Lead Antimalarial Identification Using *In Silico* Prediction Methods and Simulation

D Morris, W Woltosz, M Lawless, T Grasela, R Clark

Simulations Plus, Inc., Lancaster, CA
simulations-plus.com

ABSTRACT

Background. With increasing resistance to currently available antimalarials, new compounds with activity against resistant parasites are needed. Novel compounds were designed and first-in-human (FIH) simulations were performed, based on *in silico* predictions, to identify lead compounds. The most optimal lead compounds were then synthesized and *in vitro* experimental values were determined and compared with the *in silico* predictions.
Methods. Literature-based compounds known to inhibit dihydroorotate dehydrogenase were used to build a quantitative structure-activity model in ADMET Predictor™. Compounds active against *Plasmodium falciparum* (based on a phenotypic blood culture assay screen; PubChem Bioassay, AID 2306) were then used to identify attractive structural classes of antimalarials using MedChem Studio™. Novel compounds were generated by recombining substituents of the best compounds in the selected class. First-in-human plasma concentration (Cp) predictions in GastroPlus™, using the *in silico* predicted physicochemical properties, were used to select suitable lead compounds with acceptable dosage profiles. The selected compounds were synthesized and experimental versus *in silico* properties were compared.
Results. The synthesized lead compounds were determined to have more potent biological activity than the structurally related literature-based compounds, and the predicted and experimentally determined potencies were consistent. Experimental and predicted physicochemical properties were generally in agreement (RMSE of 0.6 log units).
Conclusion. *In silico* tools can be used to design, assess, and strategically identify potential antimalarial lead compounds with acceptable activity, risk, and human exposure profiles.

BACKGROUND

In 2015, there were approximately 214 million malaria cases and an estimated 438,000 deaths.[1] With growing resistance to current antimalarial drugs, the need for new compounds with activity against resistant parasites is critical.[2,3] *Plasmodium falciparum* (*P. falciparum*, *Pf*) is the most common species responsible for malaria infection. One characteristic that is present in humans but absent in the *Plasmodium* species is the ability to salvage preformed pyrimidine bases for pyrimidine synthesis. As such, this species solely depends on the *de novo* synthesis of pyrimidines for cell growth.[4,5] *Plasmodium falciparum* dihydroorotate dehydrogenase (*Pf*DHODH) is one of the enzymes critical in the *de novo* pyrimidine synthesis pathway for *P. falciparum*[4,5] and, thus, a potential target for antimalarial drug therapies. By combining *in silico* tools with the use of publically available *in vivo* activity data for *P. falciparum*, novel compounds were designed, synthesized, and experimentally evaluated as lead compounds for the treatment of *P. falciparum* malaria.

METHODS

Predicting *Plasmodium falciparum* Dihydroorotate Dehydrogenase Activity

In order to predict *Pf*DHODH inhibition, regression models were constructed using an artificial neural network ensemble in ADMET Predictor™. The goal of these models was to adequately predict the anti-*P. falciparum* activity (K_i, inhibitory constant) of a diverse set of published compounds known to be *Pf*DHODH inhibitors.

Identifying Attractive Classes

The regression models obtained were then used to predict anti-*P. falciparum* activity in a large set of publically available compounds screened for *in vivo* activity (red blood cells) of *P. falciparum* (3d7) growth inhibition (PubChem AID 2306), multi-drug resistant *P. falciparum* (Dd2) growth inhibition (PubChem AID 2302), and cytotoxicity (PubChem AID 2303). The *in vivo* activity data, target (*Pf*DHODH) inhibition data, and the absorption, distribution, and metabolism (ADMET) risks for these compounds were assessed. Structural classes from the PubChem dataset were then generated in MedChem Studio™ and evaluated based on favorable growth inhibition (*P. falciparum* and drug-resistant *P. falciparum*), reduced cytotoxicity, targeted inhibition with respect to *Pf*DHODH, and favorable ADMET risk profiles.

Generation and Synthesis of Lead Compounds

Substituents (R-groups) within the best structural classes were recombined to generate novel lead compounds which were evaluated on the basis of anti-*Pf*DHODH activity, ADMET risk profiles, and *in vivo* predicted profiles. Compounds predicted to have good potency as well as favorable ADMET risk and *in vivo* (pharmacokinetic [PK]) profiles were selected for synthesis. Experimental properties (*in vivo* activity, potency, and physicochemical properties) were then determined for the synthesized compounds and compared to the predicted (*in silico*) values.

RESULTS

Predicting *Plasmodium falciparum* Dihydroorotate Dehydrogenase Activity

Two models were developed for prediction of *Pf*DHODH activity. Model 1 was more conservative (fewer false positives) and Model 2 more sensitive (fewer false negatives) (**Figure 1**). However, both models did an adequate job in predicting measured *Pf*DHODH activity (**Figure 2**) and were used together for prediction of activity for potential lead compounds.

Figure 1. Model 1 Versus Model 2 Predictive Performance

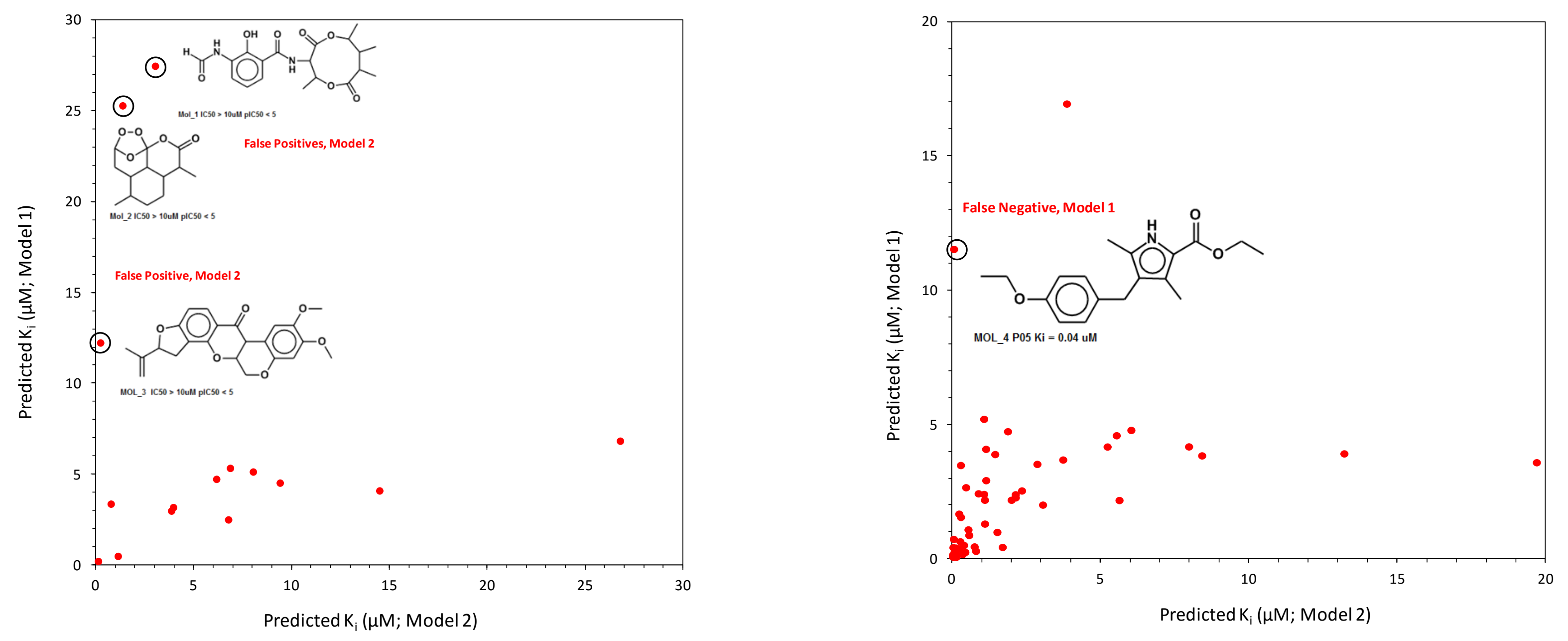
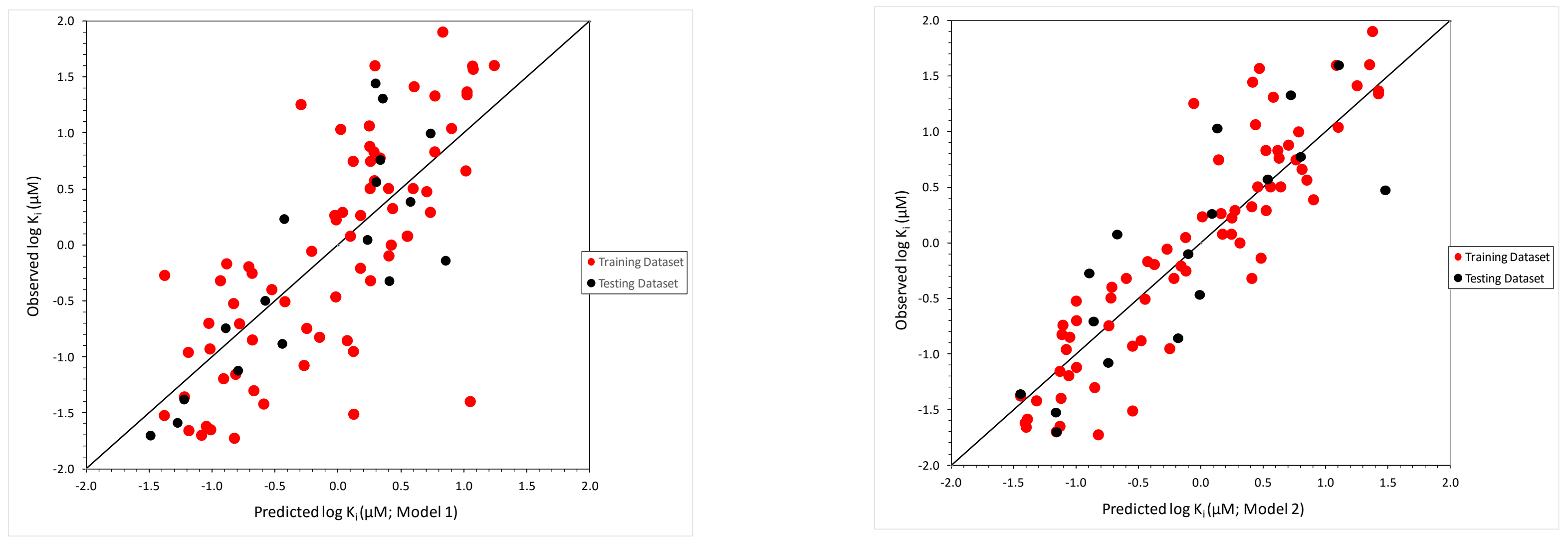


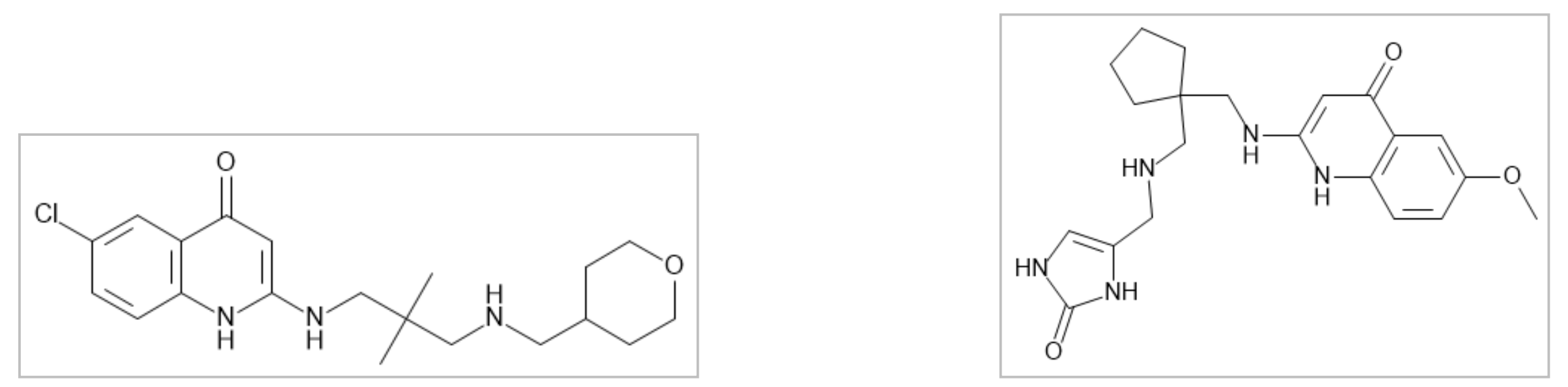
Figure 2. Comparison of Regression Models



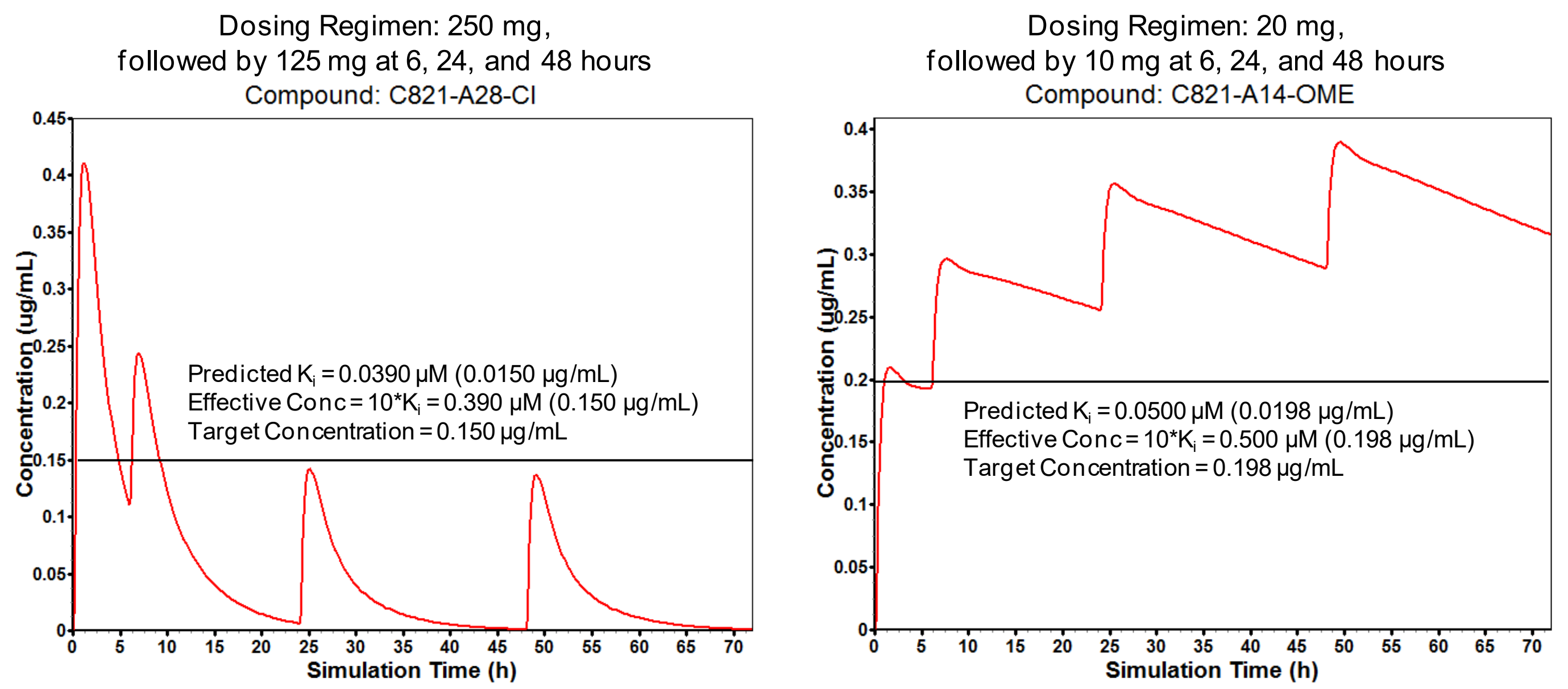
Generation and Synthesis of Lead Compounds

Twelve compounds were originally selected as possible lead compounds, however, only 8 were selected for synthesis (based on potency, ADMET risk, and *in vivo* [PK] profiles) (**Figure 3**).

Figure 3. Example of Lead Compounds



Candidate	Pred K_i	ADMET Risk
C821-A28-Cl	0.039 μM	1 (2D6)
C821-A14-OME	0.050 μM	1 (HD)



Acceptable potency and ADMET risk profile, but unfavorable PK profile; not suitable for synthesis

Acceptable potency and ADMET risk and PK profiles; suitable for synthesis

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For additional information, please contact
Denise Morris, PhD
Cognigen Corporation, a SimulationsPlus company
1780 Wehrle Drive, Suite 110, Buffalo, NY 14221
(716) 633-3463, ext. 340 or denise.morris@cognigencorp.com

Comparison of Experimental and Predicted (*in silico*) Properties of Synthesized Compounds

Biological activity of the synthesized compounds was determined under the same conditions as those used in the PubChem compound library. **Table 1** shows the experimental results obtained in asynchronous (mixed) cultures. In general, most of the synthesized compounds displayed more potent activity when compared to similar compounds from the PubChem library. The concentrations required to kill half of the organisms (XC_{50}) were greater than the concentrations predicted to provide 50% inhibition (K_i) of *Pf*DHODH. These results are consistent with *Pf*DHODH inhibition being the mode of action. The differences between the anti-*Pf*DHODH activity and functional efficacy (growth inhibition) results could be attributed to the many barriers between the drug and its site of action, for example, plasma protein binding and membrane penetration. The consistently low attenuation in potency (resistance ratio +/-, 2 to 6-fold) for the chloroquine resistant strain (Dd2) is also promising for this class and mode of action.

Table 1. Predicted Inhibitory Constants and Observed Biological Activities for Synthesized Lead Candidates

		Pred. DHODH	XC50 (μM)[a,b]		Resistance
SLP ID	Structure	K_i (μM)	3d7(-)	Dd2(+)	ratio(+/-)
0007	A0-B5-C6a	0.049	10.0	46	4.6
0008	A2-B5-C6a	0.051	1.61	6.4	3.9
0004	A0-B5-C3	0.023	0.55	2.3	4.1
0010	A3-B5-C4	0.037	0.37	1.78	4.8
0005	A0-B5-C4	0.037	0.30	1.47	5.0
0003	A0-B5-C5a	0.025	0.106	0.21	2.0
0006	A2-B5-C4	0.038	0.037	0.24	6.6
(GSK)[c]	A0-B0-C4a	0.112	0.89	4.6	5.2
(GSK)[c]	A0-B0-C4b	0.077	0.85	8.6	10.1

[a] Concentration required to reduce growth in *asynchronous* blood culture by 50%
[b] (-) and (+) denote chloroquine-susceptible and -resistant strains, resp.
[c] Close structural analogs from the PubChem Library (PubChem AID 2306 and 2303).

Select ADMET properties for the synthesized compounds were measured by Absorption Systems (Exton, PA) and compared with predictions from ADMET Predictor™, Version 6.1 (AP 6.1). Most of the observed and predicted properties were in good agreement (RMSE 0.6 log units or less, data not shown), with the exception of predicted intrinsic clearances (which were generally lower than the experimental values). These predictions were flagged as out of scope, indicating that the compounds were not within the chemical/molecular descriptor space of the ADMET model used (constructed from known compound databases [training sets]). Once ADMET Predictor™ was updated with a more expansive training set (AP 8.1), the *in silico* predictions were re-run. In general, there was even better agreement with the observed and predicted data and fewer out-of-scope predictions (**Table 2** and **Table 3**). In addition, one of the synthesized compounds (SLP-0003) was predicted to have a favorable *in vivo* profile, even with the high predicted (and confirmed) intrinsic clearance values (**Figure 4**).

Table 2. AP 8.1 Predicted and Experimental Physicochemical Properties for Synthesized Lead Compounds

Property	SLP-0003	SLP-0004	SLP-0005	SLP-0006	RMSE
S+Sw (mg/mL)	0.32	3	2	1	11-fold[a]
obsd. solubility[a]	0.76[b]	32	33	22	
S+logP	5.05	4.3	4.7	5.0	±0.63
obsd. logP	4.4	3.5	4.2	5.55	
S+pK_{a1}[c]	(5.24)[c]	(5.29)	(5.24)	(5.14)	±0.63
obsd. pK_{a1}	4.80	4.22	4.95	4.75	
S+pK_{a2}	7.86	8.08	8.65	7.96	±0.39
obsd. pK_{a2}	7.36	7.80	8.33	7.55	
S+log$D_{6.8}$	3.96	3.00	2.86	3.84	±0.54
obsd. log$D_{6.8}$	3.76	2.46	2.68	4.73	

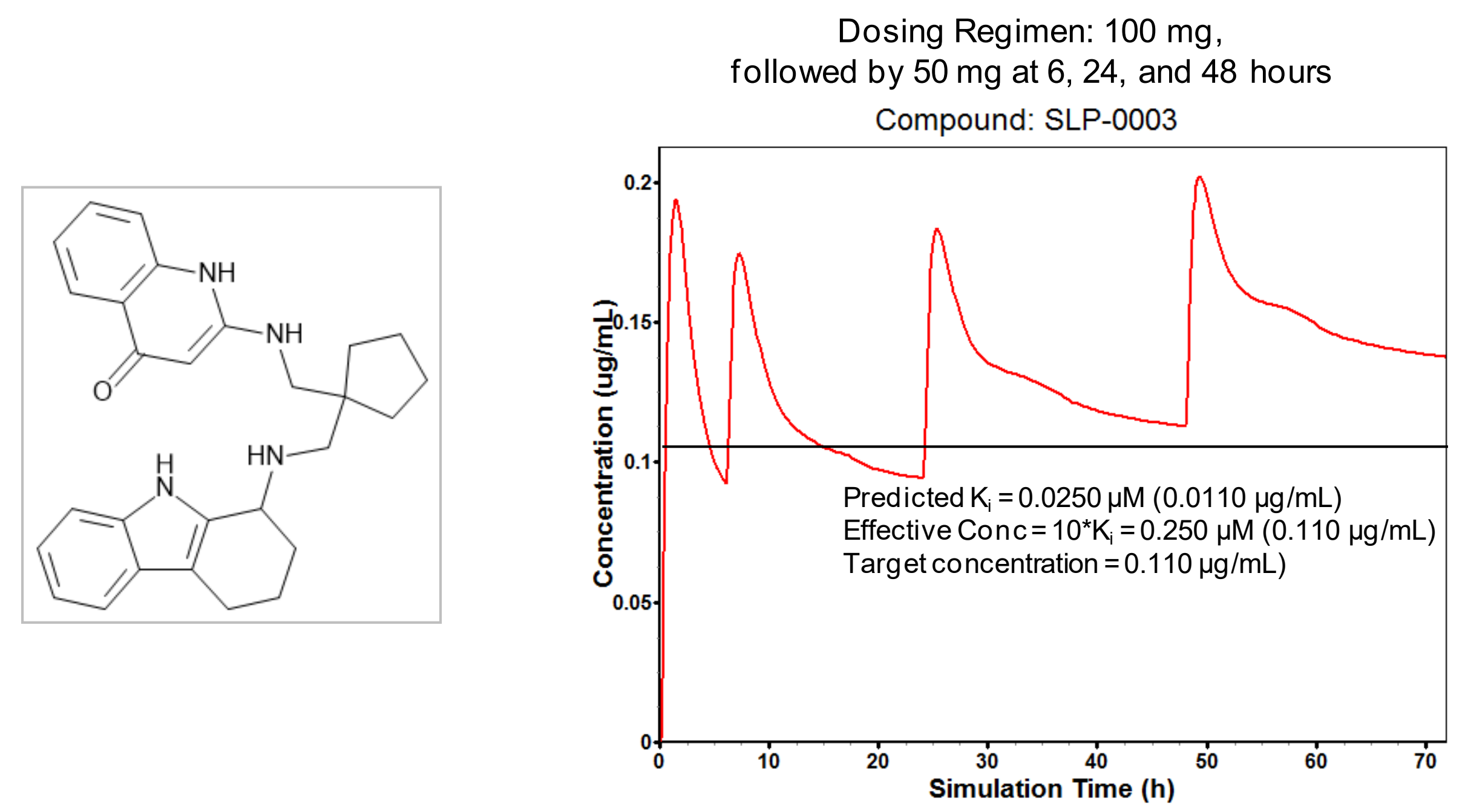
[a] Solubility of the amorphous solid, which is an upper bound on the thermodynamic solubility.
[b] Retested value; original was 0.013 μg/mL.
[c] Calculated for the hydroxy tautomer.

Table 3. AP 8.1 Predicted Recombinant CYP Intrinsic Clearance Data for Synthesized Lead Compounds

CYP	Property	SLP-0003	SLP-0004	SLP-0005	SLP-0006	
1A2	Substrate[a]	Yes (63%)	Yes (63%)	Yes (58%)	Yes (55%)	
	Pred. $CL_{1 \mu M}$[b]	1.43	1.39	2.2	2.6	5-fold
	Obsd. $CL_{1 \mu M}$	9.3[c]	7.3[c]	10	ND[d,e]	
2C9	Substrate[a]	No (64%)	No[f]	No (69%)	No[f]	
	Pred. $CL_{1 \mu M}$[b]	61	78	54	83	CL_{int} predicted as if they were substrates
	Obsd. $CL_{1 \mu M}$	ND[d,e]	1.8[d]	4.1[d]	ND[d,e]	
2C19	Substrate[a]	No (99%)	No (99%)	No (93%)	No (97%)	
	Pred. $CL_{1 \mu M}$[b]	26	118	159	74	
	Obsd. $CL_{1 \mu M}$	3.8	3.5	14.6	2.2[c]	
2D6	Substrate[a]	No (59%)	Yes (58%)	Yes (81%)	Yes (58%)	
	Pred. $CL_{1 \mu M}$[b]	115	63	121	28	4-fold
	Obsd. $CL_{1 \mu M}$	10.6	30	31	18	
3A4	Substrate[a]	Yes (98%)	Yes (98%)	Yes (98%)	Yes (98%)	
	Pred. $CL_{1 \mu M}$[b]	83	94	23	49	5-fold
	Obsd. $CL_{1 \mu M}$	179	298	256	263	
HLM	Pred. $CL_{1 \mu M}$[b]	235	208	244	359	2-fold
	Obsd. $CL_{1 \mu M}$	120	230	220	620	

[a] Predicted to be a substrate (Yes/No; percent confidence)?
[b] Clearance at 1 μM expressed as μL/min/mg HLM protein.
[c] Possibly a substrate.
[d] Unlikely to be a substrate.
[e] Not detected.
[f] Out of scope.

Figure 4. GastroPlus™ *In Vivo* Simulation Using AP 8.1 Predicted Metabolism Data



CONCLUSIONS

The synthesized lead compounds were predicted to have more potent biological activity than the structurally related literature compounds, and the experimentally determined potencies were consistent with that expectation. Updated/improved predictions for physicochemical properties and intrinsic clearances, based on a more robust training set, reinforce the use of *in silico* tools as an adequate screening approach for determining potential lead compounds. The limitations of the earlier predictions highlight the ongoing need for software tools (such as ADMET Predictor™) to continuously refine their predictive capabilities, while the positive overall outcome underscores their value in drug design.

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