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Balancing Favourable Drug-Likeness Against Respiratory and Renal Liabilities: An Integrated *In Silico* ADME–Toxicity Profile of a Novel 3{[2(Formimidoylamino)ethyl]sulfanyl}-6-(1-methoxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2ene-2-carbonyl fluoride

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Abstract

This study presents an integrated *in silico* assessment of 3-{[2-(Formimidoylamino)ethyl]sulfanyl}-6-(1-methoxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carbonyl fluoride using SwissADME and ProTox 3.0. Early drug discovery increasingly uses computational ADME and toxicity models to identify potential liabilities before committing experimental resources. However, computational outputs are model-dependent and should be interpreted as hypotheses. SwissADME calculated a molecular weight of 329.39 g/mol and topological polar surface area (TPSA) of 107.79 Å² and predicted a consensus logP of 1.14 and aqueous solubility estimates ranging from 1.72 to 7.59 mg/mL. High gastrointestinal absorption, absence of predicted blood-brain barrier permeation, and P-glycoprotein substrate status were predicted. The compound passed the Lipinski, Ghose, Veber, Egan, and Muegge filters in the SwissADME output; the platform reported no PAINS alerts and three Brenk alerts. ProTox 3.0 predicted an acute oral LD_{50} of 5000 mg/kg (toxicity Class 5), with an average similarity of 67.56 per cent and a reported prediction accuracy of 68.07 per cent. Respiratory toxicity and nephrotoxicity were predicted active with probabilities of 0.72 and 0.55, respectively, while clinical and nutritional toxicity were also predicted active. Most other reported organ-level endpoints, including hepatotoxicity, neurotoxicity and cardiotoxicity were predicted inactive within the tested model space. Tox21 pathway and molecular initiating event outputs were broadly inactive, and SwissADME predicted no inhibition of the major CYP isoforms assessed. These findings identify potentially useful drug-likeness and disposition signals but do not establish efficacy, safety, therapeutic suitability, or clinical risk-benefit. The highest-priority experimental questions are molecular identity, purity and stability. Broader CYP and toxicological investigations should then determine whether the computational signals persist under experimental conditions.

Key words: Acute oral toxicity, nephrotoxicity, pharmacokinetics, ProTox 3.0, respiratory toxicity.

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INTRODUCTION

This study presents an integrated *in silico* assessment of 3-{[2-(Formimidoylamino)ethyl]sulfanyl}-6-(1-methoxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carbonyl fluoride using SwissADME and ProTox 3.0. A recurring challenge in early pharmaceutical research is that a molecule can appear promising based on its chemical structure or preliminary biological hypotheses yet later fail because of poor solubility, inadequate exposure, unfavourable distribution, metabolic liabilities, or toxicity. Computational ADME and toxicity methods have consequently become useful first-pass approaches for prioritising compounds and guiding subsequent experimental studies. Their main value is hypothesis generation and prioritisation rather than replacing experimental pharmacology, pharmacokinetics, or toxicology.

SwissADME integrates physicochemical descriptors, aqueous solubility estimates, pharmacokinetic predictions, drug-likeness filters, and medicinal chemistry alerts within a unified computational workflow (Daina et al., 2017). ProTox 3.0 extends computational screening to acute oral toxicity, organ toxicity, toxicological endpoints, Tox21 pathways, molecular initiating events, and toxicity-associated targets using similarity-based and machine-learning approaches (Banerjee et al., 2024). The complementary scope of these platforms provides a practical framework for assessing whether a molecule shows convergent computational signals across exposure- and toxicity-related domains.

Interpreting such outputs requires particular caution. A calculated descriptor is not equivalent to a measured physicochemical property, a predicted pharmacokinetic classification is not equivalent to an observed human pharmacokinetic parameter, and a toxicity prediction is not equivalent to demonstrated organ injury. Accordingly, the present study treats every software-derived result as a computational observation unless independently established by experiment.

This study addresses a compound-specific knowledge gap. For this test compound, the available evidence in the present study is computational. No experimental dataset establishes its equilibrium solubility, permeability, transporter activity, CYP inhibition, tissue toxicity, pharmacokinetics, or therapeutic efficacy.

LITERATURE REVIEW

Computational ADME and Drug-Likeness

Drug-likeness filters are useful triage tools because physicochemical extremes are frequently associated with difficulties in absorption, permeability, and broader development performance. Lipinski et al. (2001) proposed widely used criteria involving molecular weight, lipophilicity, hydrogen-bond donors, and hydrogen-bond acceptors. Veber et al. (2002) subsequently emphasised rotatable bonds and polar surface area as complementary descriptors associated with oral bioavailability. Ghose et al. (1999), Egan et al. (2000), and Muegge et al. (2001) introduced additional descriptor-based or rule-based criteria for selecting drug-like chemical matter. These rule sets are complementary and should not be regarded as equivalent predictors of clinical performance. Passing a rule set does not establish adequate human exposure, efficacy, or safety.

SwissADME incorporates multiple filters, along with physicochemical calculations and medicinal chemistry alerts, allowing a compound to be assessed against several commonly used computational criteria in a single workflow (Daina et al., 2017). This integrated framework is useful for prioritisation but remains dependent on the definitions and assumptions underlying each embedded model.

Solubility, Permeability, and Transporter-Mediated Disposition

Aqueous solubility can influence dissolution and the amount of compound available for absorption. The ESOL model estimates aqueous solubility from molecular descriptors and provides a rapid structure-based screening estimate (Delaney, 2004). SwissADME additionally reports alternative estimates from the Ali and SILICOS-IT models (Daina et al., 2017). Agreement between models can strengthen prioritisation, whereas divergence can highlight uncertainty. None of these predictions replaces experimentally measured equilibrium solubility because observed solubility may depend on pH, ionisation, solid-state properties, polymorphism, aggregation, and chemical degradation.

Permeability and intestinal efflux should likewise be interpreted jointly. P-glycoprotein is an important transporter affecting intestinal disposition and systemic exposure, and computational classification of a compound as a P-glycoprotein substrate may indicate a potential efflux liability rather than an experimentally

demonstrated transport phenotype (Tanaka et al., 2016; Zakeri-Milani & Valizadeh, 2014).

The BOILED-Egg model provides a related computational framework for estimating gastrointestinal and brain permeation from molecular lipophilicity and polarity. It was developed as a predictive screening model for intestinal absorption and brain access, not as a replacement for experimental permeability or pharmacokinetic studies (Daina & Zoete, 2016). Consequently, predicted gastrointestinal absorption and predicted absence of brain penetration should both be interpreted as screening signals.

CYP-mediated Disposition and Drug Interaction Potential

Cytochrome P450 (CYP) enzymes are responsible for the metabolism of a large proportion of clinically used small-molecule drugs. They are an important source of interindividual variability in drug disposition (Zanger & Schwab, 2013). CYP inhibition can also contribute to clinically relevant drug-drug interactions; assessing CYP inhibition is an established component of drug development and safety evaluation (Guengerich, 2022).

Computational CYP inhibition predictions can therefore identify compounds that warrant biochemical testing. However, an inactive computational prediction does not demonstrate the absence of enzyme inhibition. Experimental interpretation should consider the relevant isoform, concentration range, mechanism of inhibition, reversibility, metabolic stability, and clinically relevant exposure. A tiered strategy in which computational screening precedes biochemical, microsomal, hepatocyte, and, where necessary, clinical investigation is therefore more appropriate than treating a computational negative as definitive evidence of negligible interaction potential.

Toxicity Prediction and Structural Alerts

Structural alert systems and predictive toxicology platforms are most useful when treated as prioritisation frameworks. Brenk and colleagues developed unwanted substructure criteria to support screening library selection and identification of potentially problematic chemical motifs (Brenk et al., 2008). PAINS filters were subsequently developed to identify substructures associated with assay interference and promiscuous screening behaviour (Baell & Holloway, 2010).

Neither a Brenk alert nor a PAINS classification demonstrates toxicity. Rather, the results identify structural features that may warrant chemical or assay-related scrutiny. In this study, we therefore interpret these alerts as chemical risk signals requiring follow-up rather than evidence of adverse biological activity.

ProTox 3.0 provides a wider toxicity prediction framework covering acute oral toxicity, organ-level endpoints, molecular initiating events, toxicological pathways, and toxicity-associated targets (Banerjee et al., 2024). Such outputs can help prioritise experimental questions, but they do not establish dose-response relationships, receptor binding, tissue exposure, or adverse outcomes in vivo.

Knowledge Gap and Rationale

Taken together, the literature supports computational ADME and toxicity methods as efficient tools for early compound triage, while also demonstrating why computational outputs must remain distinct from experimental evidence. For the present compound, the major unresolved questions concern the relationship between predicted solubility and intestinal exposure, the significance of P-glycoprotein-mediated efflux, the chemical stability of the carbonyl fluoride and imine-containing structure, and the biological relevance of predicted respiratory and renal toxicity. An integrated SwissADME and ProTox 3.0 assessment is therefore warranted not to establish a definitive development profile, but to map these uncertainties and provide a rational sequence for experimental validation.

METHODOLOGY

This study used a descriptive, single-compound computational design for early-stage pharmaceutical profiling. We performed no laboratory experiments, animal studies, or clinical investigations. The compound was the sole unit of analysis; population-based sampling and inferential statistical testing were therefore not applicable. The investigated compound was 3-{[2-(Formimidoylamino)ethyl]sulfanyl}-6-(1-methoxyethyl)-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2 carbonyl fluoride, with reported molecular formula $C_{14}H_{20}FN_3O_3S$ and molecular weight 329.39 g/mol.

The chemical structure was drawn using PubChem Sketcher through the National Centre for Biotechnology Information PubChem interface and exported as a SMILES representation. The original structure drawing was intended to retain defined stereochemical information. Where stereochemical centres were encoded, the original isomeric representation containing

explicit stereochemical descriptors should be retained as part of the supplementary raw data to support reproducibility. The supplied SwissADME report records the corresponding canonical SMILES as CC1C2C(C(OC)C)C(=O)N2C(=C1SCCNC=N)C(=O)F. Because the supplied report reproduces the canonical representation rather than the full isomeric input string, the exact original stereochemical sequence is not independently recoverable from the report. Consequently, the original isomeric SMILES file should be retained with the supplementary dataset.

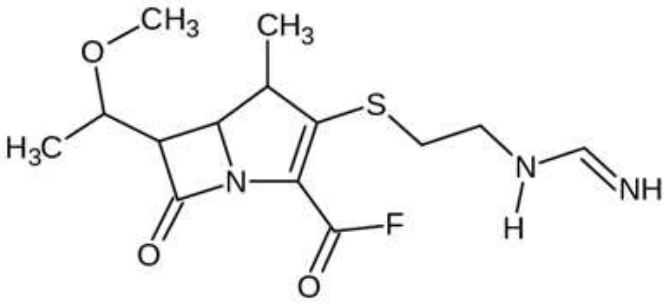

Figure 1. Chemical Structure of the Test Compound

We performed in silico ADME and drug-likeness profiling using the SwissADME web platform developed by the Swiss Institute of Bioinformatics (Daina et al., 2017). The platform was accessed at http://www.swissadme.ch. The supplied report did not specify the platform version. All predictions were executed on February 3, 2025, at 20:10 East Africa Time (EAT; UTC+3). Default server calculation parameters were retained. The analysis included molecular and physicochemical descriptors, consensus and individual logP estimates, aqueous solubility estimates from the ESOL, Ali, and SILICOS-IT models, gastrointestinal absorption, blood-brain barrier permeation, P-glycoprotein substrate status, skin permeation, CYP inhibition, oral bioavailability score, Lipinski, Ghose, Veber, Egan and Muegge filters, PAINS and Brenk alerts, lead-likeness, and synthetic accessibility.

We treated the BOILED-Egg permeability interpretation as predictive rather than experimental, consistent with its original development as a computational model for gastrointestinal and brain permeation (Daina & Zoete, 2016). We treated all descriptors and model outputs as computational results unless explicitly identified as experimental. This study included no experimental measurements.

We generated toxicity predictions using ProTox 3.0 (Banerjee et al., 2024), accessed at https://tox.charite.de/protox3/. We accessed the platform on February 3, 2025, at 20:10 East Africa Time (EAT; UTC+3) and retained the default calculation parameters. Extracted outputs comprised predicted acute oral LD_{50} and toxicity class; organ-specific and general toxicity endpoints; Tox21 nuclear receptor and stress response pathways; molecular initiating events; CYP-related endpoints; and predicted toxicity-associated targets. We recorded average similarity and reported prediction accuracy exactly as the platform displayed. We treated these values as characteristics of the model output, not as experimental confidence intervals or compound-specific experimentally demonstrated accuracy.

Endpoint selection followed the study objectives and the outputs available from the two computational platforms. For SwissADME, we interpreted drug-likeness against the platform-reported rule sets, while treating solubility, pharmacokinetic parameters, transporter status, and CYP predictions as computational estimates or classifications. For ProTox 3.0, endpoint labels of Active or Inactive and the associated probabilities were recorded as reported. We reported the predicted acute oral toxicity class and LD_{50} similarly, without reclassification. No computational label was converted into a confirmed toxicological, pharmacological, or clinical conclusion.

We extracted results into structured tables and compared them descriptively across domains. Where independent models or platforms generated conceptually related outputs, we described convergence or divergence without implying experimental validation. No inferential statistical analysis was performed. We retained numerical probabilities and similarity measures, and reported model accuracy values, as characteristics of the computational

outputs. The principal interpretation criterion was therefore whether a prediction identified a testable hypothesis or a priority uncertainty for subsequent laboratory investigation. We designate the complete raw SwissADME and ProTox 3.0 output files as Supplementary Tables S1 and S2. The original isomeric SMILES input should also be retained as part of the supplementary raw data.

FINDINGS AND DISCUSSION

Molecular and Physicochemical Characteristics

Table 1. Calculated Molecular and Physicochemical Descriptors from SwissADME

Parameter	Reported value
Molecular formula	$C_{14}H_{20}FN_3O_3S$
Molecular weight	329.39 g/mol
Heavy atoms	22
Aromatic heavy atoms	0
Fraction Csp3	0.64
Rotatable bonds	8
H-bond acceptors	5
H-bond donors	2
Molar refractivity	87.04
TPSA	107.79 Å²
Consensus logP	1.14
Ilogp	1.76
XLOGP3	0.45
WLOGP	1.18
MLOGP	0.94
SILICOS-IT logP	1.39

The SwissADME output described a nonaromatic molecule with a relatively high fraction of sp3 hybridised carbon atoms (fraction Csp3 = 0.64), eight rotatable bonds, five hydrogen-bond acceptors, and two hydrogen-bond donors. The consensus logP was predicted as 1.14, with individual logP estimates ranging from 0.45 to 1.76. These values are computational descriptors and were not experimentally measured in the present study.

Predicted Aqueous Solubility

Table 2. Predicted Aqueous Solubility from Three SwissADME Models

Model	Log S	Predicted solubility	Molar solubility	Class
ESOL	−1.64	7.59 mg/mL	0.0230 mol/L	Very soluble
Ali	−2.28	1.72 mg/mL	0.00523 mol/L	Soluble
SILICOS-IT	−2.26	1.80 mg/mL	0.00547 mol/L	Soluble

All three SwissADME models predicted soluble-range behaviour, with estimated concentrations from 1.72 to 7.59 mg/mL. The ESOL estimate was more favourable than the Ali and SILICOS-IT estimates, whereas the latter two were closely aligned. These values should be regarded as calculated estimates rather than experimental equilibrium solubilities. pH, ionisation state, solid-state properties, polymorphism, aggregation, and chemical degradation may alter experimentally observed solubility.

Predicted Pharmacokinetic Characteristics

Table 3. SwissADME Predicted Pharmacokinetic Characteristics

ADME parameter	Prediction
Gastrointestinal absorption	High
Blood-brain barrier permeation	No
P-glycoprotein substrate	Yes
Skin permeation, log Kp	−7.99 cm/s
CYP1A2 inhibition	No
CYP2C19 inhibition	No
CYP2C9 inhibition	No
CYP2D6 inhibition	No
CYP3A4 inhibition	No
Bioavailability score	0.55

SwissADME predicted high gastrointestinal absorption and no blood-brain barrier permeation. SwissADME predicted P-glycoprotein substrate status as positive, indicating a potential efflux liability. The bioavailability score of 0.55 is a platform-derived indicator and should not be interpreted as a measured fraction absorbed or measured systemic bioavailability. No inhibition was predicted for CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4.

Drug-Likeness and Medicinal Chemistry Predictions

Table 4. SwissADME Drug-Likeness Filters and Medicinal Chemistry Outputs

Criterion	Reported result
Lipinski filter	Pass; 0 violations
Ghose filter	Pass; 0 violations
Veber filter	Pass; 0 violations
Egan filter	Pass; 0 violations
Muegge filter	Pass; 0 violations
Bioavailability score	0.55
PAINS alerts	0
Brenk alerts	3: acid halide, imine_1, imine_2
Lead-likeness	1 violation: rotatable bonds >7
Synthetic accessibility	4.76/10

The compound passed all five reported drug-likeness filters. These results indicate compliance with the corresponding computational criteria, not demonstrated oral performance or therapeutic suitability. Three Brenk alerts were reported for acid-halide, imine_1, and imine_2 motifs. One lead-likeness violation was attributed to eight rotatable bonds. The synthetic accessibility score of 4.76/10 is the platform's computational estimate, not an experimentally demonstrated measure of synthetic difficulty (Brenk et al., 2008; Daina et al., 2017).

Predicted Acute Oral Toxicity

Table 5. ProTox 3.0 Predicted Acute Oral Toxicity Output

Metric	Reported result
Predicted oral LD_{50}	**5000 mg/kg**
Toxicity class	**Class 5**
Average similarity	**67.56%**
Prediction accuracy	**68.07%**

ProTox 3.0 predicted an oral LD_{50} of 5000 mg/kg and placed the compound in toxicity Class 5. The platform reported an average similarity of 67.56 per cent and prediction accuracy of 68.07 per cent. These values describe the computational output and its associated model context. They do not establish an experimentally measured LD_{50} or confirm low acute oral toxicity in vivo (Banerjee et al., 2024).

Organ-Specific and General Toxicity Predictions

Table 6. ProTox 3.0 Predicted Organ-Specific and General Toxicity Endpoints

Endpoint	Classification	Probability
Hepatotoxicity	Inactive	0.69
Neurotoxicity	Inactive	0.63
Nephrotoxicity	Active	0.55
Respiratory toxicity	Active	0.72
Cardiotoxicity	Inactive	0.69
Carcinogenicity	Inactive	0.58
Immunotoxicity	Inactive	0.79
Mutagenicity	Inactive	0.59
Cytotoxicity	Inactive	0.62
BBB barrier	Inactive	0.66
Ecotoxicity	Inactive	0.61
Clinical toxicity	Active	0.53
Nutritional toxicity	Active	0.55

The strongest organ-level positive prediction was respiratory toxicity (0.72), followed by nephrotoxicity (0.55). Clinical toxicity and nutritional toxicity were also classified as active. The model predicted hepatotoxicity, neurotoxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity, BBB-barrier toxicity, and ecotoxicity as inactive within the tested model space. An inactive classification does not mean the corresponding hazard is experimentally absent.

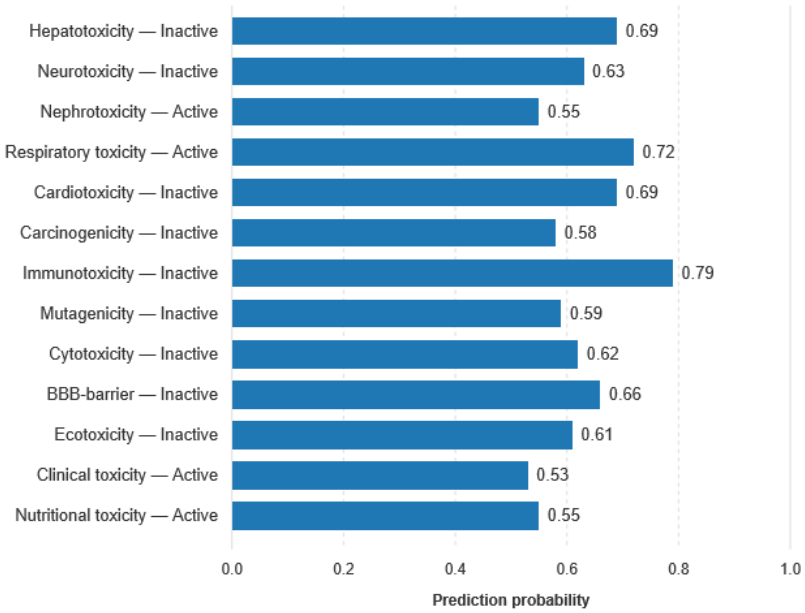

Figure 2. ProTox 3.0 Prediction Probabilities for the Reported Toxicity Endpoints. Values Represent Computational Probabilities Associated with the Classifications Reported in Table 6

Tox21 Pathways and Molecular Initiating Events

All listed Tox21 nuclear receptor pathways were predicted inactive, including aryl hydrocarbon receptor, androgen receptor, androgen receptor ligand-binding domain, aromatase, estrogen receptor alpha, estrogen receptor ligand-binding domain, and PPAR-gamma. Stress-response pathways, including nrf2/ARE, heat-shock factor response element, mitochondrial membrane potential, p53, and ATAD5, were likewise predicted inactive. Reported molecular initiating events, including THRα, THRβ, transthyretin, ryanodine receptor, GABA receptor, NMDA receptor, AMPA receptor, kainate receptor, acetylcholinesterase, constitutive androstane receptor, pregnane X receptor, NADH-quinone oxidoreductase, voltage-gated sodium channel, and Na^+/I^- symporter, were also predicted inactive. These findings indicate limited predicted activity within the particular modelled pathways and endpoints. They do not establish the absence of receptor binding, pathway perturbation, or downstream biological effects experimentally.

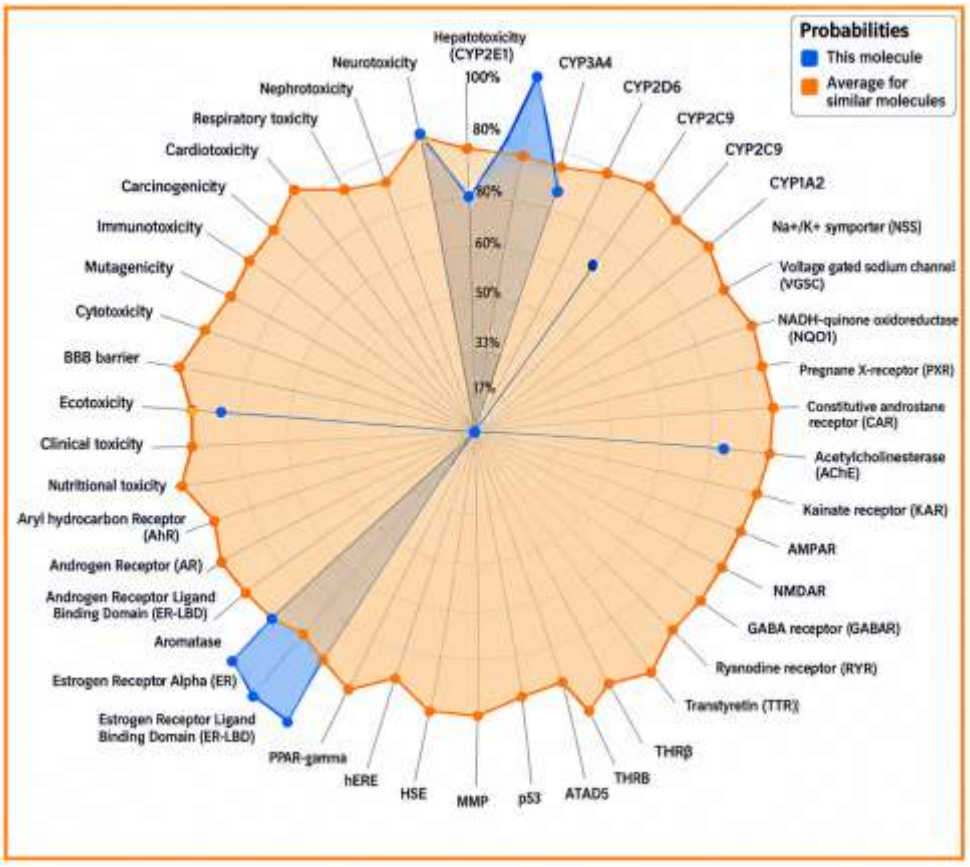

Figure 3. ProTox 3.0 Radar Visualisation of Predicted Endpoint Probabilities and Comparison with Average Probabilities for Similar Molecules. (The visualisation is a computational screening output and is not a substitute for experimental mechanistic investigation)

Discussion

Physicochemical Profile and Drug-Likeness

The SwissADME outputs place the compound within several commonly screened physicochemical ranges. Its calculated molecular weight of 329.39 g/mol is below the 500 g/mol criterion used in the Lipinski framework (Lipinski et al., 2001). The consensus logP of 1.14 represents a computational estimate of lipophilicity rather than an experimentally measured partition coefficient. The calculated TPSA of 107.79 Å² and eight rotatable bonds indicate a molecule combining relatively high polarity with moderate conformational flexibility. The compound passed the Lipinski, Ghose, Veber, Egan, and Muegge filters, which provides evidence of compliance with several computational drug-likeness criteria (Ghose

et al., 1999; Egan et al., 2000; Lipinski et al., 2001; Muegge et al., 2001; Veber et al., 2002).

However, passing these rules should not be interpreted as evidence that the compound will achieve adequate exposure, oral efficacy, or acceptable clinical safety. Drug-likeness filters are deliberately simplified screening frameworks and do not capture all determinants of absorption, metabolism, tissue distribution, target engagement, or toxicity. The lead-likeness result provides a useful complementary observation. The single lead-likeness violation resulted from more than seven rotatable bonds, suggesting that conformational flexibility may be less favourable under that particular rule set. This is a design consideration, not an indication that the compound lacks pharmaceutical potential.

Solubility and Chemical Stability

The three solubility models produced qualitatively consistent soluble-range predictions, although the ESOL value was substantially more favourable than those obtained from Ali and SILICOS-IT. Such agreement provides a reasonable screening signal but cannot substitute for experimental measurement (Delaney, 2004; Daina et al., 2017). The compound contains a formimidoylamino functionality that may contribute to pH-dependent ionisation and therefore potentially influence apparent solubility. Independent of predicted solubility, the presence of an acid-halide-related functionality raises an important chemical stability question. The Brenk acid-halide alert aligns with this structural feature and indicates that degradation should be characterised before interpreting biological observations (Brenk et al., 2008). The practical implication is that the apparent concentration of intact parent compound should be distinguished from total analytical material during subsequent solubility studies. pH-solubility measurements should therefore be accompanied by analytical stability assessment.

Predicted Gastrointestinal Absorption, P-Glycoprotein, and BBB Behaviour

The combination of high predicted gastrointestinal absorption, negative predicted BBB permeation, and positive P-glycoprotein substrate status illustrates the importance of interpreting ADME outputs collectively. The BOILED-Egg model was specifically designed to predict gastrointestinal and brain permeation using molecular polarity and lipophilicity descriptors (Daina & Zoete, 2016). Thus, the SwissADME classification of high gastrointestinal absorption should be interpreted as a computational estimate rather than a measured fraction absorbed.

Similarly, P-glycoprotein substrate status represents a predicted transporter phenotype. P-glycoprotein-mediated efflux may reduce net intestinal uptake and influence systemic exposure, making experimental bidirectional transport studies particularly informative (Tanaka et al., 2016; Zakeri-Milani & Valizadeh, 2014). The most appropriate next step is therefore experimental permeability and transporter testing using a relevant epithelial model**.**

CYP Inhibition and Metabolic Uncertainty

No inhibition was predicted for CYP1A2, CYP2C19, CYP2C9, CYP2D6, or CYP3A4 in SwissADME. The ProTox 3.0 output also included inactive CYP-related endpoints, including CYP2E1. This computational pattern is potentially reassuring as an initial screening observation, because CYP enzymes play major roles in drug metabolism and CYP inhibition can contribute to clinically important drug-drug interactions (Zanger & Schwab, 2013; Guengerich, 2022).

Nevertheless, a computational negative should be regarded as a provisional negative prediction. It does not establish the absence of biochemical inhibition, mechanism-based inhibition, time-dependent inhibition, or clinically relevant interaction potential. The present results therefore support subsequent CYP testing once the higher-priority uncertainties surrounding molecular stability, permeability, renal toxicity, and respiratory toxicity have been addressed.

Structural Alerts and Chemical Liabilities

No PAINS alerts were reported, reducing concern about common assay-interference patterns identified by the PAINS framework (Baell & Holloway, 2010). This result should not, however, be interpreted as broad evidence of biological specificity. We identified three Brenk alerts: acid halide, imine_1, and imine_2. These alerts identify structural motifs that warrant chemical scrutiny rather than demonstrating toxicity (Brenk et al., 2008). For the present compound, the structural alerts are particularly important because degradation could generate products with biological properties that differ from those of the nominal parent structure. Stability testing should therefore precede interpretation of biological assays wherever feasible.

Respiratory and Renal Toxicity Predictions

The strongest organ-specific signal in the ProTox 3.0 output was predicted respiratory toxicity (probability 0.72). Predicted nephrotoxicity followed with a probability of 0.55. These findings should not be interpreted as evidence that the compound causes lung or kidney injury. Rather, they represent model-derived prioritisation signals. Practically, the results suggest focusing experimental validation on respiratory and renal systems.

Targeted studies should establish concentration-response relationships and distinguish general cytotoxicity from organ-relevant biological effects. Appropriate endpoints may include cell viability, membrane integrity, mitochondrial function, oxidative stress, and inflammatory responses, depending on the experimental system selected.

The predicted inactivity of hepatotoxicity, neurotoxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity should likewise be interpreted cautiously. An inactive computational classification can reduce an endpoint's priority relative to stronger positive signals, but it cannot establish the absence of an experimentally relevant hazard.

Acute Oral Toxicity Prediction

The predicted oral LD_{50} of 5000 mg/kg and Class 5 classification provide a computational indication of relatively low acute oral toxicity within the ProTox model framework. Nevertheless, the result is not an experimentally determined LD_{50}. The reported average similarity of 67.56 per cent and prediction accuracy of 68.07 per cent further emphasise the need to interpret this endpoint within its model context (Banerjee et al., 2024). Accordingly, acute toxicity predictions should not be used alone to label a compound safe. Chronic toxicity, organ-specific toxicity, metabolite-mediated toxicity, exposure-related toxicity, and effects at pharmacologically relevant concentrations remain unresolved.

Integrated Interpretation

Table 7. Integrated Interpretation of Computational Evidence and Priority Uncertainties

Domain	Computational finding	Interpretive status	Priority for validation
Oral exposure	High predicted GI absorption; estimated solubility 1.72 to 7.59 mg/mL	Screening signal only	High
BBB exposure	BBB permeation predicted negative	Screening signal only	Moderate
Efflux disposition	P-glycoprotein substrate predicted	Potential liability	High
Metabolic inhibition	Major CYP inhibition endpoints predicted negative	Provisional negative prediction	Later tier
Acute oral toxicity	Predicted LD_{50} 5000 mg/kg; Class 5	Model estimate only	Later tier
Respiratory toxicity	Active prediction; probability 0.72	Priority toxicity flag	Highest
Renal toxicity	Active prediction; probability 0.55	Priority toxicity flag	Highest
Structural alerts	Acid halide, imine_1, imine_2	Chemical risk signal	Highest
Lead-likeness	One violation because of eight rotatable bonds	Design consideration	Moderate

The integrated evidence does not establish a demonstrated risk-benefit balance or development profile. Rather, it reveals a mixed computational picture. On the positive side, the compound satisfies several commonly used drug-likeness filters, shows no PAINS alerts, has predicted soluble-range behaviour, and has no predicted inhibition of the major CYP isoforms evaluated. On the liability side, P-glycoprotein substrate status, three Brenk structural alerts, and predicted respiratory and renal toxicity remain unresolved. The appropriate conclusion is therefore neither that the compound is promising because it passes multiple drug-likeness filters nor that it is unsuitable because of the predicted toxicity signals. Instead, the combined output supports hypothesis-driven experimental investigation.

Priority Experimental Validation

The computational findings support a sequential experimental validation programme. The priority is confirming molecular identity, stereochemical representation, purity, and chemical stability before drawing biological conclusions. Consider orthogonal analytical methods such as ^{1}H and ^{13}C NMR spectroscopy, high-resolution mass spectrometry, and

chromatographic purity assessment. Where stereochemistry is relevant, reconcile the analytical evidence with the original isomeric structural representation used for computational modelling.

The second priority is determination of pH-dependent solubility and chemical degradation. Measure equilibrium or kinetic solubility across physiologically relevant pH values, with simultaneous analytical monitoring of parent compound integrity. This will establish whether apparent solubility changes reflect true dissolution behaviour or chemical transformation.

The third priority is to evaluate permeability and P-glycoprotein using an appropriate epithelial model, such as Caco-2 or MDCK-MDR1. Use bidirectional transport and transporter inhibition experiments where appropriate. These experiments will directly test whether the predicted P-glycoprotein substrate behaviour is biologically observable.

The fourth priority is targeted respiratory and renal toxicity assessment because these are the strongest positive organ-level predictions. Suitable pulmonary and renal cell systems may be evaluated using concentration-response studies and endpoints selected to distinguish direct cytotoxicity from organ-specific pathway effects.

The fifth stage should address metabolic disposition and drug interaction potential. Recombinant CYP or microsomal studies, metabolic stability experiments, hepatocyte investigations, and metabolite identification can be performed after resolving foundational identity, stability, permeability, and organ toxicity questions.

Any progression to in vivo studies should be based on the resulting experimental evidence and conducted in accordance with applicable ethical, institutional, and regulatory requirements.

Limitations of the Study

The study is entirely computational and contains no experimental measurements of pharmacokinetics, equilibrium solubility, permeability, receptor binding, metabolism, toxicity, tissue exposure, or therapeutic efficacy. The predictive performance of SwissADME and ProTox 3.0 depends on their descriptors, algorithms, training data, and applicability domains. The performance characteristics of a platform across a broad chemical space cannot therefore be assumed to represent the exact uncertainty associated with an individual compound (Daina et al., 2017; Banerjee et al., 2024).

The analysis also combines outputs from computational platforms whose descriptor definitions, model architectures, and training spaces are not necessarily identical. Consequently, we interpreted apparent agreement across outputs only as qualitative convergence. The ProTox 3.0 probability values, average similarity, and reported prediction accuracy were treated as model characteristics rather than experimental confidence intervals.

The study also does not include target-based pharmacology, molecular docking, molecular dynamics, quantum chemical calculations, experimental structural confirmation, metabolite profiling, or in vivo studies. These omissions limit mechanistic inference and reinforce the hypothesis-generating role of the present analysis. Finally, the absence of experimental data means that the computationally predicted respiratory and renal signals remain unresolved hypotheses. These signals should neither be ignored nor described as confirmed toxicity until validated experimentally.

CONCLUSION

This study provides an integrated computational profile of the test compound using SwissADME and ProTox 3.0. SwissADME calculated a molecular weight of 329.39 g/mol, TPSA of 107.79 Å^2, and consensus logP of 1.14 and predicted soluble-range aqueous behaviour, high gastrointestinal absorption, no predicted BBB permeation, and P-glycoprotein substrate status. The compound passed the Lipinski, Ghose, Veber, Egan, and Muegge filters and produced no PAINS alerts but three Brenk alerts. ProTox 3.0 predicted an acute oral LD_{50} of 5000 mg/kg corresponding to Class 5 and identified respiratory and renal toxicity as the principal positive organ-level signals. The model predicted other toxicity endpoints as inactive within the tested model space.

These findings should be interpreted as computational evidence for experimental prioritisation, not as experimentally established physicochemical, pharmacokinetic, toxicological, or therapeutic properties. The most important next steps are confirming molecular identity and purity, characterising chemical stability, determining pH-dependent solubility, measuring experimental permeability and P-glycoprotein activity, and targeting respiratory and renal toxicity. Broader CYP and toxicological studies should subsequently determine

whether the computational findings persist under experimental conditions. On the current evidence alone, the compound should not be described as safe, therapeutically suitable, clinically effective, or development-ready. Its main value at this stage is providing a clearly defined set of experimentally testable hypotheses.

Data Availability

All numerical results reported in this manuscript were derived from the SwissADME and ProTox 3.0 computational outputs supplied for the compound. The study does not introduce new experimental data. The complete raw SwissADME output and ProTox 3.0 toxicity output should be deposited as Supplementary Data (Tables S1 and S2), along with the original isomeric SMILES input file and, where possible, the original structure drawing used to generate the computational representation.

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