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Pharmacokinetic and Pharmacodynamic Analysis of Protoporphyrin IX for Enhancing its Efficacy in Photodynamic Therapy

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ABSTRACT

Introduction: Protoporphyrin IX (PpIX) is a critical photosensitizer in photodynamic therapy (PDT) with applications in oncology and dermatology. Despite its clinical importance, comprehensive understanding of its pharmacokinetic profile remains limited. This study aimed to characterize the absorption, distribution, metabolism, excretion, and toxicity (ADMET) properties of PpIX using computational approaches.

Material and Methods: The molecular structure of PpIX was analyzed using two complementary computational platforms, Deep-pk and pkCSM, which utilize machine learning and deep learning algorithms trained on experimental pharmacokinetic data to predict ADMET parameters. Physicochemical properties, absorption, distribution, metabolism, excretion, and toxicity profiles were evaluated and compared between the platforms.

Results: PpIX exhibited high lipophilicity (LogP>7) with moderate hydrogen bonding capacity. Both platforms predicted good intestinal absorption (63.5-98.2%) but poor oral bioavailability, explaining the preference for topical administration in clinical settings. PpIX showed moderate tissue distribution (VDss 0.63-0.77 log L/kg) and was not predicted to be a substrate for major CYP450 enzymes, suggesting metabolic stability. However, strong inhibition of CYP1A2 (probability 0.97) and transporters (OATP1B1, BCRP) indicated potential drug interactions. The predicted short half-life (<3 hours) aligned with clinical observations. Toxicity analysis revealed non-mutagenicity and cardiac safety, but conflicting hepatotoxicity predictions and potential respiratory toxicity warrant clinical monitoring.

Conclusion: Computational analysis of PpIX confirmed pharmacokinetic properties supporting its clinical use but raised concerns about drug interactions and organ toxicity. These results provide a basis for optimizing PDT protocols and improving formulations. Differences between prediction methods highlight the need for experimental validation of key parameters to ensure clinical safety and effectiveness.

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Introduction

Photodynamic therapy (PDT) represents a promising frontier in minimally invasive cancer treatment and antimicrobial applications, hinging critically on the pharmacokinetic and pharmacodynamic properties of photosensitizers [1]. Among these, Protoporphyrin IX (PpIX) has emerged as a cornerstone molecule in clinical PDT applications due to its unique photochemical properties and selective accumulation in target tissues [2]. Despite its widespread clinical utilization, comprehensive characterization of PpIX's complex pharmacokinetic profile remains challenging using traditional experimental approaches alone [3].

The advent of computational tools has revolutionized our ability to predict and analyze pharmacokinetic parameters with unprecedented precision and efficiency. This study leverages two cutting-edge computational platforms (pkCSM and Deep-pk) to elucidate the intricate pharmacokinetic behavior of PpIX as a vital photosensitizer in PDT applications [4, 5]. While pkCSM employs graph-based signatures to predict ADMET (Absorption, Distribution, Metabolism, Excretion, and Toxicity) properties, Deep-pk utilizes deep learning algorithms to model complex pharmacokinetic relationships from molecular structures [6, 7].

By integrating these complementary computational approaches, we aim to provide novel insights into PpIX's absorption kinetics, tissue distribution patterns, metabolic pathways, and clearance mechanisms. This comprehensive pharmacokinetic profiling will address critical knowledge gaps regarding PpIX behavior in biological systems, potentially resolving persistent challenges in PDT applications such as variable photosensitizer

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accumulation, unpredictable therapeutic windows, and differential tissue responses [8].

The findings from this computational investigation hold significant implications for optimizing PDT protocols, enhancing treatment efficacy, minimizing adverse effects, and potentially expanding the therapeutic applications of PpIX-mediated photodynamic interventions. Furthermore, this study establishes a methodological framework for the computational evaluation of next-generation photosensitizers, potentially accelerating their development and clinical translation.

Materials and Methods

Computational Tools and Resources

The present study employed a dual computational approach utilizing two advanced platforms for pharmacokinetic modeling and prediction. The integration of these complementary methodologies provided comprehensive insights into the pharmacokinetic profile of Protoporphyrin IX (PpIX) as a photosensitizer in photodynamic therapy applications.

The pkCSM (predicting small-molecule pharmacokinetic properties using graph-based signatures) web server (http://biosig.unimelb.edu.au/pkCSM/) was accessed between January and March 2025. This platform implements graph-based signatures to generate predictive models of pharmacokinetic properties based on molecular structure. pkCSM has been extensively validated against diverse chemical datasets and demonstrates high accuracy in predicting ADMET parameters for small molecules and drug-like compounds [9]. In parallel, we utilized the Deep-pk platform (version 2.3), a deep learning-based computational tool that employs convolutional and recurrent neural networks to model complex pharmacokinetic relationships. Deep-pk was selected for its demonstrated ability to capture non-linear relationships between molecular structures and their pharmacokinetic behaviors, particularly for porphyrin-like compounds [10].

Molecular Structure Preparation and Optimization

The three-dimensional structure of Protoporphyrin IX (PubChem CID: 4971, molecular formula C34H34N4O4, molecular weight 562.7 g/mol) was retrieved from the PubChem database in SDF format [11]. To ensure optimal computational analysis, the molecular structure underwent rigorous preparation and optimization procedures.

Initial structure optimization was performed using Schrödinger's LigPrep module (Schrödinger Release 2024-1, Schrödinger, LLC, New York, NY) [12]. The optimized structures were converted to appropriate file formats (.mol2, .sdf, and SMILES) using Open Babel (version 3.1.1) to ensure compatibility with both computational platforms.

Comprehensive Pharmacokinetic Parameter Prediction

Absorption Parameter Analysis

A detailed absorption profile for PpIX was created using two computational platforms. Human intestinal absorption (HIA) was predicted with pkCSM's regression model, providing percentage absorption based on physicochemical properties [6]. Caco-2 cell permeability was assessed as a measure of intestinal permeability, expressed as log Papp. Additionally, P-glycoprotein substrate and inhibitor status were evaluated to understand potential active efflux mechanisms affecting PpIX absorption [13].

Parallel analysis with Deep-pk's absorption module provided complementary data on absorption rate constants (ka) under various physiological conditions, utilizing a neural network architecture that incorporated relevant molecular descriptors.

Distribution Analysis and Tissue Partitioning

The distribution profile of PpIX was characterized using computational models. The steady-state volume of distribution (Vdss) was predicted via pkCSM's regression algorithm (log L/kg). Tissue-specific partitioning was evaluated using Deep-pk's PBPK model, estimating coefficients for eight key tissues (plasma, liver, kidney, brain, skin, muscle, adipose, and tumor) while accounting for protein binding, lipid content, and inter-compartmental pH gradients [10, 14, 15].

Plasma protein binding was analyzed by both platforms: pkCSM predicted percentage binding from structural descriptors, while Deep-pk quantified affinity constants (Ka) for albumin, α1-acid glycoprotein, and lipoproteins. BBB permeability was assessed via log BB (pkCSM) and log PS (Deep-pk), the latter incorporating active transport dynamics [16, 17].

Metabolism Prediction and Enzymatic Interactions

PpIX metabolism was evaluated for Phase I/II biotransformation pathways. CYP450 substrate specificity (1A2, 2C9, 2C19, 2D6, 3A4) was assessed via pkCSM, with probability scores >0.5 indicating likely substrates. Potential CYP450 inhibition (predicted IC50) identified drug-drug interaction risks. Deep-pk predicted metabolic sites (oxidation, reduction, hydrolysis, conjugation) and derived half-life values in hepatic systems. Ferrochelatase-mediated conversion to heme was modeled, accounting for differential tumor vs. normal tissue expression to explain PpIX's neoplastic accumulation.

Excretion Analysis and Clearance Mechanisms

The excretion kinetics of PpIX were evaluated by predicting key clearance parameters. Total clearance rates (ml/min/kg) were computed using two independent platforms. Deep-pk offered enhanced resolution, differentiating hepatic, renal, and extrahepatic elimination pathways. Its model integrated physiological

variables (e.g., bile flow dynamics, enterohepatic recirculation) to refine PpIX excretion predictions.

Given porphyrins' unique pharmacokinetics, the involvement of efflux transporters (ABCG2/BCRP, ABCC2/MRP2) was assessed. Substrate potential was inferred via structural alignment with known ligands and identification of transporter-binding motifs.

Descriptive analysis

The pharmacodynamic and pharmacokinetic characteristics of Protoporphyrin IX were comparatively analyzed using descriptive analytical methods across the pkCSM and Deep-pk online platforms. Given the intricate and heterogeneous nature of the data, which encompasses both qualitative and quantitative parameters, traditional statistical testing was deemed unsuitable. Instead, a comprehensive descriptive comparative approach was employed to systematically evaluate and interpret the complex interactions and variations observed in the datasets. This methodology allowed for a nuanced examination of the drug's properties, ensuring that the subtle differences and interconnected characteristics were thoroughly explored without imposing restrictive statistical frameworks. By focusing on descriptive analysis, we were able to provide a more holistic and flexible interpretation of the pharmacokinetic and pharmacodynamic profiles derived from these two distinct computational platforms.

Results

Molecular Properties and PK Profile of PpIX

The comprehensive computational analysis of Protoporphyrin IX (PpIX) using both Deep-pk and pkCSM platforms revealed detailed insights into its pharmacokinetic behavior, which are essential for understanding its efficacy as a photosensitizer in photodynamic therapy. The molecular structure of PpIX (PubChem CID: 4971) exhibits key physicochemical properties that influence its pharmacokinetic profile, as presented in Table 1.

Absorption Parameters

The absorption characteristics of PpIX were evaluated through multiple parameters to assess its potential for various administration routes. Table 2 summarizes the absorption profile predicted by both computational platforms.

The absorption profile of PpIX reveals significant challenges for oral administration. While both platforms predict moderate to high intestinal absorption (63.5-98.2%), Deep-pk specifically indicates poor oral bioavailability at both ≥20% and ≥50% thresholds.

Table 1. Physicochemical Properties of Protoporphyrin IX

Property	Value	Platform
Molecular Weight	562.67 g/mol	pkCSM / Deep-pk
LogP	7.42 / 7.23	pkCSM / Deep-pk
Rotatable Bonds	8	pkCSM / Deep-pk
Hydrogen Bond Acceptors	4	pkCSM / Deep-pk
Hydrogen Bond Donors	4	pkCSM / Deep-pk
Surface Area	242.70 Å²	pkCSM / Deep-pk
Melting Point	306.14°C	Deep-pk
Boiling Point	554.06°C	Deep-pk
pKa (Acid)	8.04	Deep-pk
pKa (Basic)	7.15	Deep-pk

Table 2. Absorption Parameters of Protoporphyrin IX

Parameter	Deep-pk	pkCSM	Interpretation
Caco-2 Permeability	-5.49 log P_{app} (Very low)	0.169 log P_{app} (Moderate)	Major conflict: Deep-pk suggests negligible intestinal absorption (likely due to modeling active efflux or tight junctions), while pkCSM predicts moderate passive diffusion. Experimental validation needed.
Human Intestinal Absorption (HIA)	98.2% (High confidence)	63.52%	Deep-pk's high prediction may account for transporter-mediated uptake, whereas pkCSM's lower value aligns with moderate passive permeability.
Oral Bioavailability	Non-bioavailable (Medium confidence)	-	Both platforms agree on poor bioavailability, likely due to low solubility/first-pass metabolism.
P-glycoprotein Substrate	Non-substrate (Low confidence)	Substrate	Critical discrepancy: If PpIX is a P-gp substrate (per pkCSM), it may face efflux in the gut/liver, reducing bioavailability. Deep-pk's low-confidence prediction warrants caution.
P-glycoprotein Inhibitor	Non-inhibitor (Both I/II)	Non-inhibitor	Consensus: PpIX unlikely to cause P-gp-mediated drug-drug interactions.
Skin Permeability	3.10 log K_p (High)	2.73 log K_p (High)	Both agree on high skin penetration, supporting topical PpIX use (e.g., in photodynamic therapy).
MDCK Permeability	-5.43 log Papp	-	Corroborates Deep-pk's Caco-2 prediction, suggesting poor transcellular transport.

The discrepancy in Caco-2 permeability predictions between platforms (-5.49 vs. 0.169 log Papp) introduces uncertainty regarding epithelial permeability, though the negative MDCK permeability value from Deep-pk supports limited transcellular transport. Both platforms consistently predict high skin permeability (log Kp >2.7), which aligns with the successful clinical application of topical 5-aminolevulinic acid (5-ALA) for dermatological photodynamic therapy, where 5-ALA serves as a prodrug that is metabolically converted to PpIX within target tissues.

Distribution Parameters

The distribution characteristics of PpIX were assessed to understand its tissue penetration and protein binding properties, as shown in Table 3.

The distribution profile indicates that PpIX has a moderate volume of distribution (0.63-0.77 log L/kg), suggesting distribution beyond plasma into tissues. Both platforms predict significant protein binding, though with quantitative differences (1.62% vs. 11.7% unbound fraction). This high protein binding may limit free drug availability but could also provide a reservoir effect, prolonging circulation time. The blood-brain barrier penetration predictions are qualitatively consistent but differ in magnitude, with both platforms suggesting some degree of CNS distribution. This property could be relevant for potential applications in brain tumors, though the negative CNS permeability values indicate limited penetration.

Metabolism Parameters

The metabolism profile of PpIX was characterized through predictions of enzyme substrate specificity and inhibition potential, as presented in Table 4.

The metabolism results indicate that PpIX is not identified as a substrate for CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP3A4, and it is generally unlikely to be metabolized by these enzymes with high confidence. However, PpIX is identified as an inhibitor of CYP1A2, with predictions suggesting potential interactions with this enzyme. Conflicting predictions exist for the inhibition of CYP2C9 and CYP2C19. For CYP2D6 and CYP3A4, PpIX is recognized as a non-inhibitor. Additionally, PpIX may act as an inhibitor for BCRP, as well as OATP1B1 and OATP1B3, which could lead to drug interactions related to hepatic uptake and efflux.

Excretion Parameters

The excretion characteristics of PpIX were assessed to understand its elimination pathways and residence time in the body, as summarized in Table 5.

Table 3. Distribution Parameters of Protoporphyrin IX

Parameter	Deep-pk	pkCSM	Interpretation
Volume of Distribution (VDss)	0.77 log L/kg	0.632 log L/kg	Moderate tissue distribution
Fraction Unbound (Human)	1.62%	11.7%	Moderate to high protein binding
Plasma Protein Binding	40.59%	-	Moderate protein binding
Blood-Brain Barrier Penetration	Penetrable (High confidence)	Penetrable (log BB 1.648)	BBB penetration likely
CNS Permeability	-2.19 log BB	-2.687 log PS	Limited CNS permeability

Table 4. Metabolism Parameters of Protoporphyrin IX

Parameter	Deep-pk	pkCSM	Interpretation
CYP1A2 Substrate	Non-substrate (High confidence)	-	Not metabolized by *CYP1A2*
CYP2C9 Substrate	Non-substrate (High confidence)	-	Not metabolized by *CYP2C9*
CYP2C19 Substrate	Non-substrate (Medium confidence)	-	Likely not metabolized by *CYP2C19*
CYP2D6 Substrate	Non-substrate (High confidence)	Non-substrate	Not metabolized by *CYP2D6*
CYP3A4 Substrate	Non-substrate (High confidence)	Non-substrate	Not metabolized by *CYP3A4*
CYP1A2 Inhibitor	Inhibitor (High confidence)	Inhibitor	Consistent prediction of *CYP1A2* inhibition
CYP2C9 Inhibitor	Non-inhibitor (High confidence)	Inhibitor	Conflicting predictions
CYP2C19 Inhibitor	Inhibitor (Medium confidence)	Non-inhibitor	Conflicting predictions
CYP2D6 Inhibitor	Non-inhibitor (Low confidence)	Non-inhibitor	Consistent prediction of non-inhibition
CYP3A4 Inhibitor	Non-inhibitor (High confidence)	Non-inhibitor	Consistent prediction of non-inhibition
BCRP Inhibitor	Inhibitor (Medium confidence)	-	Potential interaction with *BCRP* transporter
OATP1B1 Inhibitor	Inhibitor (High confidence)	-	Likely interaction with hepatic uptake transporter
OATP1B3 Inhibitor	Inhibitor (Low confidence)	-	Possible interaction with hepatic uptake transporter

Table 5. Excretion Parameters of Protoporphyrin IX

Parameter	Deep-pk	pkCSM	Interpretation
Total Clearance	4.46 ml/min/kg	0.552 log ml/min/kg (3.57 ml/min/kg)	Moderate clearance rate
Half-Life	<3 hours (High confidence)	-	Short half-life
OCT2 Substrate	-	Non-substrate	Not affected by renal OCT2 transport
OCT2 Inhibitor	Non-inhibitor (High confidence)	-	No inhibition of renal cation transport

Table 6. Toxicity Parameters of Protoporphyrin IX

Parameter	Deep-pk	pkCSM	Interpretation
AMES Mutagenesis	Safe (High confidence)	Non-toxic	Consistent prediction of no mutagenicity
Maximum Tolerated Dose	-0.89 (log mg/kg)	0.654 (log mg/kg/day)	Moderate to low tolerated dose
hERG I Inhibition	Safe (High confidence)	Non-inhibitor	Consistent prediction of cardiac safety
hERG II Inhibition	-	Non-inhibitor	No cardiac toxicity predicted
Hepatotoxicity	Toxic (DILI I: High confidence, DILI II: Low confidence)	Non-toxic	Conflicting hepatotoxicity predictions
Skin Sensitization	Safe (Low confidence)	Non-sensitizer	Consistent prediction of low skin sensitization
Rat Acute Toxicity (LD50)	2.64 mol/kg	2.472 (mol/kg)	Consistent prediction of moderate acute toxicity
Rat Chronic Toxicity	4.21 log mg/kg	1.827 (log mg/kg_bw/day)	Moderate chronic toxicity
Carcinogenesis	Safe (High confidence)	-	No carcinogenic potential predicted
Respiratory Toxicity	Toxic (High confidence)	-	Potential respiratory concerns
T. Pyriformis Toxicity	-12047.48	0.285 (log µg/L)	Potential aquatic toxicity
Minnow Toxicity	30.31	2.285(log mM)	Mixed ecological toxicity profile

The excretion data indicates that PpIX has a moderate clearance rate (3.57-4.46 ml/min/kg) and short half-life (<3 hours), which aligns with clinical observations in photodynamic therapy where the photosensitivity window is relatively brief compared to other photosensitizers. The clearance values from both platforms are reasonably consistent. PpIX is not predicted to be a substrate or inhibitor of OCT2, suggesting that renal active secretion via this transporter is not a significant elimination pathway. The short half-life is advantageous for PDT applications as it reduces the duration of post-treatment photosensitivity, a common side effect of photodynamic therapy.

Toxicity Parameters

The toxicity profile of PpIX was comprehensively evaluated to assess potential adverse effects and safety concerns, as presented in Table 6.

The toxicity profile of PpIX presents a mixed picture. Both platforms consistently predict that PpIX is non-mutagenic (AMES negative) and lacks cardiotoxicity (hERG negative), suggesting safety in these critical aspects. However, there is significant discrepancy in hepatotoxicity predictions, with Deep-pk indicating potential for drug-induced liver injury (high confidence for DILI I) while pkCSM predicts no hepatotoxicity. Given the role of the liver in porphyrin metabolism and the known association between porphyrias and liver dysfunction, the Deep-pk prediction warrants consideration in clinical applications. The respiratory toxicity prediction from Deep-pk (high confidence) suggests potential pulmonary concerns that should be monitored, particularly in systemic applications. Both platforms predict moderate acute toxicity in rats with similar LD_{50} values (2.47-2.64 mol/kg), indicating consistency in this aspect of the toxicity profile.

Discussion

The pharmacokinetic and pharmacodynamic behaviors of Protoporphyrin IX (PpIX) as a photosensitizer in photodynamic therapy (PDT) are crucial for optimizing treatment efficacy. In this comparison, PpIX's absorption, distribution, metabolism, and excretion profiles were analyzed using two software tools, PKCSM and Deep-PK, which provided insights into its potential interactions and therapeutic window. The integration of these computational platforms enhances our understanding of PpIX's role in PDT and aids in the design of more effective therapeutic strategies.

Deep-pk predicts 98.2% human intestinal absorption (HIA) with high confidence, suggesting efficient uptake, possibly due to transporter-mediated processes. However, its Caco-2 permeability (-5.49 log Papp) indicates extremely low passive diffusion, conflicting with pkCSM's moderate prediction (0.169 log Papp). This discrepancy may arise from differences in how the models account for active transport or membrane interactions. Deep-pk's MDCK permeability (-5.43 log Papp) supports its low transcellular permeability prediction, whereas pkCSM's higher Caco-2 value aligns better with its 63.52% HIA estimate.

Both models agree that PpIX has low oral bioavailability, but Deep-pk explicitly labels it as "non-bioavailable," likely due to poor solubility or first-pass metabolism. The P-glycoprotein (P-gp) substrate prediction is highly conflicting. pkCSM classifies PpIX as a substrate, implying potential efflux in the gut/liver, while Deep-pk rejects this (albeit with low confidence). If pkCSM is correct, co-administering P-gp inhibitors (e.g., cyclosporine) could improve PpIX absorption—a hypothesis requiring experimental validation.

Both models predict high skin permeability (Deep-pk: 3.10 log Kp, pkCSM: 2.73 log Kp), supporting PpIX's use in topical therapies like photodynamic treatment for skin lesions. This consensus strengthens confidence in its dermal delivery potential.

Deep-pk predicts a VDss of 0.77 log L/kg (~5.9 L/kg linear), suggesting moderate-to-high tissue distribution, while pkCSM estimates 0.632 log L/kg (~4.3 L/kg linear), indicating slightly less extensive tissue penetration. The difference could stem from how each model accounts for PpIX's lipophilicity or tissue-

binding affinity. Deep-pk's higher value aligns better with PpIX's known accumulation in tissues like the liver and skin, which is relevant for photodynamic therapy applications.

Deep-pk reports only 1.62% of PpIX as unbound (implying 98.38% protein-bound), whereas pkCSM predicts 11.7% unbound (88.3% bound). This stark discrepancy (10-fold difference in free fraction) is critical for dosing and efficacy. Deep-pk's additional note of "40.59% plasma protein binding" contradicts its own fraction unbound value, suggesting a possible error in labeling or units. Experimental validation (e.g., equilibrium dialysis) is essential to resolve this conflict.

Both models agree PpIX can cross the BBB. Deep-pk labels it "penetrable" with high confidence, and pkCSM's log BB of 1.648 (brain/plasma ratio >1) suggests active uptake or weak efflux. This supports potential use in brain-targeted therapies (e.g., glioblastoma PDT) but raises neurotoxicity concerns. However, BBB penetrability does not guarantee effective CNS distribution. Deep-pk's log BB of -2.19 (~0.006 brain/plasma ratio) and pkCSM's log PS of -2.687 both indicate very low passive CNS permeability. This contrasts with their BBB penetrability predictions, implying that while PpIX may enter the brain, it likely fails to diffuse into deeper parenchyma. Transporter-mediated uptake (e.g., via OATP) might explain this divergence, warranting further study.

About the results of metabolism, Deep-pk demonstrates superior reliability for CYP substrate predictions (consistently high-confidence non-substrate calls for all CYPs, aligning with pkCSM's limited data) but reveals critical discrepancies in inhibition profiles. While both agree on CYP1A2 inhibition (clinically significant for drug interactions), Deep-pk's high-confidence non-inhibition calls for CYP2C9/CYP3A4 conflict with pkCSM's CYP2C9 inhibition prediction, suggesting Deep-pk may prioritize specificity (reducing false positives). However, pkCSM's lack of transporter data (e.g., OATP1B1/BCRP) limits its utility for hepatic uptake/efflux interactions—a domain where Deep-pk excels with detailed, confidence-ranked transporter inhibition predictions. For holistic ADME profiling, Deep-pk is preferable due to broader coverage (transporters, multi-enzyme consensus) and transparency in confidence metrics, though experimental validation (e.g., CYP2C9 IC50 assays) remains essential for conflicting results.

The comprehensive analysis of excretion parameters reveals a nuanced pharmacokinetic profile characterized by moderate clearance (4.46 mL/min/kg by Deep-pk, ~3.57 mL/min/kg by pkCSM) and a short half-life (<3 hours), suggesting rapid systemic elimination predominantly through hepatic metabolism. Both platforms consistently indicate the compound is not an OCT2 substrate or inhibitor, minimizing potential renal interaction risks and drug-drug interaction complexities. Deep-pk demonstrates superior predictive capabilities with high-confidence quantitative predictions, providing more granular insights into excretion mechanisms compared to pkCSM's limited data. The convergent predictions support a favorable pharmacokinetic profile with efficient elimination, reduced renal involvement, and minimal transporter-mediated interactions, though confirmatory experimental validation remains crucial for translating these computational predictions into clinical understanding.

The in silico toxicity assessment, employing Deep-pk and pkCSM, revealed a complex safety profile requiring rigorous validation. While both models consistently predicted a low risk of mutagenicity, carcinogenicity, and cardiac toxicity, significant discordance emerged regarding hepatotoxicity. Deep-pk predicted potential hepatotoxicity with high confidence, contrasting with pkCSM's non-toxic assessment, necessitating focused experimental investigation. Furthermore, both models suggested moderate acute and chronic toxicity, alongside a narrow therapeutic window indicated by Maximum Tolerated Dose predictions. The identification of potential respiratory toxicity and ecological concerns further underscores the need for comprehensive pre-clinical testing. The contrasting predictions between the two models emphasize the importance of multi-faceted computational approaches but also highlight the limitations of solely relying on in silico methods. These findings, therefore, necessitate a cautious interpretation, prioritizing experimental validation, particularly in the areas of hepatotoxicity, respiratory effects, and long-term systemic impacts, to refine the compound's safety profile and inform subsequent development decisions.

Perspective

The computational analysis of Protoporphyrin IX (PpIX) using PKCSM and Deep-pk reveals a complex pharmacokinetic profile with significant methodological discrepancies, highlighting both promising therapeutic potential and critical validation needs. While the models consistently suggest high skin permeability, potential brain-targeted applications, and low mutagenicity risks, they diverge substantially in key parameters like intestinal absorption, protein binding, and hepatotoxicity. The most striking findings include Deep-pk's 98.2% human intestinal absorption prediction, conflicting BBB penetration mechanisms, and contrasting hepatotoxicity assessments. These computational insights underscore the importance of a strategic, multi-tiered experimental validation approach, focusing on resolving model disagreements, investigating transporter-mediated interactions, and comprehensively assessing potential toxicity risks. Ultimately, the study demonstrates that computational models should be viewed as sophisticated hypothesis generators rather than definitive predictors, emphasizing the irreplaceable role of experimental validation in drug development, particularly for complex photosensitizers like PpIX in photodynamic therapy.

Conclusion

This study provides a valuable computational assessment of Protoporphyrin IX (PpIX) pharmacokinetics and pharmacodynamics, leveraging the strengths of both PKCSM and Deep-pk software to predict absorption, distribution, metabolism, and excretion (ADME) properties. The key findings highlight the potential of PpIX for topical applications due to its high skin permeability and suggest a moderate-to-high tissue distribution, which is beneficial for photodynamic therapy. However, discrepancies in predictions, particularly regarding oral bioavailability, protein binding, and hepatotoxicity, underscore the limitations of in silico approaches. These inconsistencies highlight the need for experimental validation, especially regarding P-gp interactions, protein binding, and potential liver toxicity, to refine dosing strategies and ensure patient safety. Future studies should focus on resolving these conflicting predictions through in vitro and in vivo experiments, including investigations into P-gp modulation, comprehensive liver function assessments, and detailed analysis of tissue distribution to further optimize PpIX-based photodynamic therapy protocols and enhance therapeutic outcomes.

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