with selected MDD pathways (tryptophan metabolism and serotonergic synapse) generated a hypothesis that istradefylline increased extracellular 5-HT levels (MAO-B inhibition) and reduced inflammation (KYNU, ALOX12 and PLCB2 inhibition).

Conclusion and Implications: The practically applied workflow's generated hypothesis aligns with known experimental data, validating the effectiveness of this novel computational workflow. It is a low-risk and low-cost DR computational tool

Abbreviations: 3HAA, 3-hydroxyanthranilic acid; 3HK, 3-hydroxykynurenine; AA, arachidonic acid; ACAT2, Acetyl-CoA acetyltransferase 2; ADH1C, alcohol dehydrogenase 1C; ADMET, absorption, distribution, metabolism, excretion, and toxicity; ALOX12, polyunsaturated fatty acid lipoxygenase; APP, amyloid-beta precursor protein; BBB, blood–brain barrier; CRP, C-reactive protein; CL, clearance; DILI, drug-induced liver injury scores; DR, drug repurposing; DSS, dopamine serotonin stabilizer; FDAMDD, maximum recommended daily dose; hERG, human ether-a-go-go related gene; HIA, human intestinal absorption; HK2, hexokinase 2; H-HT, human hepatotoxicity; IDO, indoleamine-2,3-deoxygenase; KEGG, Kyoto Encyclopedia of Genes and Genomes; KYNU, kynureninase; LOX, lipoxygenases; MDD, major depressive disorder; NDRI, noradrenaline-dopamine reuptake inhibitors; NMRK1, nicotinamide riboside kinase 1; PD, Parkinson's disease; PDB ID, protein data bank identification code; PLCB2, phospholipase C beta 2; PPB, plasma protein binding; ROA, rat oral acute toxicity; SARI, serotonin agonist/reuptake inhibitor; SNRI, serotonin noradrenaline reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant; VD, volume distribution.

providing a bird's-eye view for exploring alternative clinical applications of pre-approved drugs.

KEYWORDS
drug repurposing, istradefylline, KEGG database, major depressive disorder, PharmMapper, reverse pharmacophore mapping

1 | INTRODUCTION

Drug repurposing (DR) is the process where known pre-approved drugs are assigned to new clinical applications outside the original medical indication (Pushpakom et al., 2019), while traditional drug discovery, per definition, is 'the process by which new candidate drugs are discovered' (Al Qaraghuli et al., 2017). Traditional drug discovery takes approximately between 10 and 17 years from target identification to drug market availability (Parvathaneni et al., 2019), while DR takes approximately 3 to 12 years from compound identification to drug market availability (Hua et al., 2022). Advantages ascribed to DR include a low-risk strategy due to using known 'de-risk' (e.g., FDA-approved) compounds with existing clinical knowledge and drug safety; a low-cost strategy based on decreased drug development time (minimum R&D costs) and a time-saving strategy with a faster drug market availability time frame compared to traditional drug development (Hua et al., 2022; Pushpakom et al., 2019). Thus, DR offers a compelling alternative to the lengthy, resource-intensive process of traditional drug discovery. Researchers and the pharmaceutical industry increasingly recognize the potential of DR as a low-risk, cost-effective and time-saving drug development strategy.

The process of DR generally consists of hypothesis generation (identifying a candidate drug for a specific indication), assessing the candidate drug's mechanistic effect in preclinical models, efficacy evaluation in Phases II and III clinical trials, drug registration and market availability (Hua et al., 2022). Computational approaches play a crucial role in the early stages of DR. During the hypothesis-generating phase, computational techniques are employed to screen potential drug targets efficiently. These approaches include signature matching, computational molecular docking, pathway or network mapping, and retrospective clinical analysis (e.g., electronic health records and post-marketing surveillance). An in-depth discussion of the latter computational approaches has previously been reviewed (March-Vila et al., 2017; Pushpakom et al., 2019).

Among these computational methods, computational (in silico) target fishing stands out as an evolving technology (Jenkins et al., 2006) and is becoming a worthy research field with remarkable potential to advance in silico drug design and discovery (Wang & Xie, 2014). It combines machine learning algorithms, chemoinformatics tools and bioinformatics technologies to prioritize and predict potential biological targets for a given chemical compound (Wang & Xie, 2014). Choosing the right drug target is critical, as not all druggable proteins necessarily serve as effective drug targets. Therefore, the in silico target fishing concept holds a significant interest for the current pilot study.

Complementing the DR process, computational platforms play a crucial role in the in silico pharmacology approach. However, interpreting the retrieved 'large-scale' data remains a significant challenge in modern DR. To address this, a computational workflow taking large-scale data mining into consideration may improve operationalizing the hypothesis-generation step.

Accordingly, we propose a novel computational workflow that aims to predict protein targets for a pre-approved drug using reverse pharmacophore mapping. Alternative clinical applications can subsequently be explored within a DR strategy by identifying viable drug targets, including disease- and drug-target pathways. This computational workflow combines free-accessible in silico platforms (target fishing, target identification and drug-like analysis) and a retrospective literature review of the pre-approved drug intended for repurposing. Essentially, this workflow integrates existing computational tools to create a novel in silico hypothesis-generating pipeline that provides a comprehensive view of the pre-approved drug through pathway analysis for its repurposing towards a new clinical indication. We practically applied our computational workflow for validation purposes and compared the generated hypotheses to known experimental data.

What is already known?

- Large-scale data retrieval challenges the computationally aided hypothesis generation of modern drug repurposing.

What does this study add?

- A novel computational workflow addressing large-scale data demonstrates suitability of a pre-approved drug for repurposing.

What is the clinical significance?

- Istradefylline, an anti-Parkinson's drug, is an ideal repurposing candidate displaying potential antidepressant mechanisms.

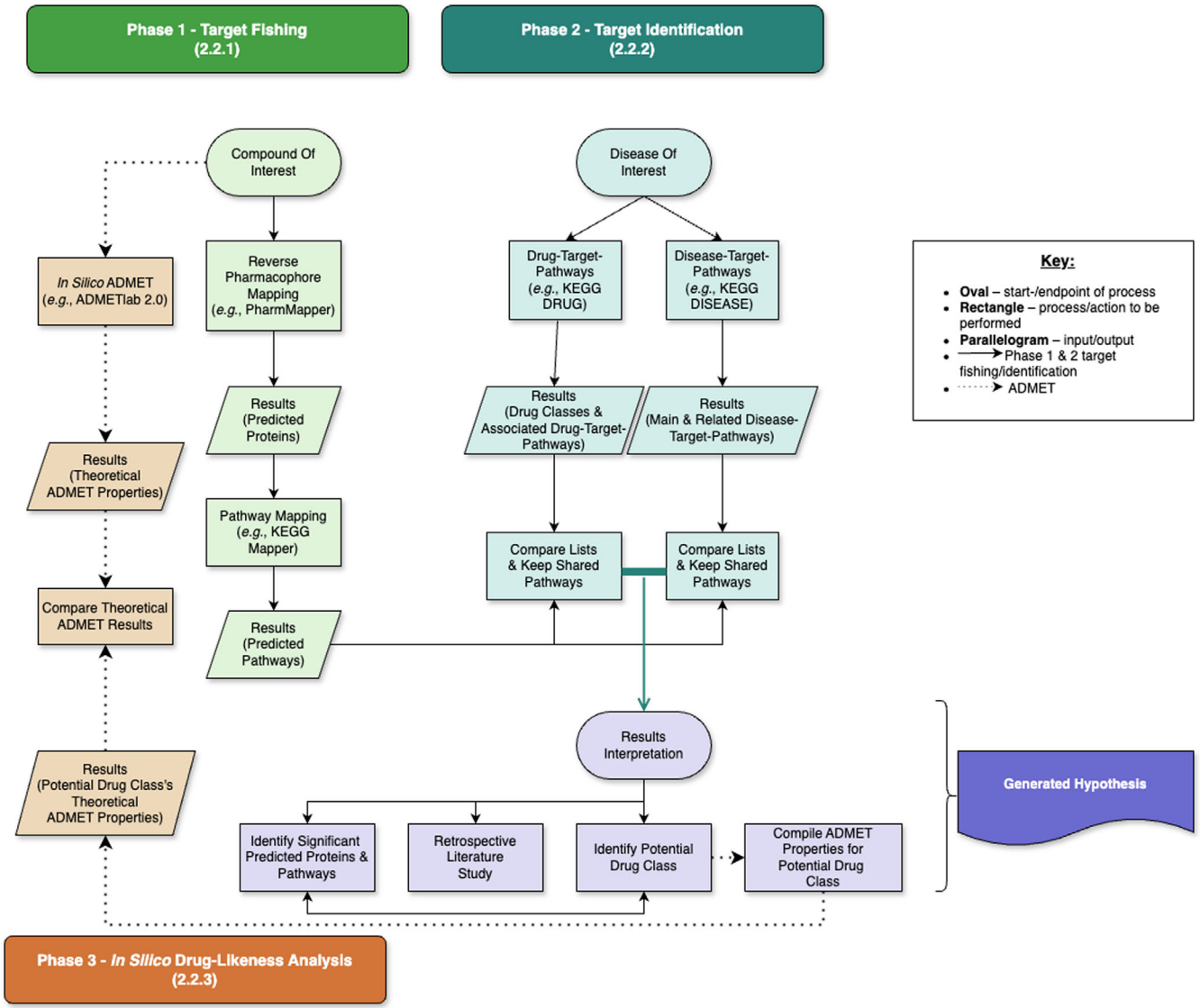
Specifically, we assessed the DR likeliness of **istradefylline** (an FDA-approved Parkinson's disease drug) towards major depressive disorder (MDD) as a 'proof-of-concept'. The computational workflow offers a novel, low-risk, low-cost and time-saving hypothesis-generating tool.

2 | METHODS

2.1 | Novel computational workflow overview—Design and development

Per definition, DR consists of the 'pre-approved' or 'de-risk' drug being investigated for an alternative clinical application outside the original medical indication. The computational workflow consists of three key phases, which are integrated and cannot stand alone; they are constantly overlapping and interactive. Each phase contributes to the overall goal of providing 'proof-of-concept' of repurposing a pre-approved drug for a specific alternative clinical application. In a sense, the computational workflow generates 'puzzle pieces' that must be pieced together to complete a picture (Scheme 1).

In Phase 1 (target fishing), the focus is on the pre-approved drug (compound of interest) under investigation. Predicted proteins associated with the pre-approved drug are retrieved using reverse pharmacophore mapping. In turn, the predicted proteins are used to gain insight into potential pathways that may be influenced by the repurposing drug. The drug's in silico ADMET properties are also determined, and a comprehensive literature review is conducted. In Phase 2 (target identification), we explore repurposing the pre-approved drug for a specific medical indication. Various computational platforms aid large-scale data interpretation, and a list of potential disease-target pathways and drug classes with associated drug-target pathways is compiled for the specific disease of interest. By comparing the theoretical pathways from Phase 1 to the disease- and drug-target pathway list in Phase 2, the drug's suitability for repurposing is assessed. Finally, Phase 3 (retrospective analysis) refines our hypothesis by considering ADMET properties and literature evidence, leading



SCHEME 1 Schematical representation of the proposed novel computational workflow. (Scheme created using drawio.com).

to a more informed decision regarding the pre-approved drug's alternative clinical application. If the pre-approved drug is potentially found to modulate specific drug-target pathways associated with a particular drug class, the computational ADMET properties of the known drugs in said drug class are obtained and recorded. By comparing the pre-approved drug's ADMET properties (Phase 1) with the average properties of drugs in the potential drug class, a comprehensive view of its suitability for repurposing as an 'ideal drug candidate' is gained. A thorough retrospective literature review of the pre-approved drug also completes the 'puzzle' and contributes to a more informed assessment.

Thus, the novel computational workflow involves piecing together these computational 'puzzle pieces' to explore alternative clinical applications for pre-approved drugs, ultimately benefiting patients and advancing the research field of DR. An in-depth description of the three key phases is to follow in Section 2.2, and for validation purposes, the computational workflow was practically implemented (Section 2.3).

2.2 | Three key phases—Computational platforms utilized

2.2.1 | Phase 1: Pre-approved drug—Theoretical protein targets, pathways and ADMET properties

The potential proteins and associated pathways that may be modulated by the pre-approved drug under investigation are theoretically predicted via various computational means. Of particular interest to the current study is the use of structure-based computational molecular docking, the so-called 'target fishing' step. The retrieved results are interpreted via pathway and network mapping, the so-called 'target identification' step. For clarity, computational target identification platforms are used for both Phase 1 and Phase 2.

Target fishing via reverse pharmacophore mapping

Structure-based computational molecular docking predicts a binding site complementary between a ligand (e.g., drug) and a therapeutic target (e.g., protein). Generally, two types of structure-based docking techniques are used:

- Conventional docking—'one target *vs*. multiple ligands'
- Inverse docking—'several targets *vs*. one ligand'

A pharmacophore describes the framework of molecular features vital for a compound's biological activity. Pharmacophore models are built using structural information about the active ligands or targets. They are developed to identify novel compounds that satisfy the pharmacophore requirements and are thus expected to be biologically active. A 3D Pharmacophore is the 3D orientation of the functional groups of a molecule that interacts with a target protein and is vital for the biological activity of a compound, while pharmacophore mapping is the process of deriving the 3D pharmacophores. A computational inverse docking strategy worth exploring is reverse pharmacophore mapping (a computational approach to finding the protein targets for a given compound). Identifying protein targets based on reverse pharmacophore mapping is gaining attention, especially after the development of free-accessible webservers, for example, PharmMapper (RRID:SCR_022604) (http://www.lilab-ecust.cn/pharmmapper/) (Liu et al., 2010; Wang et al., 2017).

For this exploratory study, we utilized the PharmMapper platform for its computational reverse pharmacophore mapping strategy to generate predicted human protein targets for the pre-approved drug under investigation. PharmMapper consists of a built-in Pharmacophore database that annotates 23,236 proteins from TargetBank, DrugBank, BindingDB, and PDTD; covers 16,159 druggable binding sites and comprises 52,431 pharmacophore models (only proteins with 3D crystal structures are included) (Liu et al., 2010; Wang et al., 2017). PharmMapper automatically finds the best mapping poses of a query compound against all the pharmacophore models in its database, and the user can retrieve a ranking list consisting of a maximum of 1000 predicted proteins for the query compound. An in-depth description of PharmMapper and its applications has already been published (Liu et al., 2010; Wang et al., 2017).

To some extent, PharmMapper still has limitations. The pharmacophore database only includes drug targets with PDB structures and corresponding co-crystallized ligands. This may lead to missed protein targets for the query compound due to the limited coverage of the database. To counter this, as with any computational work, the pharmacophore database is updated periodically to include new structures deposited in PDB and extend the database. Another limitation is the 'large-scale' data generated via PharmMapper—top hits are displayed in a ranking list based on fit scores (a maximum of 1000 predicted protein targets). Due to the 'large-scale' data output, data interpretation is complex and cannot be interpreted in isolation. Furthermore, additional computational biological target identification tools are needed to assist with interpreting the predicted protein target list. The latter is the basis for the proposed computational workflow's second step, utilizing pathway and network mapping platforms.

Target identification via KEGG MAPPER

Integrating computational target fishing information with other relevant computational and systems biology tools will assist with the manipulation and handling of target fishing data (Katsila et al., 2016; Wang & Xie, 2014). Target identification is a network-based drug discovery field where different levels of information in drug-protein and protein-disease networks are integrated to assist with data interpretation (Katsila et al., 2016). Pathway and network mapping consist of a network analysis using genetic, protein, or disease data and can aid in identifying repurposing targets. This pilot study consulted the Kyoto Encyclopedia of Genes and Genomes (KEGG; RRID:SCR_012773) database resource (http://www.genome.jp/kegg/) to aid target identification incorporated into the novel computational workflow. The KEGG database (Kanehisa & Sato, 2020) is a collection of 16 databases, which can be broadly categorized into systems information, genomic information and chemical information (Kanehisa et al., 2009).

The KEGG MAPPER database resource, consisting of a suite of KEGG mapping tools widely used for biological interpretations, is particularly interesting (Kanehisa & Sato, 2020). KEGG MAPPER was consulted to obtain pathways associated with the pre-approved drug's predicted protein targets retrieved via PharmMapper. After data retrieval, a theoretical pathway list (documenting all potential pathways associated with the predicted protein targets) was compiled (Scheme 1).

Drug-likeness analysis—Pre-approved drug

Chemical absorption, distribution, metabolism, excretion, and toxicity (ADMET) play key roles in drug discovery and development (Guan et al., 2019). Thus, in silico ADMET properties of the pre-approved drug were obtained to assist with deciding if the drug candidate was suitable for the intended clinical application. For instance, some drugs need to cross the blood–brain barrier. Predicting ADMET properties may assist with the decision of whether the pre-approved drug under investigation is suitable to continue with the DR process (Scheme 1). Various computational ADMET predicting platforms exist, utilizing different software means and strategies to estimate ADMET properties. Thus, to obtain optimal information, a combination of three ADMET docking software packages was utilized for this study, including

- ADMETlab 2.0 (Xiong et al., 2021)—A web server dedicated to the elucidation of a target compound's pharmacokinetics and toxicological properties, with these properties including '… 17 physicochemical properties, 13 medicinal chemistry properties, 23 ADME properties, 27 toxicity endpoints, and 8 toxicophore rules …' (Xiong et al., 2021). Accessible at https://admetmesh.scbdd.com/.
- SwissADME (Daina et al., 2017)—A web tool developed and maintained by the Swiss Institute of Bioinformatics, which uses in-house models to predict a target compound's physicochemical/pharmacokinetic properties, drug-likeness, and medicinal chemistry friendliness (Daina et al., 2017). Accessible at http://www.swissadme.ch.
- pkCSM (Pires et al., 2015)—A web server that predicts 30 of a given compound's properties pertaining to absorption (seven properties), distribution (four properties), metabolism (seven properties), excretion (two properties) and toxicity (10 predictors) using graph-based signatures (Pires et al., 2015). Accessible at http://structure.bioc.cam.ac.uk/pkcsm.

2.2.2 | Phase 2: Disease of interest—Disease- and drug-target pathways

The second part of the DR process is focused on the alternative clinical application for which the pre-approved drug is investigated. For the purpose of this article, the alternative medical indication is referred to as 'the disease of interest' and must be known for this computational workflow. The proposed computational workflow gathers information on disease-target pathways, drug classes and associated drug-target pathways. Furthermore, Phase 2 of the computational workflow forms part of the target identification step to aid with the target fishing data interpretation. The KEGG database was consulted, specifically the KEGG DISEASE and KEGG DRUG platforms.

Target identification via KEGG DISEASE

The KEGG DISEASE database is a collection of human diseases for the purpose of computational processing with associated disease genes and pathways (main and related pathways). Furthermore, KEGG DISEASE also contains a list of appropriate therapeutic drugs retrieved from the KEGG DRUG platform. The computational workflow proposes to retrieve the following data relating to 'the disease of interest' via the KEGG DISEASE platform:

- The main disease pathway.
- The related disease pathways.
- The associated drug classes as listed for treatment options.

The retrieved disease-target pathways (main and related pathways as derived from the KEGG database) are used to determine if the pre-approved drug potentially modulates a disease pathway. Apart from the main disease pathway, the related disease pathways (the pathways listed in addition to the main disease pathway) documented on the KEGG DISEASE database are also included in the disease-target pathway list to retrieve optimal networking information with the computational workflow. Thus, each pathway documented to play a role in the specific disease of interest is recorded and tabulated under the heading main or related disease pathway. The disease-target pathways are compared to the predicted pathway list that is based on the pre-approved drug's predicted human protein targets retrieved via PharmMapper (Phase 1). A 'hit' is recorded if one or more pathways match.

Target identification via KEGG DRUG

The KEGG DRUG database is a unified drug information resource for drugs (prescription and OTC) generated from indications in FDA drug labels (including USA and Europe). This drug database collects drug classes and the target information of the related drugs and includes appropriate links to human genes and KEGG pathway maps. Specifically, the drug-target pathways documented for each drug in the KEGG DRUG database are of interest to the computational workflow.

Subsequently, the KEGG DRUG platform is consulted to compile a second list based on the mentioned drug classes (obtained via the KEGG DISEASE database) and their related drugs' potential drug-target pathways (Scheme 1). The drug-target pathways are tabulated per drug class. The pre-approved drug's predicted pathway list is compared to the drug-target pathways tabulated per drug class. The retrieved information is used to hypothesize a potential mechanism of action for the pre-approved drug under investigation or exclude potential mechanisms of action based on the drug-target pathway hits. If the pathways match (predicted pre-approved drug pathways versus drug-target pathways), a 'hit' is recorded. Where more than

one drug-target pathway is recorded per drug class, a potential mechanism of action is hypothesized if all pathways in said drug class list were a hit. In other words, a potential drug class is excluded if not all the drug-target pathways in the drug class match.

A limitation of the current computational workflow is that the intended alternative or repurposing clinical indication (disease of interest) must be known. The current computational workflow is aimed to provide a 'proof-of-concept' and bird's eye view for the hypothesis generation step of the DR process. Another article will address such limitations, including potential diseases that may be considered for DR based on the pre-approved drug under investigation.

2.2.3 | Phase 3: In silico ADMET property analysis of the disease of Interest's known FDA-approved drugs

If a promising mechanism of action based on the drug class is gained after comparing the predicted pathway (pre-approved drug) and drug-target pathway (disease of interest) lists, the in silico ADMET properties are obtained for the drugs recorded in the specific drug class deemed of value. To ease data interpretation, where applicable, the average in silico ADMET properties is tabulated for the FDA-approved drugs of the drug class under investigation. The average ADMET properties of the FDA-approved drugs (Phase 3) are then compared to the in silico ADMET properties retrieved for the pre-approved drug (Phase 1). The comparison of the ADMET properties gives additional information to the drug-likeness analysis of the pre-approved drug vs. the drug class's drugs and as an 'ideal drug candidate'. This information may assist in strengthening the hypothesis of repurposing the pre-approved drug (Scheme 1).

Lastly, a retrospective literature search is recommended, if not performed beforehand, to gain as much information regarding the pre-approved drug and the intended alternative clinical application as possible. The literature review assists with data interpretation and strengthens the hypothesis generated via the computation workflow.

It is important to note that Phase 1, Phase 2 and Phase 3 are integrated and cannot stand alone; they are constantly overlapping and interactive.

2.3 | Application and validation of the novel computational workflow—istradefylline's repurposing towards MDD

Istradefylline, an approved drug for PD, is associated, according to existing literature, with unclear antidepressant properties. Here we have evaluated its antidepressant effects in the context of MDD. The study followed the novel computational workflow as outlined in Scheme 2, and the detailed implementation is described below as part of the validation process.

Before implementing the novel computational workflow, the following are essential considerations for practical implementation:

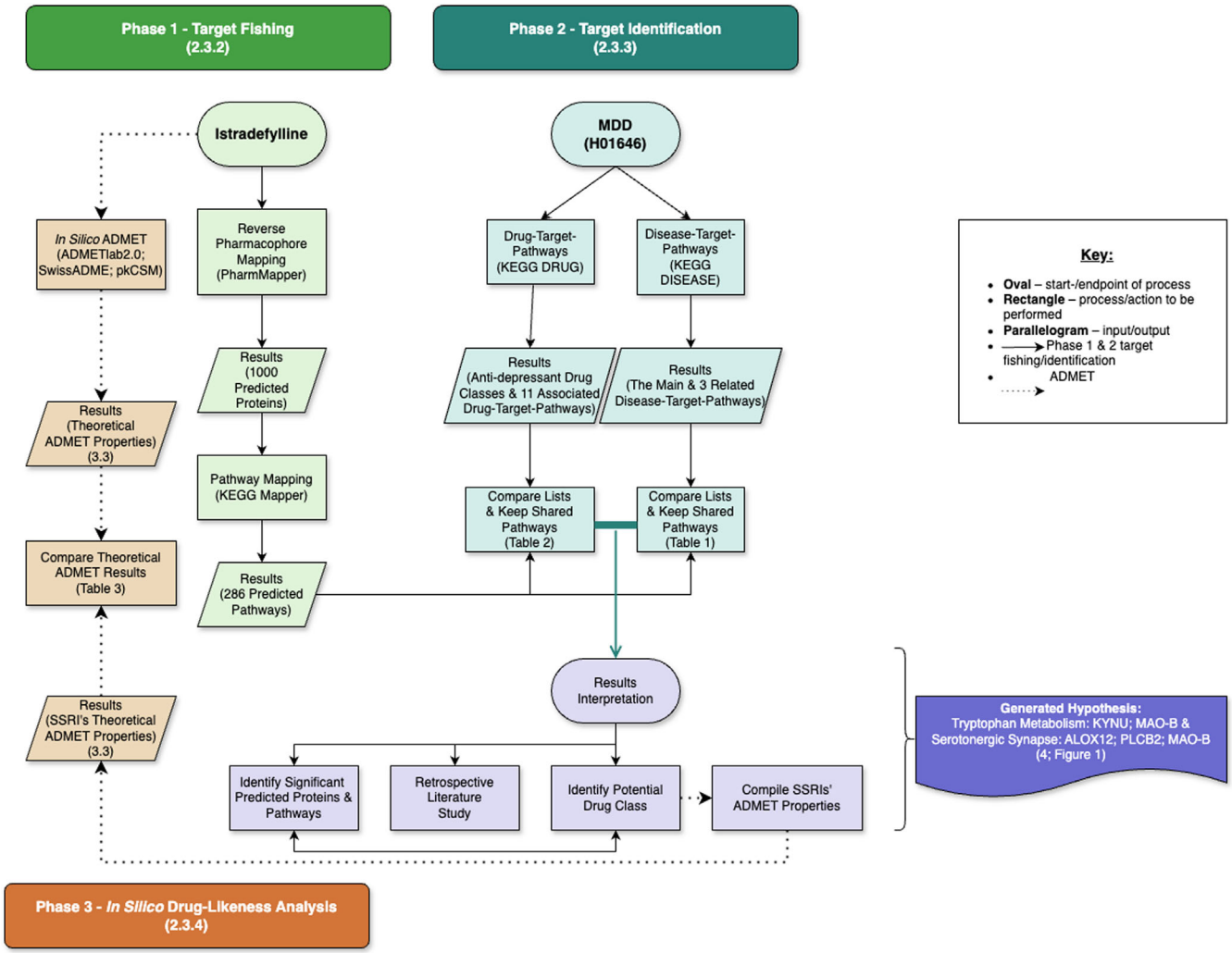
- Hypothesis-generating—Theoretical data will be generated via a combination of computational platforms and still warrants experimental validation.
- The 'input' data is used to generate the 'output' data; they are linked. Thus, the structural correctness of pre-approved drugs for submission to computational platforms for analysis is paramount to data generation.
- All computational platforms have limitations and must be considered with data interpretations, for example, 'large-scale' data retrieval and time-sensitive data.
- Free-accessible webservers are used, which may lead to unforeseen downtime.
- Computational ADMET property analysis of the pre-approved drug is highly recommended and encouraged.
- A complete literature review of the pre-approved drug will be performed and recommended.
- Phases 1, 2 and 3 are constantly overlapping and interactive.
- The proposed computational workflow provides a theoretical 'proof-of-concept' for repurposing a specific pre-approved drug for a specific clinical application.

2.3.1 | Literature background—Rationale for DR of Istradefylline

Istradefylline, also known as KW-6002, was developed as a xanthine derivative of KF17837 during the 1990s (Harper et al., 2006; Kase et al., 2004) and was the first orally active selective adenosine **A_{2A} receptor** antagonist (Harper et al., 2006), with reported K_i values of 841 and 12 nM for human **A_1** and A_{2A} receptors, respectively (Müller & Jacobson, 2011). In addition, istradefylline exhibited weak inhibition of monoamine oxidase type B (**MAO-B**), with an IC_{50} value >100 $\mu mol \cdot L^{-1}$ determined via human recombinant MAO-B, while no **MAO-A** inhibition was detected (Saki et al., 2013). Istradefylline was the first clinically approved selective A_{2A} receptor antagonist (Yamada et al., 2014), which led to it being an FDA-approved drug for treating PD-related motor symptoms. Furthermore, there were reports of the antidepressant-like activity of istradefylline, via modulation of A_{2A} receptors, in two rodent models of depression, namely a forced swim test and a tail suspension test (Yamada et al., 2013). Based on this data, it was suggested that a selective A_{2A} receptor antagonist may be beneficial as an alternative therapy for depression (López-Cruz et al., 2018; Yamada et al., 2014). Despite the promise shown by istradefylline as an antidepressant, the question remained as to whether istradefylline was a worthy DR candidate, as an antidepressant.

As we were exploring the potential of repurposing istradefylline as an alternative MDD treatment, to provide context regarding the 'diseases of interest' to the computational workflow, we also reviewed existing literature on the possible connection between PD and depression. Our findings are summarized as follows.

PD is usually described in terms of motor and non-motor symptoms (DeMaagd & Philip, 2015). Pathologically, PD is caused by the loss of **dopamine**-producing neurons, leading to a dopamine



SCHEME 2 Schematical representation of the proposed novel computational workflow applied to istradefylline as an alternative clinical application for major depressive disorder. (Scheme created using drawio.com).

deficiency and disrupting the nigrostriatal pathway, which ultimately manifests as PD's characteristic motor symptoms (Klein et al., 2019; Prell et al., 2019). These motor symptoms are well-recognized, with treatment options for tremors, rigidity and bradykinesia. The non-motor symptoms, on the other hand, include conditions such as PD-related depression and are typically poorly recognized and treated ineffectively (DeMaagd & Philip, 2015). Depression affects approximately 322 million people worldwide (WHO, 2017) and is recognized as the most common mental health disorder in the global population (Stein et al., 2020). However, classical antidepressants—such as selective serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs) and **mirtazapine**—do not seem to be as effective in some cases when used to treat PD-related depression, given their complicated shared pathophysiology (Lesenskyj et al., 2018). This inadequate treatment, in part, may be attributed to the complex pathophysiology that inevitably originates from two complex diseases coinciding.

The high prevalence of depression in PD patients (underestimated at 40%) (Leentjens, 2004; Lesenskyj et al., 2018) has gained increased interest over the past decade to link several common features of these two diseases (Klein et al., 2019; Leentjens, 2004; Lesenskyj et al., 2018). The underlying mechanisms are complex, involving an interplay of multiple neurotransmitter systems and brain regions beyond the dopaminergic system (Klein et al., 2019) with damage to these various systems likely to contribute to PD-related depression. Three neurotransmitter-focused theories have been advanced: (1) The 'dopaminergic hypothesis' proposes that degeneration of mesolimbic and mesocortical dopaminergic pathways essential for reward and motivation may contribute to PD-related depression (Leentjens, 2004; Lesenskyj et al., 2018). The latter is supported by the observation that some dopamine agonists used to treat PD motor symptoms also exhibited mood-enhancing effects (Leentjens, 2004; Lesenskyj et al., 2018). (2) The 'serotonergic hypothesis' suggests that decreased serotonergic brain activity in PD contributes to depression. Lower levels of **5-HT (serotonin)** may compensate for decreased dopamine availability in the striatum, as 5-HT can inhibit dopamine release. However, low levels of 5-HT are an independent risk factor for depression, partly explaining the higher prevalence of depression in PD, sometimes preceding motor symptoms. This hypothesis also

suggests that SSRIs may exacerbate PD motor symptoms by further disrupting the 5-HT–dopamine balance (Leentjens, 2004). (3) The 'cholinergic-adrenergic hypothesis' suggests that depression results from an imbalance between cholinergic and adrenergic activity in the brain, specifically a relative excess of cholinergic activity. While this theory is less aligned with current depression theories, it has gained renewed interest due to the mood-stabilizing effect of **donepezil**, a **cholinesterase** inhibitor (Leentjens, 2004). Beyond traditional neurotransmitter-focused theories, the elevation of inflammatory biomarkers such as **IL-6**, **IL-1ß**, **TNF-α** and CRP in PD and depression supports an inflammation hypothesis and highlights a common pro-inflammatory cytokine profile in both conditions (Miller & Raison, 2016; Prell et al., 2019). Finally, the neuroplasticity hypothesis links the overexpression of **glutamate** in depressive patients (Sanacora et al., 2012) with the **GABA** deficit often observed in PD patients (Song et al., 2021). However, further research is needed to elucidate these intricate mechanisms in the context of PD and MDD.

Lastly, based on the reviewed theories and publications, istradefylline emerged as a promising DR candidate to treat MDD. Given istradefylline's role in modulating adenosine receptors, which are involved in neuroinflammation, this drug represents a compelling avenue for exploring a DR strategy towards MDD.

2.3.2 | Phase 1: Istradefylline—Theoretical protein targets, pathways and ADMET properties

Target fishing via reverse pharmacophore mapping

The mol2 file of the pre-approved drug istradefylline was submitted to the PharmMapper server (http://www.lilab-ecust.cn/pharmmapper/) (Liu et al., 2010). The mol2 file for istradefylline was obtained by creating a two-dimensional (2D) structure via Advance Chemistry Development (ACD)/ChemSketch freeware version 12.01 (Advanced Chemistry Development Inc., 2009). A ranking list with predicted protein targets (maximum 1000) was retrieved with corresponding fit scores, and the results were ranked in descending order according to the normalized fit score (Table S1). The predicted protein targets were retrieved as protein data bank identification codes (PDB IDs). As a result, all PDB IDs were tabulated, and the retrieved PBD IDs were uploaded to the UniProt Knowledgebase (UniProtKB; RRID: SCR_002380) an expertly curated database to access integrated protein information with cross-references to multiple sources—to retrieve the corresponding UniProt identifiers (UniProt IDs). The UniProt resources are available online at https://www.uniprot.org/ (Consortium, 2022). Finally, the list of predicted protein targets was updated with the UniProtKB data, and only human protein targets were included in the predicted protein target list (Scheme 2).

Target identification via KEGG MAPPER

The retrieved list of corresponding UniProt IDs was uploaded to the KEGG MAPPER database. After data retrieval, a theoretical pathway list associated with the predicted protein targets was compiled for istradefylline and used further to compare to the disease- and drug-target pathway lists compiled for MDD (Phase 2, see Scheme 2). A total of 286 theoretical pathways based on the predicted protein targets were retrieved. The obtained KEGG pathways comprised one or more of the predicted protein targets associated with this pathway (Table S2).

Drug-likeness analysis—Pre-approved drug

Some of the primary reasons for drug development failure are linked to undesirable pharmacokinetics and toxicity of drug candidates. During drug design, evaluating absorption, distribution, metabolism, excretion, and toxicity (ADMET) in the early stages of drug development is highly recommended (Xiong et al., 2021). That said, even with the use of 'de-risked' drugs for DR, the ADMET properties must be taken into consideration. For instance, a good drug candidate for PD must cross the BBB. The question remains if the new clinical application of the drug must possess additional ADMET properties to qualify as a drug candidate for MDD. Due to patents and other legalities, the drug-likeness information of pre-approved drugs is not always available to the public. For this reason, the in silico ADMET properties of the pre-approved drug are obtained with this computational workflow. Where ADMET properties of the pre-approved drug are available in published forms, the computationally determined ADMET properties can be cross-referenced and updated. ADMETlab 2.0 (Xiong et al., 2021), SwissADME (Daina et al., 2017) and pkCSM (Pires et al., 2015) were used to determine istradefylline's theoretical ADMET properties (Scheme 2). The results were tabulated and compared to each other to gain insight into istradefylline's drug-likeness (Table 3).

2.3.3 | Phase 2: MDD—Disease- and drug-target pathways

The antidepressant-like activity of istradefylline via modulation of A_{2A} receptors in rodent models of depression has been reported and this provides a potential lead to repurposing istradefylline as an antidepressant drug (Yamada et al., 2014). However, the exact mechanism of action is unknown, with istradefylline potentially representing a 'new' class of antidepressants as A_{2A} receptor antagonists. It is also possible that istradefylline acts similarly to one of the currently recognized antidepressant drug classes. Istradefylline was subjected to the proposed computational workflow to gain insight into the likeliness of istradefylline as an alternative clinical application for MDD.

Target identification via KEGG DISEASE

Therefore, 'the disease of interest' for this computational workflow is MDD. MDD is recognized as a 'Mental and Behavioural Disorder' and is characterized by a depressed mood, loss of interest, feelings of worthlessness, and a high risk of suicide (KEGG-Disease, n.d.; Mayo-Clinic, 2018). According to the KEGG DISEASE database (KEGG-Disease, n.d.), the pathogenesis is still unknown. However, mutations in genes involved in the brain synthesis of 5-HT have been identified. It is speculated that deficiency of brain 5-HT may play a role

(Jacobsen et al., 2012). The KEGG identifier for MDD is H01646 (Scheme 2).

The current computational workflow was compiled and validated over a period of 3 years (2021–2023). Notably, during this period, the KEGG DISEASE database was updated, and the results retrieved for MDD's main disease-target pathway (as well as related pathways) were updated from the serotonergic synapse (hsa04726) to the **tryptophan** metabolism (hsa00380) pathway. Both retrieved disease-target pathway results are provided for clarity and to emphasize the importance of using up-to-date information in computational studies. Furthermore, the 2023 update yielded additional insights relevant to istradefylline's antidepressant activity, which informed our hypothesis generation (Table 1). This underscores the significance of utilizing up-to-date computational platforms to enhance data relevance and accuracy in hypothesis development. Notably, the drug-target pathways remained consistent between 2021 and 2023.

Target identification via KEGG DRUG

Subsequently, the KEGG DRUG database is consulted to retrieve a list of antidepressant drug classes and associated FDA-approved drugs. A list was compiled for drug-target pathways associated with each antidepressant drug class and tabulated (Scheme 2). The results are provided in Table 2.

2.3.4 | Phase 3—In silico ADMET property analysis of MDD's known FDA-approved drugs

The theoretical 'ideal' antidepressant

Although antidepressants have come a long way in the past few decades, none have yet delivered a pharmacological profile that would make them an ideal antidepressant. Richelson (1994) described the ideal antidepressant as having a rapid onset of action, an intermediate half-life, a defined therapeutic blood level, a high threshold of fatal overdose, and no side effects while providing a broad spectrum of efficacy and minimal drug interactions. DeVane (2000) echoed these opinions but added that it must maintain its effects with continued treatment, show little to no impairment of cognition, and provide a convenient dosing schedule and multiple elimination pathways. Discovering whether a potential drug possesses these characteristics would traditionally require clinical trials, which are both costly and time-consuming. However, using computational platforms is a more modern approach that can be employed beforehand to screen compounds for their suitability, specifically in silico ADMET docking. This involves using software to determine the potential drug's theoretical ADMET properties to gauge its possible performance before committing to animal/human trials. If the intended repurposed drug is deemed suitable to continue with the DR process, experimentally determined ADMET properties will have to be performed. With this computational workflow, an in silico ADMET property analysis is performed to determine to what degree istradefylline would theoretically meet the requirements of an 'ideal antidepressant drug' mentioned above. The results are discussed in Sections 3 and 4 (Scheme 2).

2.4 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24 (Alexander, Christopoulos et al., 2023; Alexander, Fabbro et al., 2023; Alexander, Mathie et al., 2023).

3 | RESULTS—NOVEL COMPUTATIONAL WORKFLOW PRACTICALLY APPLIED

The novel computational workflow integrates several free-accessible computational platforms to provide valuable insights into predicted protein targets (via PharmMapper), identify disease- and drug-target pathways (via KEGG database) and assess the drug-likeness (via Admelab2.0, SwissADME, pkCKM) of a pre-approved drug. This computational workflow (depicted in Scheme 2) was practically applied, and we successfully executed the three key phases outlined in the Method section. Specifically, we evaluated istradefylline's likelihood as a DR candidate for MDD. MDD's disease- and drug-target pathway lists were compared to istradefylline's theoretical pathway list (286 theoretical pathways identified). The results are documented in Tables 1 and 2, respectively.

3.1 | MDD's disease-target pathways compared with istradefylline's theoretical pathways

The main and related disease-target pathways associated with MDD (H01646) were retrieved from the KEGG DISEASE database and tabulated (Table 1; Scheme 2) with their unique KEGG identifier, as stipulated in Section 2 (Scheme 1). The results from 2021 and 2023 showed that the main disease-target pathway changed from the serotonergic synapse (hsa04726) to the tryptophan metabolism (hsa00380) pathway, which is of particular interest because tryptophan metabolism is linked to the kynurenine pathway and mental disorders (Marx et al., 2021). The related disease-target pathways also changed, with only the tryptophan metabolism pathway remaining common in both years. The other related disease-target pathways in 2021 were arachidonic acid metabolism (hsa00590), calcium signalling pathway (hsa04020), synaptic vesicle cycle (hsa04721) and bile secretion (hsa04976), while in 2023 they were glycolysis/gluconeogenesis (hsa00010), phenylalanine, tyrosine and tryptophan biosynthesis (hsa00400), and nicotinate and nicotinamide metabolism (hsa00760). The predicted protein targets that were found to hit within the disease-target pathways (main and related, 2023 results) are provided in Table 1. It is plausible that istradefylline may exert its antidepressant effects via modulation of the tryptophan metabolism pathway and its associated protein targets, such as MAO-B and **KYNU**, which may also modulate the serotonergic synapse and kynurenine pathways, respectively. An in-depth discussion of a hypothesis generated based on these pathways is to follow in the Discussion (Section 4).

TABLE 1 MDD's main and related disease-target pathways and istradefylline's potential hit disease-target pathways (results 2023).

KEGG pathway identifier	Pathway name	Predicted protein 'hits'	KEGG protein identifier
Main disease-target pathway			
hsa00380	Tryptophan metabolism	Acetyl-CoA acetyltransferase 2 (ACAT2)	hsa:39
		Monoamine oxidase B (MAO-B)	hsa:4129
		Kynureninase (KYNU)	hsa:8942
Related disease-target pathway			
hsa00010	Glycolysis/gluconeogenesis	Alcohol dehydrogenase 1C (ADH1C)	hsa:126
		Hexokinase 2 (HK2)	hsa:3099
hsa00400	Phenylalanine, tyrosine and tryptophan biosynthesis	ND[a]	
hsa00760	Nicotinate and nicotinamide metabolism	Nicotinamide riboside kinase 1 (NMRK1)	hsa:54981

[a]ND = not detected.

Please note that PharmMapper only includes proteins with 3D crystal structures in its pharmacophore database, and retrieved data include PDB IDs. Therefore, the number of predicted protein targets is limited to the number of 3D crystal structures included in PharmMapper's database. The obtained theoretical KEGG pathways comprised one or more of the predicted protein targets associated with this pathway. For the purpose of this computational workflow, the 'limited' number of predicted protein hits within a specific KEGG pathway is still considered valuable.

3.2 | MDD's drug-target pathways vs. istradefylline's theoretical pathways

The classic antidepressant drug classes were retrieved from KEGG DRUG. A comprehensive list of all FDA-approved drugs used to compile the associated drug-target pathways is reported in Table S3. Overall, a combination of 11 drug-target pathways was identified to play a role in one or more of the classic antidepressant drug classes (Table 2). Specifically, the serotonergic synapse (hsa04726) was found to play a role in all major antidepressant drug classes (except for the norepinephrine-dopamine reuptake inhibitors [NDRI] drug class) and is, therefore, of interest. Furthermore, 10 of MDD's 11 drug-target pathways corresponded with istradefylline's theoretical pathways.

It is worth mentioning that the synaptic vesicle cycle (hsa04721) was not identified as a potential hit pathway. Based on the latter, istradefylline is less likely to act similarly to the TCA, SNRI, and NDRI drug classes, for which the synaptic vesicle cycle is a drug-target pathway. For this reason, the latter drug classes' mechanisms of action were excluded as a hypothesis for istradefylline's potential antidepressant mechanism. Further support for this theory of excluding the TCA drug class is provided in a previously performed in vivo experimental study (Smith et al., 2022). The study showed that istradefylline treatment, compared with that of the TCA drug **imipramine**, did not result in similar metabolic profiles in the striata. The metabolic profiles were obtained by treating male Sprague–Dawley rats with the relevant drugs and subjecting them to a forced swim test. Brain material was collected, metabolites extracted, and analysed through untargeted metabolomics using both ^{1}H-NMR and GC-TOFMS.

In contrast, all drug-target pathways associated with the drug classes SSRI, MAO inhibitors and SARI corresponded with pathways documented for istradefylline's theoretical pathway list. Based on the results, it is plausible that istradefylline's antidepressant mechanism of action may be similar to one of these drug classes. Of particular interest is the serotonergic synapse, which is a drug-target pathway associated with most of the FDA-approved antidepressant drugs for MDD. The serotonergic synapse was found to be a hit pathway for istradefylline based on four predicted protein targets recorded to modulate the pathway. The predicted protein targets included polyunsaturated fatty acid lipoxygenase (**ALOX12**; UniProt ID P18054), amyloid-beta precursor protein (APP; UniProt ID P05067), monoamine oxidase type B (MAO-B; UniProt ID P27338) and **phospholipase C beta 2 (PLCB2**; UniProt ID Q00722). Another possibility is that istradefylline represents a 'new' antidepressant drug class, specifically incorporating the kynurenine pathway and the serotonergic synapse pathway as part of its mechanism. This theory is discussed below (Section 4).

3.3 | Istradefylline's in silico ADMET properties compared with the average ADMET properties of known SSRIs

The serotonergic synapse is a drug-target pathway listed for all classic antidepressant FDA-approved drug classes (except the NDRI class) and the only drug-target pathway listed for the SSRIs. Currently, SSRIs are the first-line treatment for MDD (Gabriel et al., 2020). Considering the above, the in silico ADMET properties of istradefylline were compared to average ADMET properties retrieved for SSRI drugs, which are deemed to be safe for MDD treatment. Each in silico ADMET platform provides its own result interpretation criteria; therefore, please

TABLE 2 The antidepressant drug classes, associated drug-target pathways and istradefylline's theoretical hit drug-target pathways.

	Antidepressant drug Class[a]											
				Atypical								
	MAO-A inhibitor	MAO-B inhibitor	Non-selective MAO inhibitor	Atypical (Nefazodone)	Atypical (DSS)	NDRI	5-HT_{1A}-receptor agonist	TCA	SNRI	SARI	SSRI	Istradefyline
Drug-target pathway[a]												
Serotonergic synapse	x	x	x	x	x		x	x	x	x	x	x
Dopaminergic synapse	x	x	x		x	x	x					x
Neuroactive ligand-receptor interaction				x	x		x			x		x
Synaptic vesicle cycle						x		x	x			
Glycine, serine and threonine metabolism	x		x									x
Arginine and proline metabolism	x		x									x
Histidine metabolism	x		x									x
Tyrosine metabolism	x		x									x
Phenylalanine metabolism	x		x									x
Tryptophan metabolism[b]	x		x									x
Drug metabolism—cytochrome P450	x		x									x

Note: Istradefylline's (pre-approved drug) theoretical pathways are based on the predicted protein targets retrieved via PharmMapper and associated with pathways as recorded in the KEGG MAPPER database.
Abbreviations: MAO-A, monoamine oxidase type A; MAO-B, monoamine oxidase type B; DSS, dopamine serotonin stabilizer; NDRI, noradrenaline dopamine reuptake inhibitor; SARI, serotonin agonist/reuptake inhibitor; SNRI, serotonin noradrenaline reuptake inhibitor; SSRI, selective serotonin reuptake inhibitor; TCA, tricyclic antidepressant.
[a]The antidepressant drug classes and associated drug-target pathways were retrieved from the KEGG DRUG database.
[b]Tryptophan metabolism pathway was also identified as a main disease pathway for MDD via the KEGG DISEASE database.

see the appropriate websites for data interpretation (ADMETlab2.0 [2020], pkCSM [2022] and SwissADME's [Daina et al., 2017]). The discussed in silico ADMET results are tabulated in Table 3, and the comprehensive retrieved data is provided in the supplementary material (Tables S4–S6).

3.3.1 | Criteria: Rapid onset of action—Absorption and distribution

The first characteristic of an ideal antidepressant - namely a rapid onset of action - was investigated. The ADMET properties that primarily affect the rapid onset of action are absorption and distribution (Table 3). When considering the in silico determined ADMET properties, istradefylline should satisfy this requirement, seeing as it theoretically possesses excellent absorption according to the Caco-2 (ADMETlab2.0 and pkCSM) and MDCK models (ADMETlab2.0).

Furthermore, istradefylline's oral bioavailability is comparable to the theoretical average of other SSRIs, and the bioavailability is expected to be greater than 30% (according to ADMETlab2.0's F_{30} test). As a result, istradefylline was the first orally active selective A_{2A} receptor antagonist on the market (Harper et al., 2006). Additionally, ADMETlab2.0 indicated that istradefylline should have excellent intestinal absorption (HIA), with pkCSM more specifically indicating that 95% of a given dose could be absorbed.

Based on the in silico ADMET results, istradefylline's distribution exhibited a theoretical plasma protein binding (PPB) of less than 75% bound (ADMETlab2.0; pkCSM), which is a slight improvement over the in silico-determined average PPB (78%) results recorded for the SSRIs. It is worth mentioning that according to the prescribing guidelines for Nourianz (Kyowa Kirin, 2019) (istradefylline's trade name), the experimental PPB is 98%. The volume distribution (VD) was found to be satisfactory and should result in istradefylline being easily distributable throughout the body.

Finally, as istradefylline's potential as an antidepressant is being investigated, it must, therefore, be able to cross the blood–brain barrier (BBB) to exhibit the desired effects. Accordingly, the theoretical ADMET properties attributed to istradefylline support it crossing the BBB (pkCSM; SwissADME). Furthermore, istradefylline and the SSRIs possess satisfactory lipophilicity (SwissADME; ADMETlab2.0). PD-related research has shown experimentally that istradefylline crosses the BBB (Müller, 2023), further supporting the retrieved theoretical ADMET properties. Based on the results shown above, istradefylline should be readily absorbed and effectively distributed. Thus, it should have a rapid onset of action and qualify for further evaluation for repurposing as an antidepressant drug.

3.3.2 | Criteria: Intermediate half-life—Metabolism and excretion

The second requirement for an ideal antidepressant is an intermediate half-life, which is important to prevent the build-up of xenobiotics and the resultant toxic effects this could lead to. The prescribed guidelines for Nourianz (by association, istradefylline) exhibit a half-life of 83 h (Kyowa Kirin, 2019). Additional requirements related to the half-life are providing a convenient dosing schedule and multiple elimination pathways. ADMETlab 2.0 indicated that istradefylline would have moderate clearance (CL) (i.e., 6.248 $ml \cdot min^{-1} \cdot kg^{-1}$), slightly lower but still excellent and comparable to the average clearance of the known SSRIs (9.230 $ml \cdot min^{-1} \cdot kg^{-1}$). Results retrieved for pkCSM support the latter observation. Nourianz's total clearance was documented as 4.6 $l \cdot h^{-1}$. Considering the latter, istradefylline should also possess a longer half-life than traditional SSRIs (Table 3).

Finally, istradefylline is highly likely to be a substrate of all the major cytochrome P450 (CYP) isozymes (ADMETlab2.0), possessing multiple elimination pathways. The ADMET docking included CYP isozymes **CYP1A2**, **CYP2C19**, **CYP2C9**, **CYP2D6** and CYP3A4. The metabolism findings are also supported by experimental ADMET properties **recorded** for Nourianz (Kyowa Kirin, 2019). Considering the above, istradefylline should theoretically meet the second-mentioned requirements for an ideal antidepressant.

3.3.3 | Criteria: Toxicity

The final requirements described for an ideal antidepressant, namely a high threshold of fatal overdose, no side effects, minimal drug interactions and little to no impairment of cognition, are mainly related to a drug's toxicologic profile and characteristics. This is a strong point of the various in silico ADMET predicting software options, with many providing theoretical profiles for the submitted query compounds. Based on the combination of retrieved in silico ADMET properties, istradefylline displayed satisfactory toxicological characteristics, generally comparable to the SSRIs or recorded with an improvement (Table 3). These theoretically satisfactory toxicological characteristics included not blocking the human **ether-a-go-go related gene channel (hERG),** as well as possessing low human hepatotoxicity (H-HT) and drug-induced liver injury scores (DILI) (ADMETlab2.0; pkCSM). Thus, istradefylline should, in theory, not negatively affect cardiac function nor lead to liver damage.

Furthermore, istradefylline showed a rat oral acute toxicity (ROA) score much lower than that of the SSRIs' average, along with a higher maximum recommended daily dose (FDAMDD), both indicating a reduced likelihood of fatal overdose (ADMETlab2.0; pkCSM). Despite these positive results, ADMETlab2.0 indicated a high AMES toxicity score (indicative of mutagenicity) and high carcinogenicity, which are immediate red flags. However, according to the official prescribing information for Nourianz (Kyowa Kirin, 2019), istradefylline showed both low mutagenicity and carcinogenicity during clinical trials. The in silico ADMET platforms have some limitations compared to clinical experimental results, suggesting the need for multiple computational ADMET software tools. However, istradefylline has a favourable toxicological profile that meets the criteria for an ideal antidepressant and deserves further investigation for DR. Using a pre-approved drug like istradefylline

TABLE 3 In silico ADMET properties of istradefylline and FDA-approved SSRIs.

		ADMETlab 2.0				pkCSM			Swissadme	
	Nourianz[a]	SSRI Avg	Istradefylline	Criteria	Interpretation	SSRI Avg	Istradefylline	Interpretation	SSRI Avg	Istradefylline
Absorption										
Caco-2		−4.6516364	−4.640000	> − 5.15: Excellent; otherwise: Poor	Excellent	1.6505	1.633	High > 0.9		
MDCK		0.0000192	0.000013	>2 × 10-6 cm·s^{-1}: Excellent; otherwise: Poor	Excellent					
HIA		0.0034545	0.010000	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Excellent	92.59%	95.49%	Excellent	High	High
F(30%)		0.0359091	0.188000	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Excellent					
Lipophilicity		3.6760000	2.776000	0 to 3 log mol·L^{-1} will be considered proper	Proper				3.40364	3.9
Distribution										
PPB	98%	78.39%	74.26%	≤90%: Excellent; otherwise: Poor	Excellent	17.78%	30.10%	% unbound		
VDss	557 l	3.038	0.713	0.04–20: Excellent; otherwise: Poor	Excellent	0.94633	−0.684	Low if below 0.71 L·kg^{-1} (log VDss < −0.15); high if above 2.81 L·kg^{-1} (log VDss > 0.45)		
BBB		0.968	0.892	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Poor	0.11717	−0.755	>0.3: Readily cross the blood–brain barrier; < −1: Poorly distributed to the brain.	Yes	Yes
Metabolism and excretion										
CL		9.230	6.248	≥5: Excellent;<5: Poor	Excellent	0.772	0.634	Numeric (log ml·min^{-1}·kg^{-1})		
CYP1A2-inh	No	0.546	0.766	Probability of being substrate/inhibitor, within the range of 0 to 1.		Yes/No[b]	Yes		Yes/No[b]	No
CYP1A2-sub	Yes	0.688	0.964							
CYP2C19-inh	No	0.509	0.268			Yes/No[b]	No		Yes/No[b]	Yes
CYP2C19-sub		0.853	0.671							
CYP2C9-inh	No	0.209	0.231			Yes/No[b]	No		Yes/No[b]	Yes
CYP2C9-sub	Yes	0.436	0.671							
CYP2D6-inh	No	0.790	0.057				No			No

TABLE 3 (Continued)

		ADMETlab 2.0				pkCSM			Swissadme	
	Nourianz[a]	SSRI Avg	Istradefylline	Criteria	Interpretation	SSRI Avg	Istradefylline	Interpretation	SSRI Avg	Istradefylline
						Yes/No[b]			Yes/No[b]	
CYP2D6-sub	Yes	0.851	0.554			Yes/No[b]	No			
CYP3A4-inh	Weak	0.548	0.344			Yes/No[b]	No		Yes/No[b]	Yes
CYP3A4-sub	Yes	0.749	0.811			Yes/No[b]	No			
Toxicity										
ROA		0.529	0.061	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Excellent	2.96467	2.206	Numeric (mol·kg^{-1})		
FDAMDD	40 mg·day^{-1}	0.683	0.022	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Excellent	0.2475	0.127	Numeric (log mg·kg^{-1}·day^{-1})		
hERG		0.786090909	0.187	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Excellent	Yes/No[b]	No			
H-HT		0.577818182	0.243	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Excellent	Yes/No[b]	No			
DILI		0.246	0.538	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Medium	Yes/No[b]	No			
AMES	No	0.167727273	0.778	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Poor	Yes/No[b]	No			
Carcinogenicity	No	0.256181818	0.783	0–0.3: Excellent; 0.3–0.7: Medium; 0.7–1.0: Poor	Poor					

[a]Nourianz is an FDA-approved drug containing istradefylline as an active ingredient; selected experimental data from the prescribing guidelines is provided (Kyowa Kirin, 2019).
[b]See Tables S4–S6 for individual SSRI results. Please note that the average values (where applicable) were reported in this table to ease data interpretation.

can reduce the risk, cost, and time of drug development, as long as the computational ADMET results are supported by published results or experimental data, where available.

4 | DISCUSSION

Identifying 5-HT (serotonin) as the sole cause of clinical depression has been intensely debated over the past 2 years and, recently, has been reviewed in-depth (Jauhar et al., 2023). It seems that unmedicated depressed patients present with decreased activity of presynaptic 5-HT-containing neurons, which is consistent with diminished tryptophan availability in the brain (Figure 1a,b). It is proposed that decreased 5-HT concentrations in the brain alone is not sufficient to result in clinical depression (Jauhar et al., 2023). Thus, the exact pathophysiology of depression is unknown. The perception that a deficiency of a single neurotransmitter causes clinical depression seems unlikely; it is rather a combination of biological and psychosocial factors that are still poorly understood.

The following hypotheses generated from disease- and drug-target pathways analysis highlight the significance of istradefylline. The tryptophan metabolism pathway (hsa00380) is valuable, focusing on predicted targets - MAO-B (PDB ID 2BK3) and KYNU (PDB ID 2HZP). The drug-target pathway of interest is the serotonergic synapse (hsa04726), with predicted protein targets **ALOX12** (PDB ID 3D3L) and PLCB2 (PDB ID 2FJU). The related pathway arachidonic acid metabolism (hsa00590), which shares the protein ALOX12, was also considered in data interpretation. The KEGG database associates tryptophan metabolism with the serotonergic synapse, linking them through the predicted protein MAO-B.

4.1 | Hypothesis 1—Increase of extracellular 5-HT levels

Istradefylline's predicted protein target MAO-B was found to modulate the disease-target pathway tryptophan metabolism, and the drug-target pathway serotonergic synapse (Figure 1c). While MAO-A inhibitors are established antidepressants (Yamada & Yasuhara, 2004), the published papers suggest MAO-B inhibitors also exert antidepressant properties (Finberg & Rabey, 2016), probably through modulation of the tryptophan metabolism. The retrospective literature search provides experimental evidence that istradefylline exerts weak inhibition of MAO-B (Saki et al., 2013). Thus, istradefylline's antidepressant capabilities can be explained, at least in part, by MAO-B inhibition, potentially increasing pre-synaptic 5-HT levels via modulation of the tryptophan metabolism pathway.

Furthermore, istradefylline is a known antagonist of the adenosine A_1 and A_{2A} receptor subtypes. Even though it is selective towards the A_{2A} receptor subtype over the A_1 receptor subtype, its antagonism of A_1 receptors is worth mentioning (Saki et al., 2013). There is evidence that antagonism of A_1 receptors leads to increased levels of extracellular 5-HT (Motohiro et al., 2001). Although its precise antidepressant mechanism remains unconfirmed, theoretically, istradefylline's antagonism of A_1 receptors could contribute to increased extracellular 5-HT levels. Various antidepressants increase 5-HT levels (Sachs et al., 2015), for example, the SSRI drug class, whose only drug-target pathway is the serotonergic synapse.

4.2 | Hypothesis 2—Anti-inflammatory theory of MDD

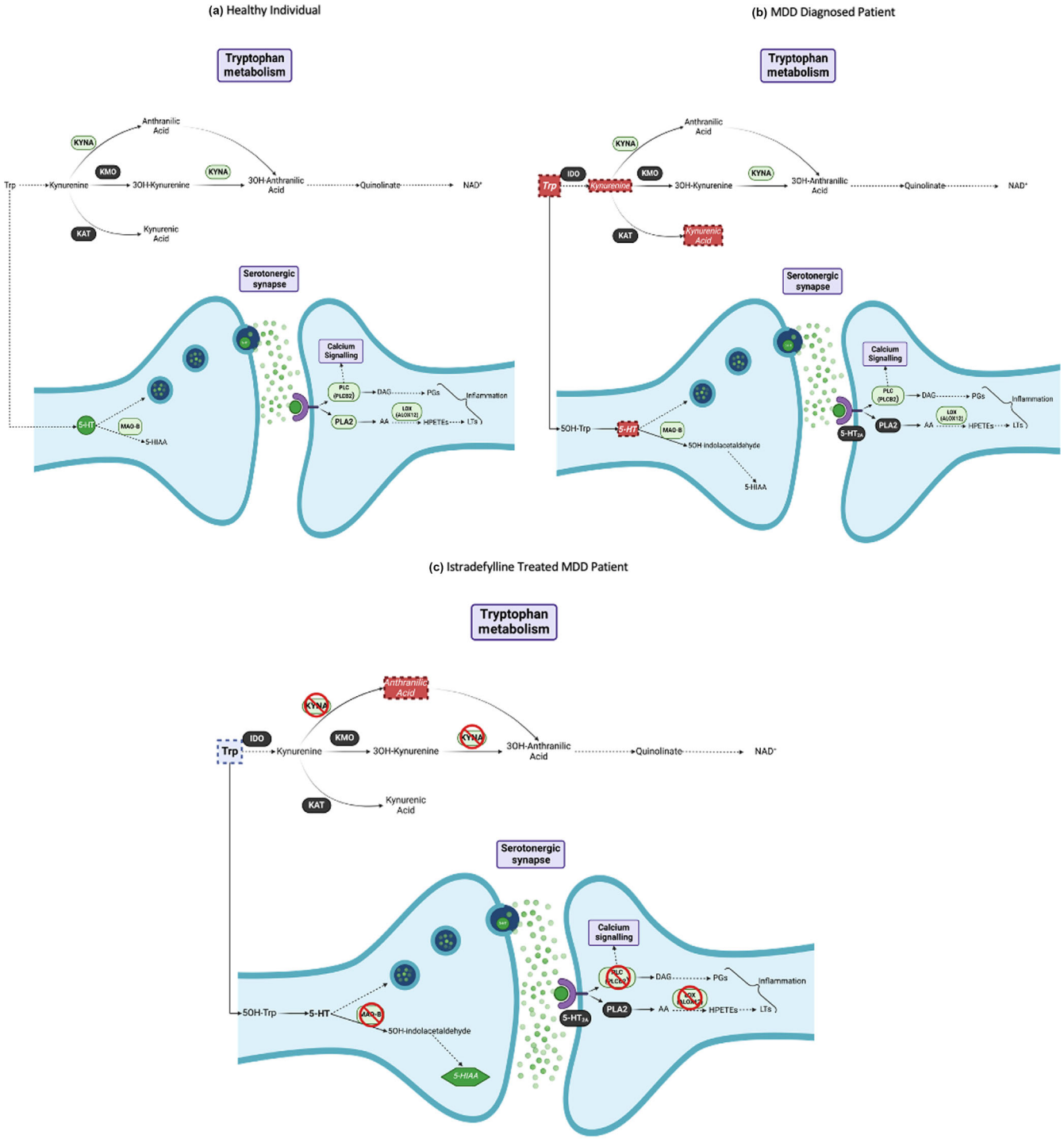
Inflammation has previously been implicated in increasing the risk of developing depression (Savitz, 2017). Acute inflammation is a beneficial process protecting against damage and spreading infection; it is a rapid and self-limiting process which is terminated by removing the insult (Regulska et al., 2021). Once this complex mechanism fails, the inflammatory process can become prolonged and chronic. A prolonged or chronic inflammatory response, in contrast to acute inflammation, within the CNS increases the risk of developing depression (Regulska et al., 2021). This complex mechanism is not fully understood; however, various pathways have previously been proposed.

Based on the computational workflow, we propose integrated mechanisms linked to inflammation as key to istradefylline's antidepressant activity. It is postulated that the kynurenine pathway and eicosanoid-related pathways, in addition to the disease- and drug-target pathways mentioned above, are modulated by istradefylline (Figure 1c).

Tryptophan is an essential amino acid from which four biosynthesis pathways originate: the synthesis of proteins, the synthesis of 5-HT, the synthesis of tryptamine, and the kynurenine pathway (Pawlowski et al., 2021). Previously, activation of the kynurenine pathway exerted depressive-like behaviour in rodents and was activated in MDD-diagnosed patients (Savitz, 2017). The kynurenine pathway is potentially activated via an inflammatory response, resulting in the formation of neurotoxic metabolites linked to the onset of depression (Pawlowski et al., 2021).

Tryptophan is metabolized to kynurenine, which in turn may be converted to neurotoxic or neuroprotective metabolites. Thus, during an inflammatory response, the breakdown of tryptophan to kynurenine is catalysed via the upregulation of **indoleamine-2,3-deoxygenase (IDO)** at the expense of 5-HT. Following this, kynurenine may either form the neuroprotective metabolite **kynurenic acid** or, alternatively, at the latter's expense, form the neurotoxic metabolite 3-hydroxykynurenine (3HK). Another metabolite, 3-hydroxyanthranilic acid (3HAA), may be synthesized from either anthranilic acid or 3HK. The kynureninase (KYNU) enzyme converts kynurenine to anthranilic acid and, subsequently, 3HAA. Non-specific hydroxylation of anthranilic acid remains the main source of 3HAA in the brain, based on 3HAA not being able to cross the blood–brain barrier (Pawlowski et al., 2021). In peripheral tissue, 3HAA is converted from 3HK via KYNU (Figure 1c).

The involvement of the kynurenine pathway was demonstrated in a meta-analysis study performed by Marx et al. (2021). The authors found a decrease in tryptophan, kynurenine, and kynurenic acid



Key: crossed out green ovals with black text– enzyme "hits" suspected to be inhibited by Istradefylline; black oval with white text – notable enzymes; red squares with white text and broken borders – metabolites with decreased concentrations; green diamond with white text – metabolites with increased concentrations.

FIGURE 1 (a) A simplified representation of the tryptophan metabolism pathway, linked to the serotonergic synapse pathway in a healthy individual. (b) A simplified representation of the tryptophan metabolism pathway, linked to the serotonergic synapse pathway, illustrating the perturbations suspected in clinical depression. (c) A simplified representation of the tryptophan metabolism pathway, with its link to the serotonergic synapse pathway and the hypothesized alterations brought on by istradefylline in MDD. Affected enzymes shown are a result of the novel computational workflow, while affected metabolites shown were reported by experimental means. *Abbreviations: MAO = monoamine oxidase; Trp = tryptophan; 5-HT = serotonin; 5-HIAA = 5-hydroxyindole acetic acid; PLC = phospholipase C; PLCB2 = phospholipase Cβ2; PLA2 = phospholipase A2; DAG = diacyl glycerols; PG = prostaglandins; AA = anthranilic acid; HPETEs = mono-hydroperoxy (e)icosatetraenoic acids; LT = leukotrienes; ALOX12 = arachidonate 12-lipoxygenase* (illustration created using Biorender.com).

concentrations in MDD-diagnosed patients, compared with healthy controls (Marx et al., 2021). Another study associated an increase in anthranilic acid concentration with MDD patients (Darlington et al., 2010; Pawlowski et al., 2021). Furthermore, a decrease in the ratio of kynurenic acid to 3-HK and kynurenic acid to kynurenine was documented for the MDD-diagnosed participants in the meta-analysis study (Marx et al., 2021). Darlington et al. (2010) documented a decrease in the ratio of 3HAA to anthranilic acid in patients diagnosed with depression. Marx et al. (2021) concluded a shift in the tryptophan metabolism from 5-HT to the involvement of the kynurenine pathway in MDD.

The kynurenine enzyme KYNU is an important predicted protein. If istradefylline theoretically acts as a KYNU inhibitor, a decrease in anthranilic acid and 3HAA or potentially an increase in the ratio of 3HAA to anthranilic acid is expected. A preliminary study performed by our research group supports the theory that istradefylline modulates the kynurenine pathway by potentially acting as a KYNU inhibitor. Decreased levels of anthranilic acid were found in istradefylline-treated rodents compared to the unmedicated control group (data unpublished; Supporting Information S7). In short, an in vivo animal study was performed as described previously (Smith et al., 2022). Rodents were subjected to an animal model of depression, treated with istradefylline, brain material collected, metabolites extracted, and analysed via a targeted metabolomics approach utilizing LC–MS/MS. Thus, 'proof of concept' is shown by the preliminary experimental data, literature analysis, and the results of the computational workflow.

Given the potential role of istradefylline via the Kynurenic pathway, as hypothesized via the computational workflow, a link towards DR may be further emphasized based on istradefylline's mechanism of action in MDD and PD treatment. According to a review by Chen and Geng (2023), selected metabolites in the kynurenic pathway are perturbed in PD patients. An increase in kynurenine, 3HK, and quinolinate was documented, while 3HAA, kynurenic acid, and NAD^+ were decreased for PD patients. Based on the computational workflow results, it may be speculated that istradefylline plays a neuroprotective role in PD by increasing kynurenic acid via inhibition of KYNU (a similar mechanism as speculated above for istradefylline's role in MDD-treated patients). In addition, a simplified theoretical representation of the latter kynurenic pathway in a healthy individual, a PD patient, and an istradefylline-treated PD patient is provided in the supplementary material, Figure S8.

The involvement of arachidonate 12-lipoxygenase (ALOX12) and phospholipase C beta 2 (PLCB2) in the serotonergic synapse is key to the current hypothesis. Briefly, arachidonic acid (AA) is an abundant polyunsaturated fatty acid from which various AA-derived metabolites (eicosanoids) are vital to numerous processes crucial in the inflammatory response (Regulska et al., 2021). The synthesis of eicosanoids is either catalysed by **cyclooxygenases (COX)**, resulting in pro-inflammatory AA-metabolites (including prostaglandins and thromboxanes) or catalysed by **lipoxygenases (LOX)** to produce leukotrienes (LTs) and anti-inflammatory derivatives (e.g., lipoxins) (Regulska et al., 2021). An imbalance between these metabolites may lead to chronic neuroinflammatory effects. Currently, eicosanoid-related pathways (specifically involving prostaglandins and lipoxins) are investigated as treatment targets for depression and, more specifically, drug-resistant depression (Regulska et al., 2021).

If istradefylline indeed raises pre-synaptic 5-HT levels (Hypothesis 1), it may activate other post-synaptic pathways in the serotonergic synapse. The calcium-dependent **cytosolic phospholipase A_2 (cPLA2)** is found at the beginning of the AA metabolic pathway and plays a role in the production of LTs (Kanehisa-Laboratories, 2023). LTs are potent activators of inflammatory responses; thus, an increase in LTs leads to an increase in inflammation. Another enzyme worth mentioning is ALOX12, which is the rate-limiting enzyme in converting AA to LTs (Wang et al., 2021). An untargeted metabolomics study utilizing a forced swim test with rodents documented increased neuronal levels of AA after istradefylline treatment (Smith et al., 2022). Based on the assumption that inhibition of ALOX12 would lead to reduced AA metabolism, istradefylline may theoretically modulate the serotonergic synapse. Thus, exerting its antidepressant activity by reducing inflammation in the arachidonic acid metabolism pathway, which is linked to the serotonergic synapse. Another predicted protein associated with the serotonergic synapse is PLCB2. PLCB2 catalyses reactions leading to the production of lipids and diacylglycerols, which themselves are further metabolized to prostaglandins (PGs) (Kanehisa-Laboratories, 2023). Thus, PGs, like LTs, play a key role in activating inflammatory responses, generally increasing inflammation (Ricciotti & FitzGerald, 2011). If istradefylline theoretically reduces PLCB2 activity (similar to ALOX12 activity), it will lead to reduced PG levels. Reduced PG and LT production could indicate that istradefylline exerts a neuroprotective action by reducing overall inflammation within the brain, which again shows a possible antidepressant mechanism.

In conclusion, we have, here, described a novel computational workflow comprising three phases is evaluated for compound identification and applied as a DR hypothesis-generating tool. The workflow leverages free-access computational platforms to predict protein targets (via PharmMapper), identify disease- and drug-target pathways (via KEGG database), and assess drug-likeness (via Admelab2.0, SwissADME, pkCKM) for a pre-approved drug. Specifically, we explore istradefylline's potential as an antidepressant for MDD.

Based on literature reviews, istradefylline, clinically approved for PD-related motor symptoms, has shown antidepressant-like effects in rodent models, suggesting potential as a DR candidate for MDD. The high prevalence of depression in PD, often poorly managed by standard antidepressants due to the complex interplay of dopaminergic, serotonergic and cholinergic-adrenergic systems, highlights the need for alternative antidepressive treatments. Additionally, both conditions share elevated levels of inflammatory biomarkers (IL-6, IL-1ß, TNF and CRP), supporting an inflammation hypothesis. Given istradefylline's anti-inflammatory potential and its effect on adenosine receptors, it is a promising candidate for repurposing as an MDD treatment, offering a novel therapeutic approach that warrants further exploration via the DR computational workflow.

Theoretically, based on the computational workflow, istradefylline exerts its antidepressant properties via integrated mechanisms of action linked to increased levels of 5-HT and reduced inflammation. Considering the above, proceeding to the next step of the DR process, where the pre-approved drug's mechanistic effect is assessed, is recommended.

The following considerations need to be considered with practically implementing the computational workflow: (1) Limited database coverage: Predicted proteins are limited to PharmMapper's pharmacophore database, which includes only drug targets with 3D crystal structures. Predicted drug- and disease-target pathways are limited to the knowledge database of KEGG. Drug-like analysis of the pre-approved drug is limited to the use of Admelab2.0, SwissADME and pkCKM. (2) Large-scale data complexity: 'Large-scale' data are generated; interpreting these data is complex and cannot be done in isolation. Although the workflow assists with streamlining interpretation via complementary computational target identification tools, a solid knowledge of the pre-approved drug and its intended repurposing clinical indication is still needed. (3) Database updates and biases: The proposed computational workflow relies on existing databases, which are continually being updated. However, data retrieved can become outdated if too much time passes between retrieval and processing. Additionally, biases within a database affect the extracted data. Therefore, staying informed about the database updates and considering the limitations will enhance the effectiveness of this computational workflow.

However, incorporating 'pathways' as part of the novel computational workflow offers several benefits over focusing solely on a specific predicted protein: (1) Broad impact: Pathways involve multiple proteins and interactions. A 'holistic' view of a broader therapeutic effect may be gained by considering a pathway. (2) Redundancy and Compensation: Pathways often have redundant components. If one protein is inhibited, compensatory mechanisms may activate other proteins. Therefore, one may identify potential compensatory pathways by understanding and considering the entire pathway. (3) Network effects: Proteins do not act in isolation; they interact within networks. Pathway analysis considers these network effects, providing a holistic view of disease mechanisms and potential interventions.

In conclusion, proof-of-concept is provided that the novel computational workflow, utilizing reverse pharmacophore mapping in combination with in silico target identification platforms, is a low-risk, time-saving and low-cost DR hypothesis-generating computational tool—it is recommended for a bird's-eye view for repurposing pre-approved drugs.

AUTHOR CONTRIBUTIONS

Conceptualization— Mietha van der Walt; **data generation: PharmMapper**— Mietha van der Walt; **KEGG databases**— Mietha van der Walt and Arnold Smith; **Admelab2.0, SwissADME & pkCKM**— Arnold Smith; **literature search:** Mietha van der Walt and Arnold Smith; **Data mining** (analysis, interpretation and validation)— Mietha van der Walt and Arnold Smith; **writing of manuscript** (original draft preparation, review and editing)— Mietha van der Walt and Arnold Smith; **supervision and project administration**— Mietha van der Walt. All authors have read and agreed to the submitted version of the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data available on request from the authors. The data that support the findings of this study are available from the corresponding author upon reasonable request.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

The author(s) affirm that this article provides an honest, accurate and transparent account of the study being reported. All significant aspects of the study have been included, and it was conducted with the utmost scientific rigor. The raw data supporting the study's findings, which is not provided in the supplementary material, can be obtained from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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