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RESEARCH ARTICLE

Baricitinib and tofacitinib off-target profile, with a focus on Alzheimer's disease

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Abstract

INTRODUCTION: Janus kinase (JAK) inhibitors were recently identified as promising drug candidates for repurposing in Alzheimer's disease (AD) due to their capacity to suppress inflammation via modulation of JAK/STAT signaling pathways. Besides interaction with primary therapeutic targets, JAK inhibitor drugs frequently interact with unintended, often unknown, biological off-targets, leading to associated effects. Nevertheless, the relevance of JAK inhibitors' off-target interactions in the context of AD remains unclear.

METHODS: Putative off-targets of baricitinib and tofacitinib were predicted using a machine learning (ML) approach. After screening scientific literature, off-targets were filtered based on their relevance to AD. Targets that had not been previously identified as off-targets of baricitinib or tofacitinib were subsequently tested using biochemical or cell-based assays. From those, active concentrations were compared to bioavailable concentrations in the brain predicted by physiologically based pharmacokinetic (PBPK) modeling.

RESULTS: With the aid of ML and in vitro activity assays, we identified two enzymes previously unknown to be inhibited by baricitinib, namely casein kinase 2 subunit alpha 2 (CK2-α2) and dual leucine zipper kinase (MAP3K12), both with binding constant (K_d) values of 5.8 μM. Predicted maximum concentrations of baricitinib in brain tissue using PBPK modeling range from 1.3 to 23 nM, which is two to three orders of magnitude below the corresponding binding constant.

CONCLUSION: In this study, we extended the list of baricitinib off-targets that are potentially relevant for AD progression and predicted drug distribution in the brain. The results suggest a low likelihood of successful repurposing in AD due to low brain permeability, even at the maximum recommended daily dose. While additional research is needed to evaluate the potential impact of the off-target interaction on

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AD, the combined approach of ML-based target prediction, in vitro confirmation, and PBPK modeling may help prioritize drugs with a high likelihood of being effectively repurposed for AD.

KEYWORDS
Alzheimer's disease, Janus kinase inhibitors, machine learning, off-target, physiological based pharmacokinetic modeling, target prediction

Highlights

- This study explored JAK inhibitors' off-targets in AD using a multidisciplinary approach.
- We combined machine learning, in vitro tests, and PBPK modelling to predict and validate new off-target interactions of tofacitinib and baricitinib in AD.
- Previously unknown inhibition of two enzymes (CK2-a2 and MAP3K12) by baricitinib were confirmed using in vitro experiments.
- Our PBPK model indicates that baricitinib low brain permeability limits AD repurposing.
- The proposed multidisciplinary approach optimizes drug repurposing efforts in AD research.

1 | INTRODUCTION

Repurposing Janus kinase (JAK) inhibitor drugs for Alzheimer's disease (AD) has received increasing attention.[1,2] Members of this class were designed to target the JAK family (JAK1, JAK2, JAK3, and non-receptor tyrosine-protein kinase TYK2) inhibiting the production of multiple pro-inflammatory cytokines, such as interleukin (IL)-6, IL-10, and interferon (IFN)-γ.[3,4] As AD progression and severity are associated with immune-driven neuroinflammation,[5,6] the potential role of some JAK inhibitors in treating AD is supported by their capacity to reduce neuroinflammation and modulate immunoregulatory processes.[7,8]

Two JAK inhibitors, baricitinib and tofacitinib, currently approved to treat rheumatoid arthritis, were recently identified among potential drug candidates for repurposing in AD.[1,2] Besides the potential effects of baricitinib and tofacitinib in AD due to pharmacological inhibition of one or more targets of the JAK family, these drugs are known to have additional off-targets (i.e., protein targets different than the primary therapeutic target).[1,9,10] The unintended off-target activity of JAK inhibitor drugs adds to the evidence that they may modulate additional targets on specific pathways and cellular processes in other diseases.

Previous work has explored the association between JAK inhibitors' target profile and AD in the context of potential repurposing using a multi-step machine learning (ML) framework.[1] The framework first identified potential associations between a list of genes and AD severity and then produced a ranked list of possible repurposing drugs. Then, the highly rated drugs were evaluated for common trends among their targets (i.e., primary targets and additional drug–target interactions previously identified). Therefore, although baricitinib and tofacitinib were previously identified as promising candidates for repurposing in AD, the potential influence of previously unknown targets in AD progression remains unclear.

In this work, we aimed to identify previously unknown drug–target interactions of baricitinib and tofacitinib that may be a factor in the use of these drugs in the context of AD. We used a multidisciplinary approach that combined experimental ligand-based ML for target prediction (Target Inference Generator [TIGER][11]), in vitro testing of predicted targets, and physiologically based pharmacokinetic (PBPK) modeling. A similar approach recently allowed us to profile tofacitinib and baricitinib, focusing on targets related to thrombosis and viral infections, and led to the identification of two previously unknown off-targets.[9]

2 | METHODS

2.1 | Macromolecular target prediction and selection

TIGER v. 19.07[11,12] software was used for target activity prediction. TIGER is a ligand-based target prediction approach that leverages the chemical similarity principle — stating that molecules sharing similar structural features (potential pharmacophore points) are likely to have similar bioactivity[13]—to perform target prediction. Moreover, TIGER benefits machine learning methodologies and cheminformatics.[14,15] While cheminformatics plays a pivotal role in handling and structur-

TABLE 1 Target predictions for potential baricitinib and tofacitinib drug–target interactions suggested by TIGER relevant for Alzheimer's disease (AD).

Drug	Predicted targets associated with AD
baricitinib	Metabotropic glutamate receptor 1 (MGlu1)
	Dual leucine zipper kinase (MAP3K12)
	Casein kinase II subunit β (CK2-β)
	Carbonic anhydrase II (CA2)
	PI3-kinase P110 subunit α (PIK3CA)
	Ras-related protein Rab-7a (RAB7a)
tofacitinib	Phosphodiesterase 8B (PDE8A)
	Glutaminyl cyclase (GC)
	Inducible nitric oxide synthase (iNOS)

Abbreviation: AD, Alzheimer's disease.

ing chemical data by calculating molecular descriptors and assessing chemical similarity between ligands, machine learning is used for model training, feature selection, and making predictions regarding potential ligand–target interactions.

Baricitinib and tofacitinib were provided as simplified molecular input line entry system (SMILES)[16] strings and pre-processed in KNIME v3.7.2,[17] using the MOE v.2019.0102[18] "wash" function for structure standardization (using the following options: "disconnect salts," "remove lone pairs," "deprotonate strong acids," "remove minor component," "protonate strong bases," and "add hydrogen"). Chemically advanced template search version 2 (CATS2)[19] descriptors and two-dimensional MOE descriptors ("QSAR descriptors" node of KNIME; "Forcefield" = MMFF94*) were calculated for all generated molecules and used as input for target prediction. Targets with TIGER score > 1 were retained for follow-up analysis. The cut-off value on the TIGER score was chosen based on recent prospective studies in which predicted targets were successfully confirmed experimentally.[11,20,21] Targets were further filtered based on their relevance on AD after screening scientific literature. Finally, targets that were not previously identified as off-targets of baricitinib or tofacitinib were selected for in vitro testing.

2.2 | In vitro characterization

Baricitinib (99.97% purity) and tofacitinib (99.96% purity) were purchased from MedChem Express LLC[22] and Biosynth Carbosynth.[23] Both drugs were tested in vitro on a selection of protein targets associated with AD. The protein targets (Table 1) were chosen using the TIGER target prediction. Glutaminyl cyclase and Ras-related protein RAB-7 experiments were conducted by directly visualizing protein inhibition. The concentrations of baricitinib and tofacitinib were increased incrementally, up to a maximum concentration of 200 μM (see Table S1 in supporting information). Other targets were performed on a fee-for-service basis at Eurofins.[24] In these in vitro biochemical assays, if the drug showed inhibition or stimulation exceeding 25% at a concentration of 30 μM in two separate measurements, binding constants (K_d) were determined. Assay details are included in supporting information.

RESEARCH IN CONTEXT

1. **Systematic review:** We searched the literature for reports investigating the repurposing of Janus kinase (JAK) inhibitors for Alzheimer's disease (AD), particularly due to activity on unintended off-targets. Much remains to be identified on the potential effects of JAK inhibitors in AD.
2. **Interpretation**: To explore the potential AD progression associated with off-targets of baricitinib and tofacitinib, we used a machine learning-based target prediction tool. Additionally, we conducted in vitro experimental characterization of the predicted targets and used physiologically based pharmacokinetic (PBPK) modeling for estimating drug concentration in the brain. Our approach led to the identification of previously unknown putative drug–target interactions of baricitinib. However, target affinities appear to be low compared to anticipated drug bioavailability at the target, suggesting a low likelihood of successfully repurposing baricitinib in AD.
3. **Future directions**: The combination of multiple approaches can identify and characterize previously unknown drug–target interactions of other approved drugs potentially relevant for AD progression.

2.3 | Brain concentration prediction

Due to the lack of existing information on baricitinib's potential to cross the blood–brain barrier in humans, a permeation assay was performed by Eurofins in the MDCKII cell line. The mean permeability of baricitinib from the apical to the basolateral side (A to B) was 4.5×10^{-6} cm/s, and the B to A was 5.5×10^{-6} cm/s. According to Palmer and Alavijeh,[25] this moderate permeability value falls within the acceptable range for a desired target profile of a central nervous system (CNS) drug candidate.

A PBPK model, with seven compartments, among them brain vasculature and brain tissue, for baricitinib was developed in Berkeley Madonna 10.[26] The PBPK model structure is presented in Figure S1 in supporting information, and the modeling script, including annotations of parameters, is provided as supporting information (Modelling Script). Physiological parameters included organ volumes and blood flow rates for a standard human male.[27–30] Blood-to-tissue partition coefficients were estimated in silico from Rodger & Rowland's algorithm based on log *K*, *pKa*, and molecular weight.[31] Absorption rates and clearance values were from a previous baricitinib model.[32]

Administration was modeled as a single oral dose of 4 mg (maximum recommended daily dose) baricitinib. From the gut, uptake to the liver was modeled with a first-order rate constant determined in a previous study,[28] then distributed to systemic circulation. Estimates of baricitinib concentrations in blood plasma over time were validated with a previous model.[32] Other organs were categorized as slowly perfused (i.e., muscles, adipose, bone, skin) or rapidly perfused (i.e., heart, lung, spleen, kidneys) tissue. Urinary excretion was modeled based on previously established clearance values.[32]

Concentrations of baricitinib in the brain were computed by different approaches (Figure S1). First, it was estimated to be 0.91% of blood concentration, as suggested by the Quantitative Structure–Activity Relationship (QSAR) tool of the PreADMET webserver).[33,34] This estimation is further referred to as "Prediction 1 [QSAR]." The second approach, "Prediction 2 [Mouse exp.]", relied on a brain-to-plasma concentration ratio of 20%, which was experimentally observed in mice.[35] Finally, the third approach involved modeling of the blood–brain barrier (BBB) permeation using the quantitative in vitro–in vivo scaling methodology developed by Ball et al.[30] This last estimation of baricitinib concentration in the brain tissue is herein mentioned as "Prediction 3 [QIVIVE BBB]."

The impact of parameter deviation on the model's predictions was assessed by a sensitivity analysis based on the method by Evans and Andersen[36] (see Table S2 in supporting information). For this, each model parameter was individually increased by 5% and the associated impact on maximal brain tissue concentrations (Prediction 1 [QSAR], Prediction 2 [Mouse exp.], and Prediction 3 [QIVIVE BBB]) was computed. Oral administered dose was maintained at 4 mg. Normalized sensitivity coefficients (SC) were determined by using Equation 1:

$$SC = \frac{(C' - C)}{(P' - P)} \times \frac{P}{C} \quad (1)$$

C and C' refer to the maximal concentration of baricitinib in brain tissue (Prediction 1 [QSAR], Prediction 2 [Mouse exp.], or Prediction 3 [QIVIVE BBB]) with unchanged parameters or one elevated parameter, respectively, P and P' to the value of the unchanged or elevated parameter of interest.

To address the impact of parameter uncertainty on these predicted concentrations of baricitinib in the brain, their calculation has been iteratively repeated 1000 times, with the most sensitive parameters (having the greatest influence on the results) being re-sampled in each iteration. Monte Carlo simulations were performed with Berkeley Madonna 10 associated functions for all the parameters found with an absolute value of normalized sensitivity coefficient > 0.1 for at least one brain concentration (Prediction 1 [QSAR], Prediction 2 [Mouse exp.], or Prediction 3 [QIVIVE BBB]). Parameter simulated distributions were determined according to literature.[32,37,38] One thousand simulations were performed, and results were analyzed by comparing first quartile, median, and third quartile values for each time point for each of the brain concentrations (Prediction 1 [QSAR], Prediction 2 [Mouse exp.], and Prediction 3 [QIVIVE BBB]).

3 | RESULTS

The list of 78 potential drug–target interactions predicted with TIGER score > 1 (baricitinib [n = 31]; tofacitinib [n = 47]) was published elsewhere.[9] Of those 78, we selected 9 potential drug–target interactions which are known to be relevant for AD (Table 1, [baricitinib [n = 6]; tofacitinib [n = 3])[39–44] and experimentally validated these 9 predictions using biochemical or cell-based assays.

Of the nine drug–target interactions tested, CK2-α2 and dual leucine zipper kinase (MAP3K12) were inhibited by > 25% and were characterized further by quantification of K_d (Table 2). The K_d values were in the micromolar range (two-point measurements, CK2-α2-baricitinib K_d = 5.8 μM [5.4 μM; 6.1 μM]; MAP3K12-baricitinib K_d = 5.8 μM [5.5 μM; 6.1 μM], in, respectively, Table S4 and S5 in supporting information). Concentration-dependent inhibition profiles for CK2-α2-baricitinib and MAP3K12-baricitinib are shown in, respectively, Figure S2 and Figure S3 in supporting information.

Among estimations of the concentration of baricitinib available in the brain, Predictions 2 [Mouse exp.] and 3 [QIVIVE BBB] were similar (see concentration vs. time profiles in Figure S4 in supporting information). The distribution of maximal brain concentrations predicted by Monte Carlo simulations of the PBPK model is presented in Table 3. Median C_{max} values range from 1.3 nM (for Prediction 1 [QSAR]) to 23 nM (for Prediction 2 [Mouse exp.] and Prediction 3 [QIVIVE BBB]), which is, respectively, > 4000 times and 200 times lower than the minimum K_d value from in vitro experiments (Table 2).

4 | DISCUSSION

In this work, we used a multidisciplinary approach to explore the off-target effects of JAK inhibitors in the context of AD. We combined ligand- and machine learning–based target prediction to identify previously unknown drug–target interactions of baricitinib and tofacitinib. We subsequently conducted in vitro experiments to confirm the predicted drug–target interactions. This led to the identification of two enzymes previously unknown to be inhibited by baricitinib (CK2-α2 [K_d = 5.8 μM]; MAP3K12 [K_d = 5.8 μM]). Additionally, we predicted concentrations of baricitinib in brain tissue using PBPK modeling. The predicted maximum concentrations were found to be between 1.3 and 23 nM, which is two to three orders of magnitude below the corresponding binding constant. The putative off-target effect of baricitinib adds to the evidence that the drug potentially modulates the activity of additional proteins on pathways and cellular processes involved in the pathogenesis of AD. Nevertheless, target affinities are too low compared to anticipated drug bioavailability at the target.

CK2 is an active serine–threonine protein kinase that modulates multiple signaling pathways.[45,46] Abnormal CK2 signaling is associated with several diseases, including numerous neurological conditions.[47] The high activation of CK2 in AD is associated with abnormal phosphorylation of tau protein.[48] This abnormal phosphorylation contributes to the formation of neurofibrillary tangles, which are linked to the

TABLE 2 In vitro characterization of baricitinib and tofacitinib for inhibiting the selected macromolecular targets.

Drug	Predicted target	Assay type	K_d (μM)
baricitinib	Casein kinase II subunit α2 (CK2-α2)[a,b]	binding	5.4, 6.1[c]
	Carbonic anhydrase II (CA2)[d]	binding	inactive
	PI3-kinase P110-α subunit (PIK3CA)[d]	binding	inactive
	Metabotropic glutamate receptor 1 (MGlu1)[a,b]	cell-based	inactive
	Dual leucine zipper kinase (MAP3K12)[b]	binding	5.5, 6.1[c]
	Ras-related protein rab-7a (RAB7a)	binding	inactive
tofacitinib	Glutaminyl cyclase (GC)	binding	inactive
	Inducible nitric oxide synthase (iNOS)[d]	binding	inactive
	Phosphodiesterase 8A (PDE8A)[d]	binding	inactive

Abbreviation: K_d, binding constant.
[a]Antagonistic effect.
[b]The drug was tested in multiple concentrations (top concentration of 100 μM).
[c]K_d determination with $N = 2$. No averaging was made, and both values were presented.
[d]The drug was tested at a concentration of 30 μM.

TABLE 3 Predictions of maximal baricitinib concentrations in the brain by 1000 Monte Carlo simulations of the PBPK model after 4 mg oral intake.

		Model brain tissue concentrations		
		Prediction 1[QSAR]	Prediction 2 [Mouse exp.]	Prediction 3 [QIVIVE BBB]
C_{max} (nM)	First quartile	1.1	19	18
	Median	1.3	23	23
	Third quartile	1.6	27	29

Abbreviations: C_{max}, maximum concentration of baricitinib in the brain; Mouse exp, experimentally observed in mouse; QIVIVE BBB, quantitative in vitro-in vivo extrapolation of blood-brain barrier permeation; QSAR, quantitative structure–activity relationship.

progression of the disease. In addition, elevated CK2 activity may also enhance β-secretase (BACE1) transcription, which is the first and rate-limiting step in the production of amyloid beta (Aβ), the main constituent of amyloid plaques.[42,43]

While inhibition of BACE1 cleavage of amyloid precursor protein (APP) seemed to be an attractive approach to treat AD, potent BACE1 inhibitors, such as atabecestat, verubecestat, and lanabecestat, were developed and tested regarding efficacy and safety during clinical studies in patients with AD.[49–52] However, these compounds failed later phases of randomized clinical trials due to the lack of efficacy or safety reasons. Although the complete pharmacological inhibition of BACE1 activity leads to detrimental adverse events in the randomized controlled trials, it remains to be established if only a low degree of BACE1 inhibition levels (as a result of the off-target inhibition of CK2 activity by baricitinib, for example) may be needed to decrease Aβ production.

Another critical signaling pathway in neurological disorders is the c-Jun N-terminal kinase (JNK). This family of protein kinases plays a crucial role in neuronal plasticity, regeneration, cell death, and regulation of cellular senescence.[37] MAP3K12 works as an injury sensor that initiates the JNK-dependent stress response in neurons to mediate context-dependent axon re- and degeneration.[38] Notably, inhibition of MAP3K12 is suggested to selectively regulate a JNK pathway that mediates neuronal degeneration and apoptosis. Therefore, there is considerable interest in identifying MAP3K12 inhibitors for use in chronic neurodegenerative indications.[27,39–41]

Moving forward, to further investigate baricitinib as a potential treatment in AD, we predicted its brain concentration using a simple PBPK model. Implementing PBPK models is a key aspect of drug development to predict in vivo absorption, distribution, metabolism, and excretion (ADME) processes, for a large variety of applications while reducing costs, time, and ethical issues associated with animal experimentation.[58] Due to the difficulty in measuring in vivo human BBB permeability, this effort is especially relevant for CNS drug candidates, which have a higher failure rate.[59,60]

The binding affinities between baricitinib and each target (CK2-α2 and MAP3K12) were measured and reported as K_d values, representing its half saturation/occupancy binding concentration for the putative target. For CK2-α2 and MAP3K12, K_d values were in the micromolar range (Table 2), suggesting that to achieve sufficient binding of the ligand to the target to trigger a biological effect, the concentration of baricitinib in the brain would be around or above the K_d value. To estimate the anticipated concentration that could be reached in the brain, we used PBPK modeling to perform quantitative in vitro to in vivo extrapolation (QIVIVE). These results suggested a maximal concentra-

tion of baricitinib in the brain in the nanomolar range (1.3 to 23 nM), ≈ 4000 times and 200 times lower than the K_d value for CK2-α2 and MAP3K12, respectively. Because the ligand concentration in the brain is anticipated to be orders of magnitude lower than the K_d, binding of baricitinib with either target after a 4 mg oral dose would be limited or even negligible, and thus, a pharmacological effect is unlikely to occur. Furthermore, although both CK2-α2 and MAP3K12 are expressed in different tissues,[46,61] with the low plasma concentration of 97 nM measured in clinical studies,[27] off-target effects outside the brain are also not likely.

While the evidence is limited, our findings align with the existing real-world evidence, which has failed to identify a significant association between the use of JAK inhibitors and AD. An observational study using electronic health records (EHRs) was recently conducted to test whether the use of JAK inhibitors was associated with the risk of AD in a large population. The study found no differences in the risk of AD in patients treated with tofacitinib compared to those treated with abatacept, a tumor necrosis factor (TNF)-α inhibitor drug.[62] However, it is worth noting that the mean follow-up duration was relatively short, only 6 months. As AD is a slow, progressive disease, it is likely that the short follow-up period resulted in limited statistical power to detect smaller differences in magnitude and was not able to detect delayed treatment outcomes. While the authors of the study suggest the null finding could be due to the short follow-up duration in their study, thereby limiting the statistical power and ability to detect delayed treatment outcomes, the findings are in line with our experimental evidence.

While our findings reveal a low likelihood of successfully repurposing tofacitinib and baricitinib in AD due to off-target effects, recent studies suggest that tofacitinib and baricitinib may modulate neuroinflammation and neurodegeneration processes due to on-target effects (i.e., via modulation of JAK-STAT pathways).[1,63] The use of baricitinib in patients with neurodegenerative diseases, including AD, is currently under clinical investigation (NCT05189106) to assess whether the JAK inhibitor reaches therapeutic levels in the CNS and may suppress neuroinflammation. Therefore, potential repurposing of baricitinib to treat neurodegeneration-related conditions should not be ruled out.

Although the combined use of computational and experimental approaches allowed us to identify and characterize previously unknown off-target interactions for baricitinib (CK2-α2 and MAP3K12), adding to the known target space of baricitinib, there are a few limitations in our approach. First, there might be additional targets of relevance that were not predicted by the TIGER computational tool. Moreover, although TIGER encompasses multiple protein families, it is limited by the manual annotation of the molecules' target information in the collection of biologically active reference molecules.[64] Second, we acknowledge that the activity of small-molecule drugs using in vitro assays does not always translate into activity in the cellular environment. Thus, the results should still be interpreted cautiously and treated as preliminary evidence for the off-target binding of baricitinib and tofacitinib. Third, as the predictive accuracy of the PBPK model is highly dependent on available pharmacokinetic data for baricitinib, the computation of drug concentration in the brain could be improved if information related to the unbound fraction in the brain or intrinsic transcellular permeability were available.[60] Moreover, the PBPK model prediction only addresses the concentration of baricitinib in a compartment of interest, not its pharmacological effect. A pharmacodynamic model capable of integrating ligand-binding interactions would provide additional justification for predicting drug effects.[65]

Finding potential drug candidates among already approved medication for other indications to address the urgent need for disease-modifying pharmacological treatments for AD remains an important goal. By synergistically integrating multiple approaches used in drug development, we have successfully identified previously unknown drug–target interactions for baricitinib. We further assessed the brain distribution of baricitinib and found that its low permeability considerably reduces its suitability for repurposing in AD. While additional research is needed to evaluate the implications of potential baricitinib off-target in the context of AD, this comprehensive approach can help optimize drug repurposing efforts by increasing the chances of successful potential candidates for repurposing in AD.

5 | CONCLUSION

Due to recent interest in JAK inhibitors as promising drug candidates for treatment of AD, we designed a multidisciplinary approach to investigate this potential effect for baricitinib and tofacitinib. Using machine learning, we predicted new off-targets of baricitinib related to AD. Previously unknown inhibition of two enzymes (CK2-α2 and MAP3K12) by baricitinib was confirmed using in vitro experiments. While our PBPK model suggested a low likelihood of successfully repurposing this drug in AD due to low brain permeability even at the maximum recommended daily dose, we have demonstrated the added benefit of a multidisciplinary approach that combines ML target prediction, in vitro confirmation, and PBPK modeling that may optimize efforts in drug repurposing in AD.

AUTHOR CONTRIBUTIONS

Andrea M. Burden, Maria L. Faquetti, Francesca Grisoni, and Shana J. Sturla devised the concept. Andrea M. Burden, Gisbert Schneider, and Shana J. Sturla provided resources. Petra Schneider and Gisbert Schneider developed the target prediction methods. Francesca Grisoni and Maria L. Faquetti designed the computational workflow for target prediction. Hélène Bigonne and Georg Aichinger designed and performed the PBPK model. Maria L. Faquetti, Petra Schneider, Laura Slappendel, Georg Aichinger, and Hélène Bigonne performed the experiments. Maria L. Faquetti, Laura Slappendel, Hélène Bigonne, Georg Aichinger, Gisbert Schneider, Shana J. Sturla, and Andrea M. Burden analyzed and interpreted results. Maria L. Faquetti, Laura Slappendel, and Hélène Bigonne wrote the original draft of the manuscript. All authors reviewed and edited the manuscript.

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CONFLICT OF INTEREST STATEMENT

P.S. and G.S. declare a potential financial conflict of interest as co-founders of inSili.com LLC, Zurich, and in their role as scientific consultants to the pharmaceutical industry. M.L.F., L.S., H.B., G.A., S.J.S., and A.M.B. declare no conflicts of interest. Author disclosures are available in the supporting information.

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SUPPORTING INFORMATION

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

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