

## Confidence-Aware *In Silico* ADME–Toxicity Profiling of 3,4-dithia-1-azabicyclo[3.2.0]heptan-7-one: Integrating Drug-Likeness, Pharmacokinetics, and Prediction Confidence

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### Abstract

This study evaluated the ADME and toxicity characteristics of the compound using a confidence-aware, single-compound in silico framework. The compound was evaluated from supplied SwissADME and ProTox 3.0 reports, with emphasis on cross-endpoint consistency and the evidential weight of confidence-related indicators. The predicted physicochemical profile was generally favourable with moderate polarity, low-to-mild calculated lipophilicity and consistently favourable predicted aqueous solubility. High predicted gastrointestinal absorption, absence of predicted P-glycoprotein substrate behaviour and negative predictions for CYP1A2, CYP2C19, CYP2C9, CYP2D6 and CYP3A4 inhibition supported a generally favourable preliminary computational ADME profile. The compound passed Lipinski, Veber and Egan filters but failed Ghose, Muegge and lead-likeness criteria largely because of its unusually small size; a disulfide-associated structural alert identified chemical reactivity as a specific uncertainty. ProTox 3.0 estimated an acute oral LD<sub>50</sub> of 500 mg/kg (class 4). Still, the reported ProTox 3.0 compound-specific prediction-accuracy indicator was 23 per cent and the reported average structural similarity to reference compounds was 31.74 per cent. Materially, limiting confidence in the point estimate ProTox 3.0 returned positive classifications for neurotoxicity, respiratory toxicity, BBB-related activity and ecotoxicity, while clinical toxicity was borderline at the reported probability threshold, whereas several other organ and pathway endpoints were inactive. The integrated computational profile supports consideration of the compound for targeted experimental validation for experimental validation rather than a definitive safety or development conclusion. The principal contribution is a confidence-aware evidence framework that converts computational agreement and discordance into explicit experimental decision questions; no analogue generation, molecular optimisation, docking, molecular dynamics or SAR modelling was performed.

**Key terms:** Acute oral toxicity, applicability domain, computational toxicology, disulphide, drug-likeness.

## 1.0 INTRODUCTION

This study reports a confidence-aware, single-compound *in silico* ADME–toxicity profile of 3,4-dithia-1-azabicyclo[3.2.0]heptan-7-one (C<sub>4</sub>H<sub>5</sub>NOS<sub>2</sub>; molecular weight 147.22 g/mol), a compact bicyclic scaffold comprising a β-lactam and ring-embedded disulfide. Computational ADME and toxicity assessment is widely used in early-stage drug-discovery workflows in early drug-discovery workflows because it can identify physicochemical, pharmacokinetic and safety liabilities before resources are committed to extensive synthesis and biological testing. Nevertheless, the usefulness of an *in silico* result depends not only on the apparent quality of the prediction but also on the model's provenance, the chemical space represented in its training data, the endpoint definition, and the uncertainty associated with the query molecule. Recent ADMET modelling literature increasingly emphasises prediction reliability, calibration and applicability-domain assessment when interpreting molecular predictions rather than optional reporting details (Catacutan et al., 2024; Venkataraman et al., 2025; Parrondo-Pizarro et al., 2026).

The compound investigated in this study, the test compound, was selected as a chemically distinctive test case because it combines a highly compact bicyclic β-lactam scaffold with a ring-embedded disulfide. The supplied structure, represented by the isomeric SMILES O=C1C[C@@H]2N1CSS2, is non-aromatic, rigid and unusually small (147.22 g/mol). At the same time, its sulfur-containing ring introduces a chemical-reactivity dimension that size and lipophilicity alone do not capture. β-Lactam-containing bicyclic systems are well established in medicinal chemistry, and sulfur-containing heterocycles continue to contribute substantially to modern therapeutics; however, the exact parent scaffold has not been extensively characterised through an integrated ADME–toxicity framework in the literature identified for this study.

Its low molecular weight places the molecule within a fragment-scale chemical space that warrants interpretation using both fragment and conventional drug-discovery criteria. Fragment-based drug discovery deliberately exploits low-molecular-weight structures because they can sample chemical space efficiently and provide tractable starting points for later growing, linking or merging. Importantly, fragment selection is a multidimensional decision that includes physicochemical properties, chemical tractability, binding behaviour and ADMET considerations; failure of a generic size-based filter does not, by itself, establish poor suitability for further investigation (Erlanson et al., 2016; Yoo et al., 2025; Grosjean & Biggin, 2026).

An integrated, confidence-aware analysis is therefore justified because no single endpoint can capture the development relevance of this compact sulfur-containing scaffold. Solubility and gastrointestinal absorption address exposure potential; permeability and efflux address distribution; CYP predictions address possible metabolic interaction; structural alerts and sulfur reactivity address chemical liability; and acute, organ-level, pathway-level and target-associated toxicity predictions address complementary safety questions. Interpreting these outputs jointly is more informative than treating any one prediction as a stand-alone verdict, particularly when discordant endpoints identify uncertainty that should be resolved experimentally.

The study objectives were consequently to (i) characterise the predicted physicochemical, solubility, pharmacokinetic and drug-likeness profile of the compound; (ii) integrate predicted acute, organ-level, pathway-level and target-associated toxicity endpoints with the reported confidence

indicators; and (iii) translate the combined evidence into a transparent compound-prioritisation framework that distinguishes favourable attributes, liabilities and unresolved questions and specifies an appropriate sequence for experimental validation. The intended contribution is therefore a confidence-weighted in silico evidence synthesis to prioritise a single compound, not de novo molecular design, analogue optimisation, or establishment of a validated structure–activity relationship.

## 2.0 LITERATURE REVIEW

### Computational ADME, AI/ML Prediction and Endpoint Integration

Rule-based drug-likeness filters remain useful first-line tools for evaluating oral-absorption potential, and frameworks derived from molecular size, lipophilicity, polarity and flexibility remain embedded in many screening workflows (Lipinski et al., 2001; Ghose et al., 1999; Veber et al., 2002; Egan et al., 2000; Muegge et al., 2001). SwissADME integrates several such criteria with consensus physicochemical and pharmacokinetic predictions, making it useful for rapid multiparameter screening (Daina et al., 2017). However, contemporary ADMET research increasingly incorporates machine-learning and graph-based models, while also recognising that accuracy alone does not establish prediction reliability. Recent studies show that calibration, uncertainty quantification, and a query molecule's relationship to the model's training chemical space can materially affect the value of an ADMET prediction (Catacutan et al., 2024; Li et al., 2024; Venkataraman et al., 2025; Schliephacke et al., 2026). For the present compound, whose molecular weight is far below that of many conventional drug-like molecules, this distinction is important because apparently unfavourable rule-based outputs may partly reflect domain boundaries rather than a biological liability.

### Fragment-Scale Chemistry and the Relevance of the Scaffold

Low-molecular-weight molecules are central to fragment-based drug discovery because they can sample chemical space efficiently and serve as tractable starting points for systematic growth, linking or merging. Recent reviews emphasise that modern fragment workflows are inherently multi-objective: initial fragment selection and hit progression consider not only binding but also physicochemical properties, synthetic tractability, selectivity and ADMET-related risks (Erlanson et al., 2016; Yoo et al., 2025; Grosjean & Biggin, 2026). Accordingly, a compound that fails a lower molecular-weight boundary in a generic drug-likeness filter should not be rejected automatically when it is being considered as fragment-scale chemistry. This perspective is directly relevant to the test compound because its 147.22 g/mol size, rigid bicyclic topology and absence of rotatable bonds place it at the small, structurally constrained end of chemical space. The scaffold is therefore scientifically useful as a test case for examining whether a compact sulfur-containing bicyclic structure can show a coherent ADME profile while retaining unresolved chemical and toxicological uncertainties.

### AI/ML Toxicology, Uncertainty and Applicability Domain

Computational toxicology is increasingly used as a tiered prioritisation approach that combines structural similarity, machine-learning models, pathway-level evidence and mechanistic hypotheses before experimental testing (Judson et al., 2010; Kavlock et al., 2012; Raies & Bajic, 2016; Tice et al., 2013). OECD guidance and QSAR best practice require attention to model definition, validation, applicability domain, and reliability when predictions support scientific or regulatory decisions

(OECD, 2007, 2023; Tropsha, 2010). Recent molecular-ML work reinforces this principle: models can perform well overall while still producing unreliable estimates for out-of-domain compounds, and uncertainty measures can provide information that a point prediction alone cannot capture (Li et al., 2024; Parrondo-Pizarro et al., 2026). This implies that a positive toxicity call should be interpreted as a prioritisation signal rather than proof of hazard, while a negative call should not be equated with demonstrated safety.

## **ProTox 3.0 as a Multi-Endpoint Toxicity Framework**

ProTox 3.0 extends computational toxicity screening beyond a single acute endpoint by combining toxicity-class prediction with structural-similarity and machine-learning approaches across multiple endpoint categories, including organ toxicity, general toxicity, pathways, molecular initiating events and toxicity-associated targets (Banerjee et al., 2024). This breadth is particularly useful for a single-compound investigation because it lets you evaluate apparently convergent and divergent signals within one framework. At the same time, a wide endpoint panel does not remove uncertainty: the evidential weight of a prediction depends on the model, chemical-space support and endpoint definition. This study therefore treats the supplied ProTox 3.0 report as complementary evidence and confidence indicators, not as experimental confirmation of biological activity or toxicity.

## **Sulfur-Containing Heterocycles and Disulfide Reactivity**

Sulfur-containing heterocycles occupy an important position in medicinal chemistry because sulfur can alter polarity, lipophilicity, redox behaviour, conformational properties and target interactions. Recent reviews document the continuing presence of sulfur-containing motifs in therapeutic compounds and highlight their value as structurally and pharmacologically diverse building blocks (Mustafa & Winum, 2022; Tiz et al., 2025; Shamsudin & Rullah, 2025). For the present molecule, however, the relevant issue is the specific presence of a ring-embedded disulfide rather than sulfur content in the abstract. Disulfides can participate in reduction and thiol–disulfide exchange, with rates governed by the local chemical environment and reaction conditions (Fernandes & Ramos, 2004; Nagy, 2013; Sevier & Kaiser, 2002). The constrained disulfide in this scaffold could therefore behave differently from flexible acyclic disulfides, but the present computational outputs cannot quantify its reactivity. This makes chemical stability and exchange behaviour priority experimental questions rather than conclusions that can be inferred from a structural alert alone.

## **Specific Knowledge Gap Addressed**

Collectively, the cited literature supports the importance of integrated ADMET profiling, fragment-aware interpretation, sulfur-specific chemical analysis and uncertainty assessment. Still, these strands were not identified together for the exact parent scaffold in the targeted literature search undertaken for this study. This study addresses that gap by combining the supplied SwissADME and ProTox 3.0 outputs into a single evidence map in which endpoint agreement, endpoint discordance, confidence indicators, and chemical plausibility jointly determine prioritisation. A targeted literature search did not identify a peer-reviewed study reporting an integrated ADME–toxicity characterisation of the exact parent scaffold. The originality claimed here is consequently methodological and evidential: the study demonstrates how a single, chemically distinctive compound can be prioritised for experimental validation without overstating computational predictions as demonstrated molecular design.

## 3.0 METHODOLOGY

A descriptive, single-compound computational profiling design was used to integrate physicochemical, ADME and toxicity predictions with reported confidence indicators. The workflow comprised five steps: (1) recording the baseline physicochemical, ADME and toxicity outputs from the supplied reports; (2) grouping findings into favourable attributes, potential liabilities and unresolved or discordant signals; (3) assigning each finding an evidential weight according to cross-model consistency and the confidence information reported by the software; (4) mapping each high-priority uncertainty to a specific experimental question; and (5) using the resulting evidence map to define criteria for whether the compound should progress to experimental testing. No analogue generation, de novo design, docking, molecular dynamics, SAR modelling or optimisation algorithm was used. The term compound prioritisation is therefore used deliberately to distinguish the present workflow from validated molecular design.

**Table 1. Confidence-Aware Compound Prioritisation Map for the Test Compound**

| Design domain                     | Observed computational signal                                                   | Interpretive implication                                                            | Rationale                                                                                        |
|-----------------------------------|---------------------------------------------------------------------------------|-------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------|
| <b>Solubility / exposure</b>      | All three solubility models favourable; high predicted GI absorption            | Retain as a favourable hypothesis                                                   | Small size and moderate polarity support a favourable exposure hypothesis.                       |
| <b>Metabolic interaction</b>      | No predicted inhibition of CYP1A2, CYP2C19, CYP2C9, CYP2D6 or CYP3A4            | Retain as a favourable hypothesis                                                   | Avoid structural changes that introduce strong CYP inhibition or unstable metabolic motifs.      |
| <b>Efflux</b>                     | Not predicted to be a P-gp substrate                                            | Preserve / monitor                                                                  | The efflux liability was not indicated by the supplied model.                                    |
| <b>CNS exposure</b>               | SwissADME: no BBB permeation; ProTox: BBB-barrier activity 0.79                 | Resolve through endpoint-specific experimental testing                              | The two endpoint definitions are not equivalent; the discordance is mechanistically informative. |
| <b>Neurorespiratory liability</b> | Neurotoxicity 0.62; respiratory toxicity 0.56                                   | Reduce / validate                                                                   | Signals are above the ProTox decision threshold but remain low-moderate and model-dependent.     |
| <b>Disulfide reactivity</b>       | One structural alert associated with disulfide functionality                    | Contextualise; interpret contextually rather than as a binary development criterion | The ring-embedded S–S bond is the most chemically distinctive potential reactivity liability.    |
| Acute toxicity                    | LD <sub>50</sub> 500 mg/kg; class 4; prediction accuracy 23%; similarity 31.74% | Validate before exclusion                                                           | The point estimate is low-confidence and should be treated as hypothesis-generating.             |
| Fragment scale                    | MW 147.22 g/mol; Ghose/Muegge/lead-likeness failures due to size                | Contextualize, not automatically correct                                            | Size-based failures may reflect intended fragment character rather than poor developability.     |

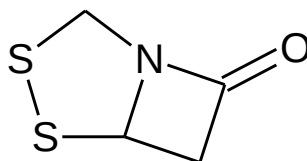
The study adopted a descriptive, single-compound computational profiling design. The sole analytical unit was 3,4-dithia-1-azabicyclo[3.2.0]heptan-7-one, represented by the supplied isomeric SMILES O=C1C[C@@H]2N1CSS2. The molecular structure was generated using the PubChem Sketcher interface and represented using the supplied stereochemical isomeric SMILES. We exported them as Isomeric SMILES strings to retain exact stereochemical information, including explicit @ and @@ chiral indicators where wedge/dash bonds were defined. No laboratory experiments were conducted and no new predictive model was trained. The analysis integrated physicochemical descriptors, aqueous-solubility estimates, pharmacokinetic predictions, medicinal-chemistry filters, acute oral toxicity, organ-level toxicity, pathway-level signals and model-confidence information. We selected this multiparameter scope because ADMET liabilities are interdependent and discordant endpoint predictions can reveal uncertainty missed by single-endpoint interpretation.

In silico ADME and toxicity profiles were evaluated using SwissADME (<http://www.swissadme.ch>; Swiss Institute of Bioinformatics) and ProTox 3.0 (<https://tox.charite.de/protox3/>). Predictions were generated on 3 February 2025 at 20:10 EAT (UTC+3) using the default platform parameters available at the time of analysis. Numerical findings were transcribed from the resulting SwissADME and ProTox 3.0 reports. SwissADME outputs included molecular and physicochemical descriptors, lipophilicity estimates, solubility predictions from ESOL, Ali and SILICOS-IT, gastrointestinal absorption, blood-brain barrier prediction, P-glycoprotein substrate classification, skin permeation, CYP inhibition predictions, bioavailability score, drug-likeness filters and synthetic-accessibility information. ProTox 3.0 outputs included acute oral LD<sub>50</sub> and toxicity class, average similarity to reference compounds, reported prediction accuracy, organ-specific and general toxicity endpoints, Tox21 pathway predictions, molecular initiating events, metabolic endpoints and predicted toxicity-associated targets (Banerjee et al., 2024; Daina et al., 2017). Complete raw export files are provided as Supplementary Data, including the full SwissADME CSV predictions and ProTox 3.0 toxicity output summary tables, as Supplementary Data (Tables S1 and S2).

Predictions were interpreted hierarchically. We first recorded point estimates or categorical outputs without modification; then assessed agreement and disagreement across available models; finally, used platform-reported confidence indicators to determine evidential weight. Importantly, the study did not independently calculate a formal applicability domain, uncertainty interval or calibration curve for the underlying proprietary models. Instead, reported prediction accuracy, structural similarity and cross-model consistency were treated as descriptive indicators of prediction support and were not interpreted as formal uncertainty quantification, consistent with current recommendations that data-space distance and model-based uncertainty be considered jointly when evaluating molecular predictions (OECD, 2007, 2023; Li et al., 2024; Parrondo-Pizarro et al., 2026). No inferential statistical analysis was undertaken because the study involved a single compound without experimental replication; therefore, the reported probabilities and model metrics were treated descriptively.

## 4.0 FINDINGS AND DISCUSSION

### Molecular and Physicochemical Characteristics



**Figure 1. Structure of the Test Compound (isomeric SMILES: O=C1C[C@@H]2N1CSS2)**

Source: Structure prepared from the PubChem Sketcher-derived isomeric SMILES input.

**Table 2. Molecular and Physicochemical Characteristics of the Test Compound**

| Parameter                    | Value                                          |
|------------------------------|------------------------------------------------|
| Molecular formula            | C <sub>4</sub> H <sub>5</sub> NOS <sub>2</sub> |
| Molecular weight             | 147.22 g/mol                                   |
| Heavy atoms                  | 8                                              |
| Aromatic heavy atoms         | 0                                              |
| Fraction Csp <sup>3</sup>    | 0.75                                           |
| Rotatable bonds              | 0                                              |
| H-bond acceptors (SwissADME) | 1                                              |
| H-bond donors                | 0                                              |
| Molar refractivity           | 39.31                                          |
| TPSA                         | 70.91 Å <sup>2</sup>                           |
| Consensus logP               | 0.72                                           |
| iLOGP                        | 1.37                                           |
| XLOGP3                       | 0.15                                           |
| WLOGP                        | 0.52                                           |
| MLOGP                        | 0.46                                           |
| SILICOS-IT logP              | 1.11                                           |

The predicted physicochemical profile indicates a small, rigid and non-aromatic molecule with moderate polarity and low-to-mild calculated lipophilicity. The zero-rotatable-bond count is consistent with its fused bicyclic topology. Although calculated logP values varied across algorithms, all estimates remained within a relatively low-to-moderate lipophilicity range. The Csp<sup>3</sup> fraction of 0.75 further reflects the scaffold's saturated, three-dimensional character. The key medicinal-chemistry observation is its very small molecular size relative to conventional small-molecule drug property spaces, which helps explain subsequent lower-bound filter failures (Bickerton et al., 2012; Kerns & Di, 2008).

## Aqueous Solubility

**Table 3. Predicted Aqueous Solubility across Three SwissADME Models**

| Model      | Log S | Predicted solubility  | Class        |
|------------|-------|-----------------------|--------------|
| ESOL       | -0.85 | 20.9 mg/mL (0.142 M)  | Very soluble |
| Ali        | -1.20 | 9.38 mg/mL (0.0637 M) | Very soluble |
| SILICOS-IT | -0.18 | 97.9 mg/mL (0.665 M)  | Soluble      |
|            |       |                       |              |

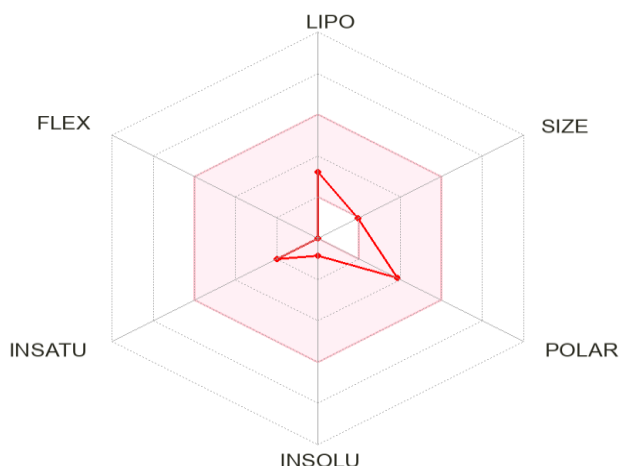
All three SwissADME models predicted favourable aqueous solubility, although the estimated values varied substantially among models, with estimated concentrations of 9.38–97.9 mg/mL. ESOL predicted 20.9 mg/mL, Ali predicted 9.38 mg/mL, and SILICOS-IT predicted 97.9 mg/mL. Agreement in qualitative direction across the three models provides convergent screening-level support, but does not establish experimental solubility compared with reliance on a single algorithm, although it does not replace experimental equilibrium-solubility measurement. Actual solubility can be influenced by crystal packing, ionisation, solid-state form and measurement conditions, particularly for a very small heterocycle (Delaney, 2004; Kerns & Di, 2008).

## Predicted Pharmacokinetic Characteristics

**Table 4. Predicted Pharmacokinetic Characteristics**

| ADME Parameter                 | Prediction |
|--------------------------------|------------|
| Gastrointestinal absorption    | High       |
| Blood–brain barrier permeation | No         |
| P-glycoprotein substrate       | No         |
| Skin permeation, log Kp        | -7.09 cm/s |
| CYP1A2 inhibition              | No         |
| CYP2C19 inhibition             | No         |
| CYP2C9 inhibition              | No         |
| CYP2D6 inhibition              | No         |
| CYP3A4 inhibition              | No         |
| Bioavailability score          | 0.55       |

The supplied SwissADME analysis predicted high gastrointestinal absorption, no P-glycoprotein substrate behaviour and no inhibition of CYP1A2, CYP2C19, CYP2C9, CYP2D6 or CYP3A4. Together, these predictions indicate no major liability within the specific endpoints assessed by the SwissADME models, but they remain computational hypotheses. The absence of predicted BBB permeation is also compatible with the compound's moderate polarity and low calculated lipophilicity under conventional passive-permeability concepts (Daina et al., 2017; Fischer et al., 1998; Pajouhesh & Lenz, 2005). The bioavailability score of 0.55 should be interpreted as a screening indicator rather than a measured fraction absorbed.



**Figure 2. SwissADME Physicochemical Property Radar for the Test Compound (LIPO, SIZE, POLAR, INSOLU, INSATU, FLEX)**

Source: Supplied SwissADME report.

## Drug-Likeness and Medicinal-Chemistry Profile

**Table 5. Drug-Likeness Filters and Medicinal-Chemistry Alerts**

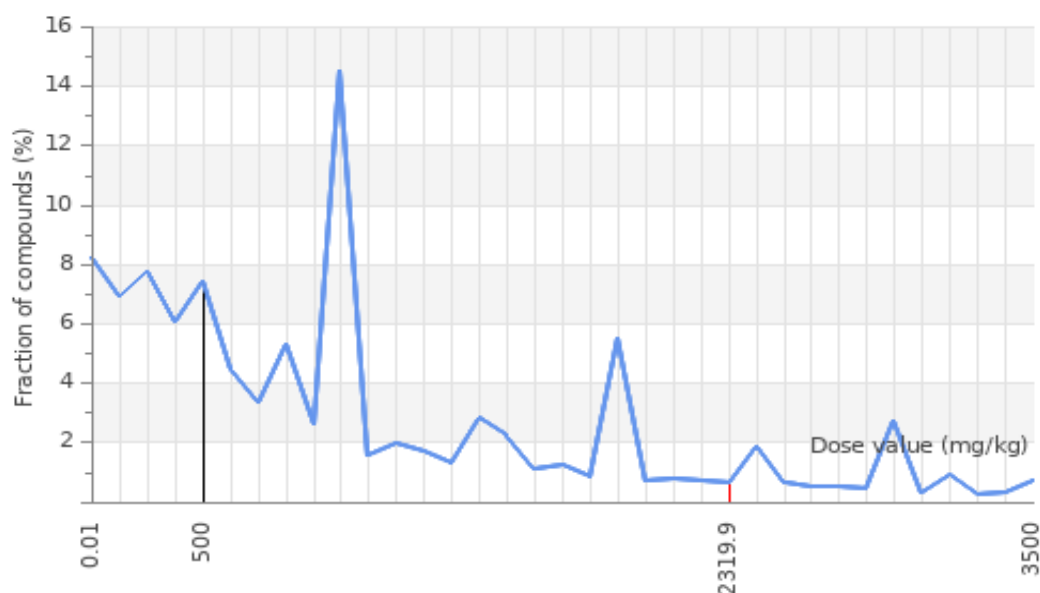
| Criterion                   | Result                           |
|-----------------------------|----------------------------------|
| Lipinski                    | Pass; 0 violations               |
| Ghose                       | Fail; MW <160, MR <40, atoms <20 |
| Veber                       | Pass                             |
| Egan                        | Pass                             |
| Muegge                      | Fail; MW <200, carbon count <5   |
| PAINS alerts                | 0                                |
| Brenk-type structural alert | 1; disulfide                     |
| Lead-likeness               | Fail; MW <250                    |
| Synthetic accessibility     | 3.01/10                          |
| Bioavailability score       | 0.55                             |

The compound passed the Lipinski, Veber and Egan filters but failed the Ghose, Muegge and lead-likeness criteria because its molecular weight and atom/carbon counts fall below the lower-size boundaries embedded in those rules. This pattern is more appropriately interpreted as evidence of fragment character than as a generalised failure of drug-likeness. Contemporary drug-likeness research cautions against treating hard thresholds as universal measures of developability and supports multiparameter optimisation instead (Bickerton et al., 2012; Erlanson et al., 2016; Kerns & Di, 2008; Wager et al., 2010). The single Brenk-type alert associated with the disulfide functionality highlights chemical reactivity as a more specific design question than simple filter compliance.

## Acute Oral Toxicity and Prediction Confidence

**Table 6. ProTox 3.0 Acute Oral Toxicity Prediction and Confidence Indicators**

| ProTox 3.0 metric                         | Result    |
|-------------------------------------------|-----------|
| Predicted oral LD <sub>50</sub>           | 500 mg/kg |
| Toxicity class                            | Class 4   |
| Average similarity to reference compounds | 31.74%    |
| Prediction accuracy                       | 23%       |



**Figure 3. Distribution of Predicted LD<sub>50</sub> Values among ProTox 3.0 Reference Compounds, with the Compound Positioned at 500 mg/kg**

Source: Supplied ProTox 3.0 report.

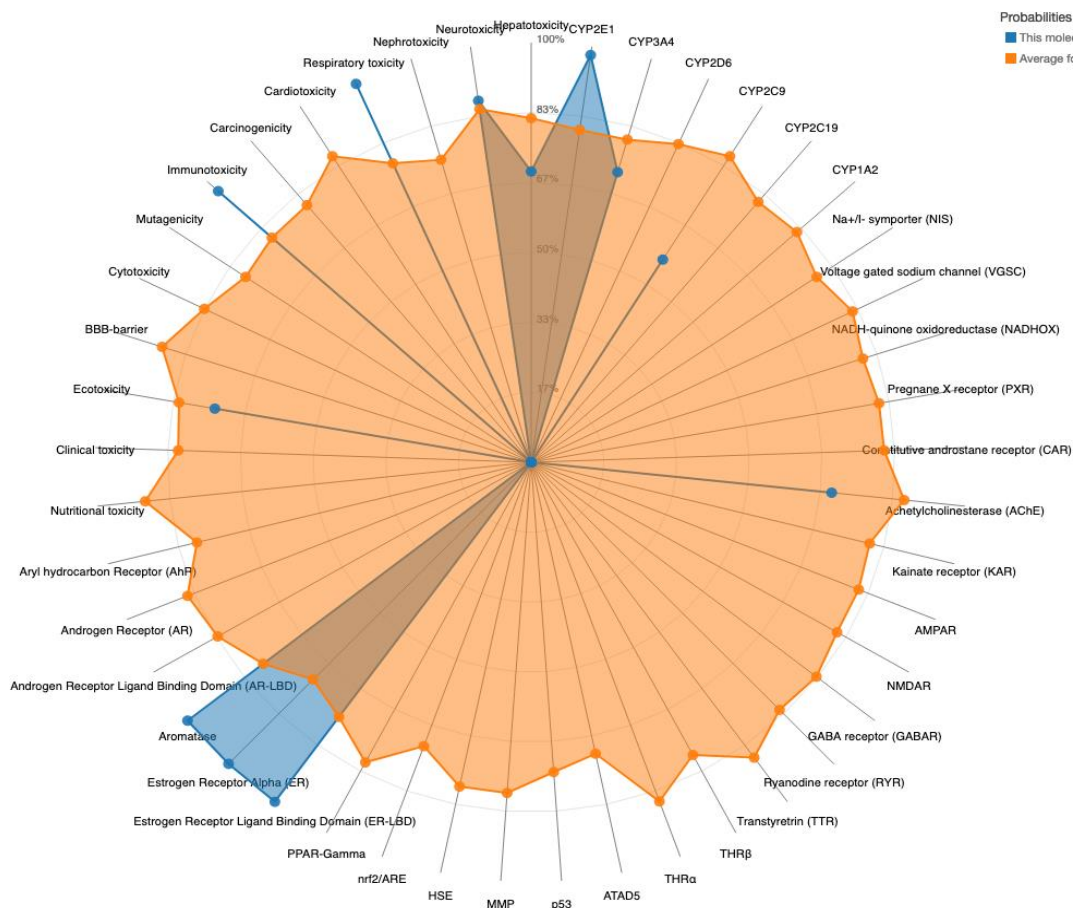
The predicted acute oral LD<sub>50</sub> of 500 mg/kg corresponds to ProTox 3.0 toxicity class 4. However, the associated prediction accuracy was only 23 per cent and the average structural similarity to reference compounds was 31.74 per cent. These indicators materially reduce confidence in the point estimate. The most defensible interpretation is therefore that ProTox 3.0 has identified a potential acute-toxicity concern under limited analogue support, not that the compound has been experimentally established as a class-4 toxicant. This interpretation is consistent with model applicability-domain principles (OECD, 2007, 2023; Raies & Bajic, 2016; Tropsha, 2010).

## Organ-Level and General Toxicity Endpoints

**Table 7. Organ-Specific and General Toxicity Endpoints Predicted by ProTox 3.0**

| Endpoint             | Classification | Probability |
|----------------------|----------------|-------------|
| Hepatotoxicity       | Inactive       | 0.68        |
| Neurotoxicity        | Active         | 0.62        |
| Nephrotoxicity       | Inactive       | 0.72        |
| Respiratory toxicity | Active         | 0.56        |
| Cardiotoxicity       | Inactive       | 0.75        |
| Carcinogenicity      | Inactive       | 0.53        |
| Immunotoxicity       | Inactive       | 0.99        |
| Mutagenicity         | Inactive       | 0.71        |
| Cytotoxicity         | Inactive       | 0.63        |
| BBB-barrier activity | Active         | 0.79        |
| Ecotoxicity          | Active         | 0.66        |
| Clinical toxicity    | Active         | 0.50        |
| Nutritional toxicity | Inactive       | 0.65        |

Five endpoints were classified as active: neurotoxicity (0.62), respiratory toxicity (0.56), BBB-barrier activity (0.79), ecotoxicity (0.66) and clinical toxicity (0.50). The clinical-toxicity value is exactly at the nominal decision threshold and is therefore best treated as indeterminate rather than strongly positive. The strongest positive signal was BBB-barrier activity, followed by ecotoxicity, neurotoxicity and respiratory toxicity. Hepatotoxicity, nephrotoxicity, cardiotoxicity, carcinogenicity, immunotoxicity, mutagenicity, cytotoxicity and nutritional toxicity were classified as inactive. These negative calls do not establish the absence of hazard; rather, they indicate that the model did not identify sufficient evidence for activity within its learned chemical space (National Research Council, 2007; Raies & Bajic, 2016; Tice et al., 2013).



**Figure 4. ProTox 3.0 Toxicity Radar Comparing Endpoint Probabilities for the Compound with Class-Average Values**

Source: Supplied ProTox 3.0 report.

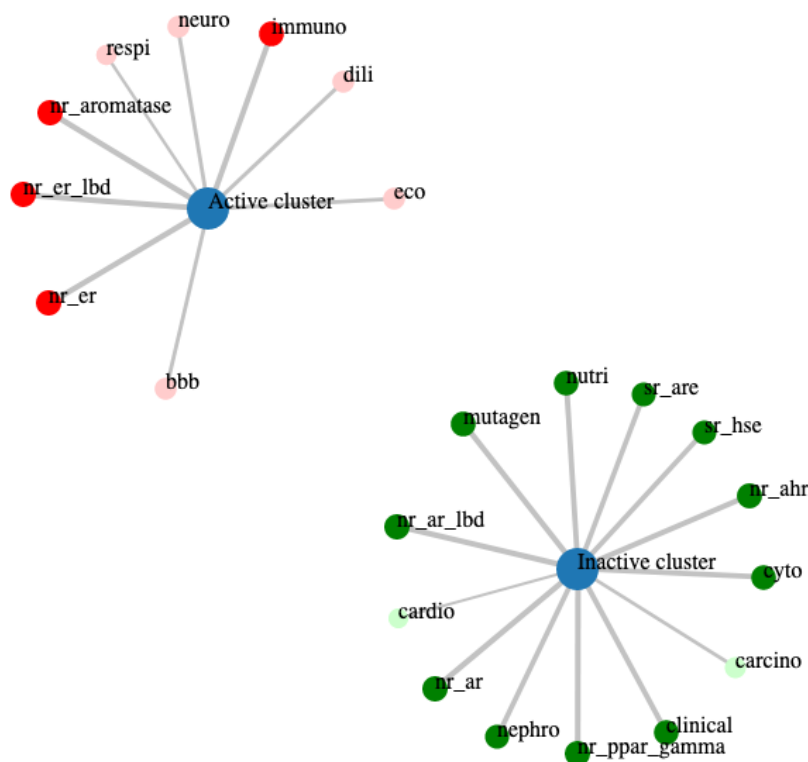
## Tox21 Pathways and Molecular Initiating Events

All reported Tox21 nuclear-receptor and stress-response pathways were predicted inactive, including AhR, AR, AR-LBD, aromatase, ER, ER-LBD, PPAR- $\gamma$ , nrf2/ARE, HSE, mitochondrial membrane potential, p53 and ATAD5. All reported molecular initiating events were likewise inactive, including THRA, THRB, 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<sup>+</sup>/I<sup>-</sup> symporter. This broad pattern provides limited computational support for conventional receptor- or stress-pathway-driven toxicity within the tested model space (Judson et al., 2010; Kavlock et al., 2012; Martin et al., 2010; National Research Council, 2007; Tice et al., 2013).

## Predicted Toxicity-Associated Targets

The supplied ProTox 3.0 report included possible toxicity-associated targets such as AA2AR, ADRB2, ANDR, AOFA, CRFR1, DRD3, ESR1, ESR2, GCR, HRH1, NR1I2, OPRK, OPRM, PDE4D, PGH1 and PRGR. No specific probable-binding calls were returned for the compound. Accordingly, view this target-

level output as hypothesis-generating rather than evidence of confirmed target engagement or mechanism.



**Figure 5. ProTox 3.0 Network Visualisation Clustering Active and Inactive Predicted Endpoints for the Test Compound**

Source: Supplied ProTox 3.0 report.

## Integrated Risk–Benefit Interpretation

**Table 8. Integrated Computational Risk–Benefit Interpretation and Prioritisation**

| Domain                         | Integrated interpretation                                               | Priority                                   |
|--------------------------------|-------------------------------------------------------------------------|--------------------------------------------|
| <b>Physicochemical profile</b> | Favourable: compact, rigid, moderately polar, low-to-mild lipophilicity | Preserve                                   |
| <b>Solubility</b>              | Consistently favourable across three models                             | Preserve; confirm experimentally           |
| <b>Oral exposure</b>           | High predicted GI absorption                                            | Preserve; confirm experimentally           |
| <b>CYP interaction</b>         | Negative for five assessed CYP isoforms                                 | Preserve; confirm experimentally           |
| <b>Efflux</b>                  | No P-gp substrate prediction                                            | Preserve / monitor                         |
| <b>CNS exposure</b>            | Uncertain because SwissADME and ProTox outputs differ                   | High priority to resolve                   |
| <b>Neurorespiratory safety</b> | Positive low-moderate computational flags                               | High priority to validate                  |
| <b>Acute oral toxicity</b>     | Class 4 estimate, but low model confidence                              | Do not use alone for go/no-go              |
| <b>Structural reactivity</b>   | Ring-embedded disulfide alert                                           | High priority to characterize              |
| <b>Fragment status</b>         | Small-size filter failures largely size-driven                          | Contextualize rather than force compliance |

The integrated profile is favourable at the physicochemical and preliminary ADME levels but considerably less certain at the toxicological level. The most defensible development interpretation is therefore conditional: preserve the favourable solubility, oral-exposure hypothesis, low CYP-inhibition liability and non-P-glycoprotein-substrate prediction, while prioritising chemical reactivity, acute-toxicity uncertainty and the neurorespiratory/CNS signals for validation.

## Discussion

The computational profile suggests that the scaffold occupies a useful but unusual physicochemical region. Its compact, rigid and non-aromatic topology is compatible with fragment-scale chemistry. At the same time, the combination of moderate polarity and low calculated lipophilicity plausibly explains the favourable solubility and preliminary oral-exposure predictions. The important interpretive point is not that the compound is intrinsically “drug-like”, but that its physicochemical attributes are sufficiently coherent to justify experimental testing despite its atypically small size. This aligns with modern fragment-based practice, where property windows are interpreted in context rather than enforced as universal cut-offs (Yoo et al., 2025; Grosjean & Biggin, 2026).

The lower-bound failures in the Ghose, Muegge and lead-likeness filters are best understood as rule-domain effects. Because these filters were designed around distributions characteristic of larger drug-like molecules, their failure by a 147-Da scaffold does not establish that the molecule is unsuitable for medicinal chemistry. For this compound, the more informative question is whether increasing size during any future analogue programme would deliver a measurable gain in biological activity or selectivity

without eroding solubility, permeability control or metabolic behaviour. Thus, the present results support contextualising the fragment character rather than forcing compliance with arbitrary molecular-weight thresholds.

The ring-embedded disulfide is the most chemically distinctive source of uncertainty because it can participate in redox and thiol–disulfide exchange chemistry. A generic disulfide structural alert is therefore mechanistically plausible but insufficiently specific to predict the magnitude, kinetics or biological consequence of reactivity for this constrained scaffold. The highest-value next experiment is consequently chemical characterisation of stability and exchange behaviour in physiologically relevant conditions. Such measurements would determine whether the alert reflects a manageable chemical feature or a genuine liability that should constrain further development.

The toxicity outputs better illustrate why prediction confidence must be separated from the numerical result itself. The acute-toxicity estimate has low reported accuracy and limited reference-set similarity, so the point estimate should carry less decision weight than a prediction supported by a well-populated local chemical neighbourhood. This interpretation is aligned with recent uncertainty research showing that molecular distance from the training domain and model-derived uncertainty provide complementary information, particularly under distribution shift (Li et al., 2024; Parrondo-Pizarro et al., 2026). The practical implication is that low-confidence toxicity predictions should trigger orthogonal testing rather than automatic exclusion.

The organ-level pattern is internally heterogeneous, supporting endpoint-specific validation rather than a single global toxicity label. The neurotoxicity and respiratory signals warrant targeted follow-up, while the threshold-level clinical-toxicity result is more appropriately regarded as indeterminate. The apparent disagreement between the SwissADME BBB-permeation prediction and the ProTox BBB-barrier activity output should not be treated as a contradiction without examining endpoint definitions: one is framed around predicted permeability, whereas the other belongs to a toxicity-associated endpoint model. Their divergence nevertheless identifies a real decision uncertainty because any experimentally demonstrated CNS exposure would change the interpretation of the neurotoxicity signal. This is precisely the type of cross-endpoint inconsistency that an integrated profiling strategy is intended to expose rather than conceal.

The broad inactivity across the reported Tox21 pathways and molecular initiating events provides limited reassurance, but it does not demonstrate the absence of biological hazard. Mechanistic screens can reduce uncertainty about particular modes of action, yet negative computational calls remain conditional on model scope, data quality and chemical-space representation. The most defensible interpretation is therefore convergent but incomplete evidence: several canonical toxicity pathways were not flagged, while separate endpoint models still identified neurorespiratory and CNS-related concerns. Experimental testing should exploit this information to focus on the most decision-relevant uncertainties rather than reproduce every computational endpoint indiscriminately.

## Compound Prioritisation Implications

Taken together, the findings support conditional prioritisation of the compound for experimental study, but not a claim of readiness for therapeutic development. The most robust attributes are the internally coherent physicochemical and solubility predictions and the absence of predicted inhibition across the five

assessed CYP isoforms. The principal uncertainties are chemical reactivity, toxicological confidence and the interpretation of CNS-related signals. This creates a rational decision rule: retain the compound as an experimental candidate while requiring chemical, permeability, metabolic and toxicity evidence before any progression to analogue optimisation.

The current evidence can also define a future optimisation boundary without claiming that optimisation has already been performed. Any subsequent analogue programme should avoid increasing size or lipophilicity solely to satisfy generic drug-likeness filters and should instead test whether each structural change improves a measured biological objective. Evaluate modifications around the sulfur-containing ring specifically for their effects on chemical stability and thiol–disulfide exchange. Future computational ranking should retain uncertainty and structural-similarity information so apparent improvements in predicted toxicity are not mistaken for genuine risk reduction when model applicability weakens. These are prospective decision rules, not validated SAR relationships.

## Experimental Validation and Decision Sequence

Experimental validation should proceed in a staged sequence that resolves the highest-value uncertainties first. Confirm chemical identity and purity using appropriate spectroscopic and chromatographic methods; then measure chemical stability and sulfur reactivity in relevant buffers and biological matrices, including thiol–disulfide exchange kinetics where justified. Measure equilibrium solubility under controlled pH and temperature, then assess permeability and metabolic stability, including CYP inhibition verification using recombinant enzymes or microsomes. In parallel, conduct targeted cell-based cytotoxicity and focused assays addressing the predicted neurotoxicity and respiratory-liability signals. Assess BBB permeability only with an endpoint explicitly matched to the scientific question. Results from this first validation tier should determine whether a second tier of more resource-intensive studies is warranted. This staged approach converts the computational profile into testable decision gates while avoiding premature escalation based on low-confidence predictions.

## Limitations of the Study

The principal limitation is that all findings are computational and were derived from supplied software reports rather than new experiments. The study therefore cannot establish pharmacological activity, target engagement, metabolic fate, exposure, dose–response relationships or in vivo safety. The acute-toxicity estimate is additionally weakened by its reported 23 per cent prediction accuracy and 31.74 per cent average structural similarity to reference compounds. Importantly, the study did not independently compute applicability domains, calibrate the underlying models or estimate formal predictive intervals; reported similarity and confidence information should therefore not be interpreted as a substitute for full uncertainty quantification. The supplied reports also showed cross-platform descriptor differences, illustrating why direct numerical equivalence across computational implementations should be avoided. Finally, no analogue series or experimental activity data were available, so the study does not establish SAR and should not be described as molecular optimisation.

## 5.0 CONCLUSION AND RECOMMENDATIONS

**Conclusion:** The test compound presents a compact, rigid and physicochemically coherent screening profile with favourable predicted solubility and gastrointestinal exposure and no predicted P-glycoprotein substrate behaviour or inhibition of the five assessed CYP isoforms. Its apparent failures of several drug-likeness filters largely stem from its unusually small size. They should therefore be interpreted in the

context of fragment-scale chemistry rather than as automatic evidence of poor developability. The ring-embedded disulfide remains the most important chemically specific feature requiring experimental characterisation.

The toxicological evidence is materially less certain than the physicochemical and preliminary ADME evidence. The reported acute-toxicity estimate has low accuracy and limited structural-similarity support, while separate models identify neurotoxicity, respiratory toxicity, and BBB-related signals. Treat these findings as prioritisation signals whose decision weight depends on model support and endpoint definition, not as definitive demonstrations of hazard or safety. The study therefore supports progression to targeted experimental validation rather than an immediate go/no-go conclusion.

The principal contribution is a confidence-aware, single-compound evidence framework that integrates complementary ADME and toxicity outputs, distinguishes prediction favourability from prediction reliability, and converts uncertainty into explicit experimental questions. The work does not demonstrate molecular design or optimisation; instead, it provides a defensible basis for deciding whether and how to characterise the compound before any future analogue programme experimentally. The next stage should prioritise chemical identity and stability, disulfide reactivity, experimental solubility, permeability, metabolic behaviour and focused toxicity testing, with subsequent computational redesign undertaken only after empirical evidence becomes available.

## Data Availability

All numerical findings in this manuscript derive from the SwissADME and ProTox 3.0 reports generated for the test compound on February 3, 2025, at 20:10 EAT (UTC+3). The complete raw export files, including full SwissADME CSV predictions and ProTox 3.0 toxicity output summary tables, are available as Supplementary Data Tables and charts. We generated no new experimental data or newly trained predictive models in this study.

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