Research Article

In silico metabolism and toxicity prediction using a knowledge-based approach

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Abstract

The application of *in silico* approaches for predicting metabolic pathways and toxicity profiles has significantly advanced drug discovery and chemical risk evaluation. By harnessing developments in cheminformatics, machine learning, and expert-driven platforms, these computational techniques enable the early assessment of how candidate molecules may be metabolized and their possible toxic effects, often prior to laboratory synthesis or experimental testing. In this study, knowledge-based algorithms were utilized to predict the metabolism and toxicity of a previously identified dual-acting pyrrole-based compound using *in silico* methods. The licensed Lhasa software suite (Lhasa Limited, Leeds, UK), specifically the Meteor and Derek modules, was employed for these analyses. Toxicity assessment indicated that compound 7b has a plausible potential to cause skin irritation or corrosion in mammalian systems. However, it was computationally predicted to be inactive in bacterial mutagenicity assays (Ames test) and did not trigger any alerts across 58 other evaluated toxicity endpoints. The analysis also identified the closest metabolic analogues of compound 7b, revealing that the compound is most likely to undergo hydrolysis of its acyclic carboxylic ester, followed by hydroxylation of the tryptophan ring. These *in silico* findings provide valuable insights, but further validation through *in vitro* and *in vivo* studies should be carried out.

Keywords

metabolism, toxicity prediction, knowledge-based approach, pyrrole

Introduction

The integration of *in silico* methods into the prediction of metabolism and toxicity has transformed the landscape of drug discovery and chemical safety assessment (Iliev et al. 2023). These computational approaches leverage advances in cheminformatics, machine learning, and expert systems to forecast how small molecules are metabolized and to anticipate their potential toxicological effects before they are synthesized or tested experimentally. By simulating metabolic pathways and predicting adverse outcomes, *in silico* tools play a crucial role in guiding early-stage compound selection, optimizing lead structures, and reducing the reliance on costly and time-consuming *in vitro* and *in vivo* studies (Kazmi et al. 2019; Agahi et al. 2020).

Drug metabolism profoundly influences the pharmacokinetic and pharmacodynamic profiles of compounds, affecting their absorption, distribution, efficacy, and safety. Enzymatic biotransformations can result in the activation or inactivation of therapeutic agents, as well as the formation of reactive or toxic metabolites. *In silico* metabolism prediction tools, such as those based on knowledge-driven rules or machine learning algorithms, can identify likely sites and routes of metabolism, predict the involvement of specific enzymes (e.g., cytochrome P450 isoforms), and propose plausible metabolite structures.

These predictions help researchers anticipate metabolic liabilities, design compounds with improved metabolic stability, and prioritize experimental validation for the most promising candidates (Alqahtani 2017). Lhasa's Meteor software module is widely utilized in pharmaceutical research to predict potential metabolites and identify metabolic "soft spots" early in drug development, thereby supporting the design of compounds with enhanced metabolic stability. *In silico* studies can aid in the identification and analysis of metabolites during experimental research, helping to streamline workflows and minimize manual effort (Boyce et al. 2022).

In parallel, *in silico* toxicity prediction has become an essential component of chemical risk assessment and drug development. Computational toxicity profiling utilizes a variety of data sources and modeling techniques to estimate the likelihood of adverse effects, including acute toxicity, organ-specific toxicity, genotoxicity, and carcinogenicity. By analyzing structural features associated with known toxicities and integrating mechanistic information, these tools can flag potential "hot spots" for toxicity within a molecule, inform structure-activity relationship studies, and support regulatory submissions. Importantly, *in silico* toxicity models contribute to the principles of the 3Rs (Replacement, Reduction, and Refinement) by minimizing the need for animal testing and focusing experimental efforts on compounds with favorable safety profiles (Noga et al. 2024).

The synergy between *in silico* metabolism and toxicity prediction enables a more holistic evaluation of chemical entities at the earliest stages of research and development. Therefore, this study focuses on the metabolism evaluation of a hit structure identified by our research group using an *in silico* knowledge-based approach. The toxicity was also evaluated by applying numerous toxicity endpoints.

Materials and methods

Software and configuration

Toxicity predictions were performed using Derek Nexus (Knowledge Base: v6.4.1; Nexus: v2.7.2; Lhasa Limited, Leeds, UK), a knowledge-based expert system for mechanistic toxicity assessment. The software's rule-based framework incorporates structural alerts derived from empirical toxicological data, peer-reviewed literature, and regulatory guidelines. Metabolic predictions were performed using Meteor Nexus (Lhasa Limited, Leeds, UK), a knowledge-based expert system for Phase I/II biotransformation analysis. The software integrates empirical data, peer-reviewed literature, and proprietary metabolic rules to predict potential metabolites and their accumulation pathways.

Compound input and setup

The pyrrole-based hit compound (structure detailed in Table 1) was input into Derek Nexus using its canonical SMILES string, generated and validated via ChemAxon's MarvinSketch (v22.15). To ensure reproducibility, the compound's stereochemistry and tautomeric forms were explicitly defined prior to analysis.

Endpoint selection

All toxicity endpoints available in the Derek Prediction Set Up menu were evaluated, including carcinogenicity (e.g., IARC Group classifications); genotoxicity (mutagenicity, clastogenicity); organ toxicity (hepatotoxicity, nephrotoxicity, neurotoxicity); reproductive/developmental toxicity; sensitization (skin, respiratory); and miscellaneous endpoints – phototoxicity and phospholipidosis. Predictions were generated for bacteria (primarily for Ames test relevance) and mammals (rat, human) to assess species-specific toxicological profiles. The prediction confidence levels were categorized as probable: strong structural or mechanistic evidence (≥70% likelihood); plausible: moderate evidence (30–69% likelihood); Equivocal: Insufficient or conflicting evidence (<30%); negative predictions (no alerts) were assigned when the compound lacked structural motifs associated with toxicity in Derek's knowledge base (e.g., no phototoxic arylpropanone or polycyclic aromatic fragments).
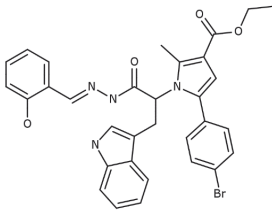
Metabolic parameters

Metabolic pathways were visualized hierarchically as metabolic trees, annotating parent compounds and their derivatives with reaction types (e.g., hydroxylation, ester hydrolysis). Mechanistic rationales, such as CYP3A4 affinity for aromatic oxidation, and literature citations supporting predicted biotransformations were curated. Competing reactions, such as glucuronidation versus sulfation, were flagged for further analysis.

Results and discussion

Our previous studies identified a novel hit compound featuring a pyrrole core. This compound demonstrated dual inhibitory activity against MAO-B and AChE, along with promising antioxidant properties (Table 1) (Mateev et al. 2024). In the present study, we investigated the metabolic profile of this hit structure using an *in silico*, knowledge-based approach. Additionally, its toxicity was assessed by evaluating multiple toxicity endpoints with the licensed Lhasa software.

The structure was selected based on our extensive studies aimed at discovering novel multi-target MAO-B/AChE inhibitors with a pyrrole-based scaffold. The ligand presented in Table 1 was identified as a potential dual-acting inhibitor, also demonstrating favorable radical-scavenging properties. Additionally, its blood-brain barrier permeability coefficient was found to be within a moderate range. Therefore, it was applied for further *in silico* evaluations.

Table 1. Pyrrole-based compound with dual MAO-B/AChE inhibitory activity used in this study.

Code	7b
Formula	$C_{32}H_{29}BrN_4O_4$
Exact Mass	612.1372
Average Molecular Mass	613.5
Smiles	BrC=1C=CC(=CC=1)C5=CC(C(OCC)=O)=C(C)N5C(CC2=CNC3=CC=CC=C23)C(N/N=C/C4=CC=CC=C4O)=O
MAO-B	21% (1 µM) (Mateev et al. 2024[a])
AChE	31% (10 µM) (Mateev et al. 2024[a])
DPPH	61.27% (250 µM) (Mateev et al. 2024[b])
ABTS	90.49% (250 µM) (Mateev et al. 2024[b])

In silico toxicity assessment

The initial calculations were aimed at determining the chemical toxicity after in-depth evaluations with the Derek Nexus module of Lhasa. The module provides data about the potential safety concerns of the tested structures. These assessments are vital in pharmaceutical development and regulatory submissions, as they identify chemical features – known as toxicophores – that are linked to harmful biological effects (Anwar et al. 2021). Several studies have demonstrated a strong correlation between experimental results and predictions made by Derek Nexus, supporting the reliability and established status of the module (Judson et al. 2013). Derek Nexus utilizes data from both the Ames bacterial reverse mutation assay and transgenic rodent mutation models to inform its toxicity predictions.

Derek Nexus assesses chemical compounds across more than 50 toxicological endpoints, providing a likelihood rating for each prediction that ranges from high certainty to more tentative assessments. Negative results are classified from "inactive" to "improbable." For example, if a compound shares structural features with known mutagens, it may be designated as a "probable mutagen" and assigned a specific confidence level. The platform also offers mechanistic explanations for its predictions, such as detailing cytochrome P450-mediated metabolic pathways in cases of hepatotoxicity or referencing established assays for endpoints like skin sensitization. Notably, the analysis indicated that compound **7b** did not show toxicity across any of the 57 evaluated endpoints, with the full list of endpoints summarized in Table 2.

Moreover, compound **7b** was evaluated using the Carcinogenic Potency Categorization Approach (CPCA) for N-nitrosamines. This approach is a structure-activity relationship (SAR)-based framework that categorizes N-nitrosamine impurities into potency categories, each associated with an acceptable intake limit. The categorization is based on the presence of specific structural features within the molecule that are known to influence carcinogenic potential. The CPCA is particularly relevant for N-nitrosamines containing a carbon atom on both sides of the N-nitroso group and is widely used to predict carcinogenic risk when experimental data are unavailable or limited.

During the assessment of compound **7b**, special attention was given to the identification of dialkyl N-nitrosamine substructures, as these are recognized as highly potent carcinogens due to their metabolic activation pathways. Structural analysis of **7b** demonstrated that it does not contain any dialkyl N-nitrosamine motifs, which are typically associated with a higher risk of carcinogenicity. The absence of these hazardous structural features in compound **7b** suggests a lower predicted risk for nitrosamine-related carcinogenicity.

Several structural alerts were identified during the *in silico* toxicity assessment of compound **7b** using the Derek Nexus platform, with most of these alerts related to the skin sensitization endpoint. Derek Nexus is a knowledge-based expert system that predicts toxicity by identifying chemical substructures, known as toxicophores, which are associated with specific toxicological outcomes. Fig. 1 illustrates the specific alert generated for the substituted phenol ring in compound **7b**, highlighting the correspondence between this structural feature and the system's prediction.

The primary alert triggered for compound **7b** is associated with the presence of a substituted phenol ring. Phenolic structures are recognized as potential skin sensitizers, and their presence in a molecule often prompts further evaluation. Derek Nexus incorporates this knowledge into its alert system, flagging compounds that contain such substructures due to their potential to induce skin sensitization reactions. This is based on evidence that phenol derivatives can undergo metabolic activation or direct interaction with skin proteins, ultimately leading to immune responses characteristic of sensitization.

Table 2. Toxicity endpoints not firing any alerts at the selected reasoning level.

5alpha-Reductase inhibition	Methaemoglobinaemia
Adrenal gland toxicity	Mitochondrial dysfunction
Anaphylaxis	Mutagenicity in vivo
Androgen receptor modulation	Nephrotoxicity
Bladder disorders	Neurotoxicity
Bladder urothelial hyperplasia	Non-specific genotoxicity in vitro
Blood in urine	Non-specific genotoxicity in vivo
Bone marrow toxicity	Occupational asthma
Bradycardia	Ocular toxicity
Carcinogenicity	Oestrogen receptor modulation
Cardiotoxicity	Oestrogenicity
Cerebral oedema	Peroxisome proliferation
Chloracne	Phospholipidosis
Cholinesterase inhibition	Photo-induced chromosome damage in vitro
Chromosome damage in vitro	Photo-induced non-specific genotoxicity in vitro
Chromosome damage in vivo	Photo-induced non-specific genotoxicity in vivo
Cumulative effect on white cell count and immunology	Photoallergenicity
Cyanide-type effects	Photocarcinogenicity
Developmental toxicity	Photomutagenicity in vitro
Glucocorticoid receptor agonism	Phototoxicity
Hepatotoxicity	Pulmonary toxicity
HERG channel inhibition in vitro	Respiratory sensitization
High acute toxicity	Skin sensitization HPC
Irritation (of the eye)	Splenotoxicity
Irritation (of the gastrointestinal tract)	Teratogenicity
Irritation (of the respiratory tract)	Testicular toxicity
Kidney disorders	Thyroid toxicity
Kidney function-related toxicity	Uncoupler of oxidative phosphorylation
Lachrymation	Urolithiasis

Alert Description Image   **Match with query compound**
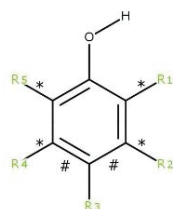
R1 = H, CH, F, Cl, Br, I
R2, R4 = H, F, Cl, Br, I, CH2 or C (sp2 or sp hybridised) except C(=O)OR6
R3, R5 = H, F, Cl, Br, I, C except C(=O)OR7
R1-R5 cannot all be hydrogen
R6, R7 = C, H

Atoms marked % require a double bond and cannot be attached to additional heteroatoms
All ring fusions allowed except bonds marked # cannot be fusion bonds
Bonds marked * can be single or part of an aromatic fusion
Dashed bonds can be either single or double
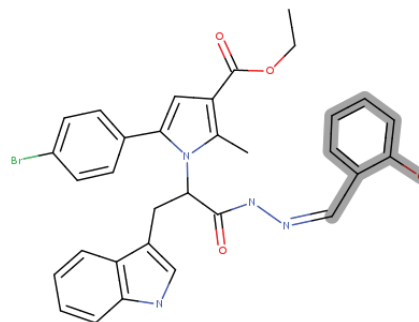
Figure 1. Description of the alert image and its occurrence in **7b** (highlighted in grey).

While phenol itself has generally not shown skin sensitization in animal studies, several of its substituted derivatives have demonstrated sensitizing potential. For instance, compounds such as 2,5-dimethylphenol, 3,4-dimethylphenol, pentachlorophenol, and 2,4,5-trichlorophenol have been identified as skin sensitizers in the local lymph node assay (LLNA) (Ashby et al. 1995). Similarly, other substituted phenols, including 4-styrylphenol (also known as 4-hydroxystilbene) and chloroxylenol (4-chloro-2,3-dimethylphenol), have produced positive results in the guinea pig maximization test (GPMT) (Cronin and Basketter 1994). However, it is important to recognize that not all substituted phenols act as sensitizers; some, such as 4-tert-butylphenol, 2-methylphenol, and 3,4,5-trimethylphenol, have yielded negative results in both the GPMT and LLNA, indicating variability in sensitization potential among phenol derivatives, as discussed by Yamano et al. (2007).
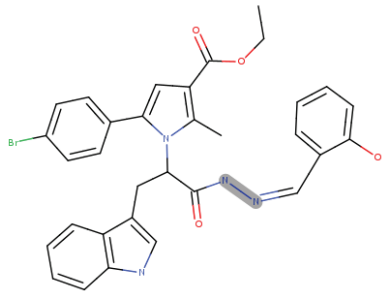
Alert Description Image	**Match with query compound**
N —— N Nitrogen atoms cannot be aromatic or be attached to additional heteroatoms	

Figure 2. Description of the alert image and its occurrence in **7b** (highlighted in grey).

Hydrazine and phenylhydrazine are classified as significant contact allergens (category A) based on an analysis of the available human and animal data, and positive human patch test results were reported for hydrazine and selected derivatives (Foulds and Koh 1991). Conversely, some human patch test studies have reported negative results (Rothe 1988). Hydrazides such as carbonohydrazide showed positive activity in the GPMT, although the structural analogue adipohydrazide was negative in the LLNA. The alert description image and its match with the evaluated structure, **7b**, are given in Fig. 2.

In the context of skin sensitization, research by Chilton et al. (2018) has validated the use of confidence metrics for non-sensitizer predictions, drawing on data from established assays such as the Local Lymph Node Assay (LLNA) and the Human Repeat Insult Patch Test (HRIPT). These studies confirm the reliability of Derek Nexus in predicting sensitization potential based on a chemical structure.

The structural configuration of the query molecule does not correspond to any known structural alerts or reference compounds linked to bacterial in vitro mutagenicity in the Derek knowledge base. Additionally, the compound lacks any unclassified or ambiguous features, further supporting the computational prediction that it will be inactive in the bacterial reverse mutation (Ames) assay.

Overall, analysis using Derek indicated that the title compound **7b** is predicted to have plausible potential for skin irritation or corrosion in mammalian models. However, the compound was predicted to be inactive with respect to *in vitro* mutagenicity in bacterial assays and showed no activity in 58 other evaluated toxicity endpoints. Therefore, it could be used for future hit-to-lead and lead optimization processes.

In silico metabolic profiling

Meteor Nexus is a commercially available metabolite prediction software that is part of a larger suite of tools developed by Lhasa Ltd. Meteor generates metabolites using a knowledge-based expert system, which identifies structural patterns within the parent compound and correlates these patterns to encoded biotransformations. The Meteor software employs a knowledge-based approach, using curated scientific data and reasoning algorithms to predict possible metabolic transformations (Phase I and II) for a given chemical structure. This approach is distinct from data-driven or purely statistical models, as it relies on encoded expert knowledge and mechanistic rules to generate its predictions (Boyce et al. 2022). Moreover, recent validation studies published in journals such as Xenobiotica and the Journal of Medicinal Chemistry support the integration of Derek Nexus with metabolic prediction platforms like Meteor Nexus. These studies highlight the advantages of combining mechanistic toxicity predictions with metabolic fate analysis, demonstrating that such an approach can improve early risk assessment in drug discovery by providing a more comprehensive evaluation of both parent compounds and their metabolites. The results provided by Meteor are generalized in Fig. 3.

The most probable metabolic pathway for compound **7b** is hydrolysis of the acyclic carboxylic ester located at the 4th position of the pyrrole core motif (Fig. 4). Ester hydrolysis is a common metabolic reaction, typically catalyzed by carboxylesterase enzymes in various tissues, especially in the liver. This process involves the cleavage of the ester bond, resulting in the formation of a carboxylic acid and an alcohol as the primary metabolites. These hydrolysis products are generally more polar than the parent ester, which increases their hydrophilicity and promotes their excretion via the kidneys (Laizure et al. 2013).

For compound **7b**, hydrolysis at the 4th position would introduce a polar carboxylic group, likely increasing water solubility and reducing membrane permeability. This transformation may impact the pharmacokinetic profile of **7b** by decreasing its bioavailability while facilitating its elimination. Thus, the presence of the acyclic carboxylic ester at this position represents a key metabolic liability that should be taken into account during further development and optimization of the compound. Table 3 presents the chemical structures identified as the closest metabolic analogues to compound **7b**. The reference compounds were selected based on their structural similarity to **7b**, particularly with respect to core motifs and key functional groups
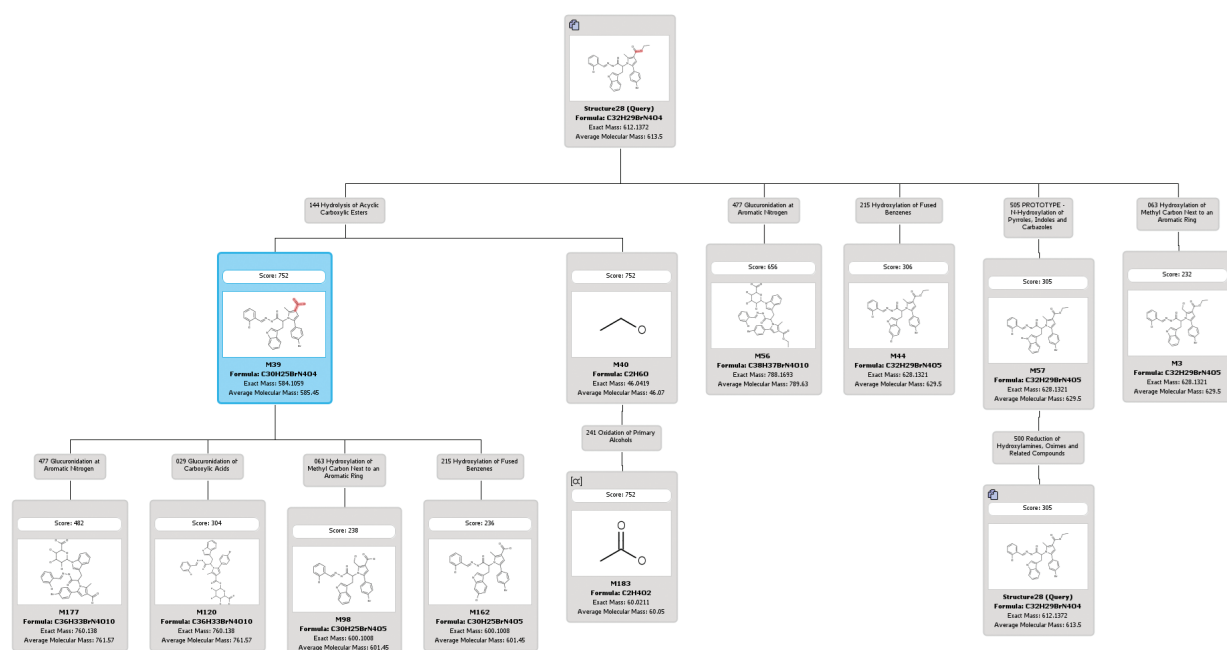
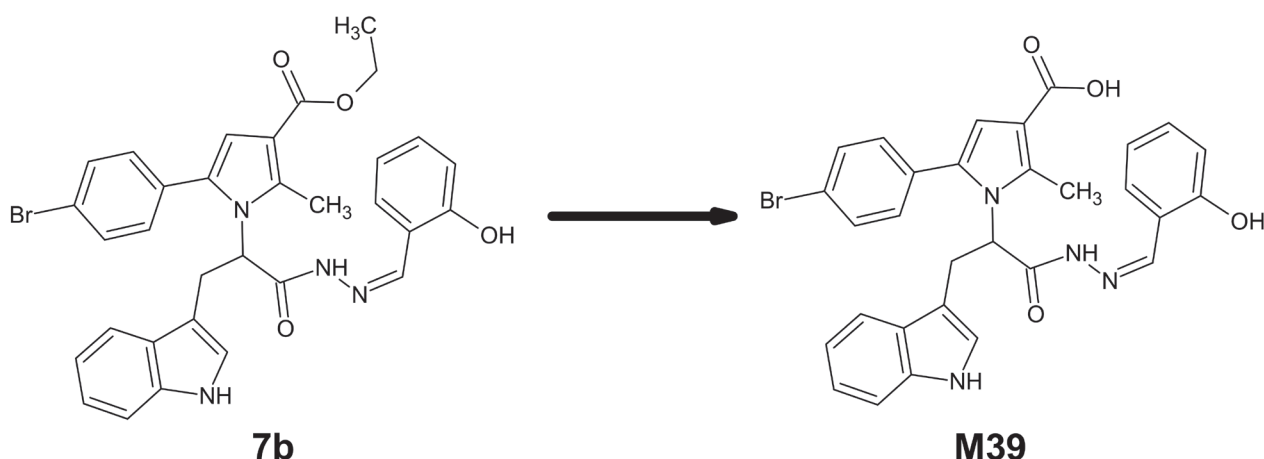**Figure 3.** Predicted biotransformation pathway of **7b**.

Figure 4. Hydrolysis of the ester group in **7b**.

relevant to metabolic transformation. The comparative analysis of these analogues enables a more informed prediction of the metabolic pathways that the pyrrole-based compound **7b** may undergo.

Furthermore, the predictions generated by the Meteor software indicated a high likelihood for the initial hydrolysis of the acyclic carboxylic ester moiety in compound **7b**, followed by subsequent hydroxylation of the indole ring (Fig. 5). The reliability of these predicted metabolic transformations is supported by the knowledge-based algorithms employed by Meteor, which utilize curated mechanistic rules and expert-derived data to forecast plausible biotransformation pathways.

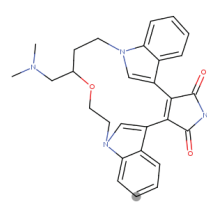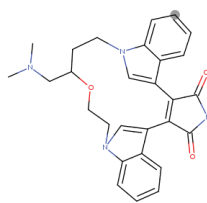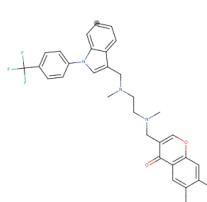The initial hydrolytic cleavage of the ester group is a well-recognized Phase I metabolic reaction, typically mediated by carboxylesterase enzymes, resulting in the formation of a more polar carboxylic acid derivative. Following this transformation, the indole ring is predicted to undergo hydroxylation, a common oxidative process catalyzed by cytochrome P450 enzymes. Such hydroxylation reactions generally increase the hydrophilicity of the molecule, further facilitating its excretion.

The sequential nature of these metabolic events – beginning with ester hydrolysis and proceeding to aromatic hydroxylation – reflects typical metabolic routes for compounds containing both ester and indole functionalities. This metabolic profile provides valuable insight into the likely biotransformation fate of compound **7b** and highlights potential sites for structural modification to improve metabolic stability or modulate pharmacokinetic properties. Table 4 presents the chemical structures identified as the closest metabolic analogues to compound **7b**.

Table 3. Nearest metabolic analogues of compound **7b** undergoing hydrolysis of the acyclic carboxylic ester.

	19%	Positive	Ma et al. 2005
	17%	Positive	Roberts et al. 2012
	16%	Positive	Roberts et al. 2012

Table 4. Representative compounds undergoing ester hydrolysis and subsequent tryptophan ring hydroxylation analogous to **7b**.

	66%	Positive	Barbuch et al. 2006
	65%	Positive	Barbuch et al. 2006
	62%	Negative	Sun and Yost 2008
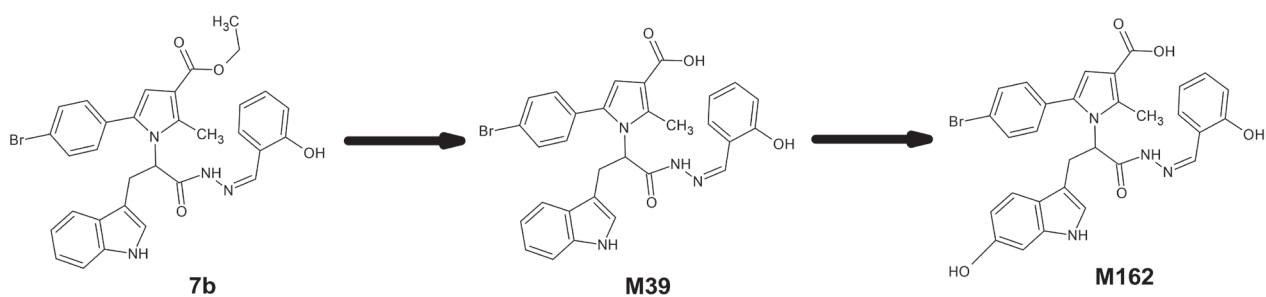
Figure 5. Hydrolysis of the ester group followed by hydroxylation of the tryptophan ring in **7b**.

By examining the documented metabolic profiles of these structurally related compounds, it is possible to anticipate potential metabolites, assess metabolic liabilities, and guide the rational design of derivatives with enhanced metabolic stability. This approach provides a valuable framework for understanding the likely biotransformation routes of **7b** and supports the overall strategy for optimizing its pharmacokinetic properties.

Conclusion

The *in silico* analysis using Derek Nexus suggested that compound **7b** exhibits plausible potential for skin irritation or corrosion in mammalian models. However, it was computationally predicted to lack activity in bacterial mutagenicity assays (Ames test) and showed no alerts across 58 additional toxicity endpoints. The structural configuration of **7b** lacks alignment with established mutagenic alerts or reference compounds within the Derek knowledge base. Furthermore, the absence of ambiguous or misclassified structural features supports its predicted inactivity in bacterial reverse mutation assays. By analyzing the metabolic profile of **7b**, this study identified potential metabolites, metabolic liabilities, and opportunities to rationally design derivatives with improved metabolic stability. The integrated *in silico* framework presented here provides a robust strategy for predicting biotransformation pathways and optimizing the pharmacokinetic properties of pyrrole-based lead compounds.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

The authors declared that no experiments on animals were performed for the present study.

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

Author contributions

E.M. – idea, writing, visualization; M.G. – writing, data analysis; A.M. – data analysis, visialization; A.Z. – supervision.

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Data availability

All of the data that support the findings of this study are available in the main text.

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