Poster T3038

Novel Antimalarial Drug Candidates Generated *in silico* by Analysis of Public HTS Data

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Abstract

Purpose: Carry out a prospective experiment to demonstrate the ability of *in silico* drug design tools to design new lead drug candidates from phenotypic screening data.

Methods: We developed quantitative structure-activity relationships (QSARs) for two potential target enzymes using ADMET Predictor™ (AP), then used those models to predict target enzyme activities for the 13,533 compounds identified as active in a high-throughput antimalarial screening program recently made public by GlaxoSmithKline (Pubchem biossay AID 2306). Applying these models and the class generation technology in MedChem Studio™ to those data enabled us to identify a particular class of chemistry as likely to inhibit one of our chosen targets, *Plasmodium falciparum* dihydroorotate dehydrogenase (PfDHODH). Combinatorial elaboration followed by *in silico* screening based on a wide variety of ADMET liabilities (ADMET Risk™) produced a range of candidate molecules that were then further elaborated to include substituents not reported by GSK.

Results: Several novel compounds designed on the basis of their predicted biological activities and ADMET properties were successfully synthesized and assayed. Two of the first four candidates synthesized exhibited half-maximal growth inhibition at concentrations below 100 nM when assayed against wild-type parasites in blood culture. Potencies were markedly less (though still sub-micromolar) when measured against chloroquine-resistant strains of the parasite. pKa, logD, solubility and cytochrome P450 (CYP) susceptibilities were reasonably well-predicted by AP and rank order estimates of half-lives were confirmed by measurements made using human liver microsomes.

Conclusions: The results indicate that the most potent NCEs have half-lives that are too short for them to be clinical candidates. The *in silico* design methods were remarkably successful, however, in identifying and advancing a promising area of antimalarial chemistry with a very limited expenditure of time and money. Further work remains before compounds from this structural class will make it to the clinic, but additional analogs may address the shortcomings of the compounds tested to date.

References

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Screening Data

PubChem AID 2306 contains data from a screen for growth inhibition of a chloroquine-susceptible ("wild type") malaria strain (3d7) cultured in parasitized red blood cells. Data and structures for 13,456 compounds that were active when tested at 2 μM were donated to the public domain by GlaxoSmithKline [2].

AID 2302 contains data from a 2 μM counter-screen against a chloroquine-resistant strain (Dd2).

Building Predictive Models

Two parasite enzymes were chosen as potential targets: dihydroorotate dehydrogenase (DHODH), which is central to the *de novo* pyrimidine synthesis pathway critical to parasite survival [3,4]; and (Pfmrk), a regulatory cyclin-dependent kinase (CDK) [4,5].

Literature data on diverse sets of inhibitors were used to construct artificial neural net ensemble (ANNE) regression models in ADMET Modeler™ (a module of ADMET Predictor™); data from the PubChem screens were not used. Figure 1 illustrates the predictive performance of one of two models subsequently used to predict PfDHODH inhibition. A second, complementary model was built that accounted for the highlighted outlier.

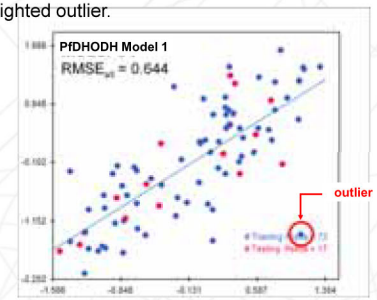

Figure 1: Predictive performance of PfDHODH Model 1. **Blue points** represent compounds from the training set **Red points** represent compounds in the external test set.

Identifying Candidates

Structural classes within the PubChem data set were generated and assessed for *in vivo* activity, predicted target inhibition and violations of 24 default ADMET Risk™ criteria using MedChem Studio™ and ADMET Predictor.

R-groups within the best classes were recombined and the best candidates produced were evaluated individually as shown in Figure 2.

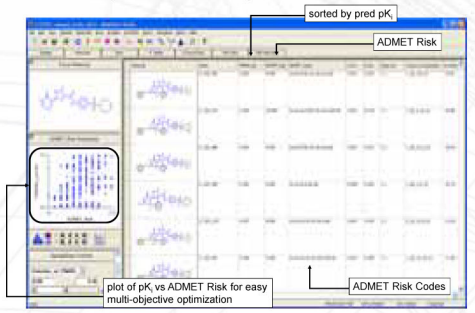

Figure 2: Survey of candidate Pfmrk inhibitors

Synthesis

One PfDHODH inhibitor scaffold comprised of three substructures was found to be particularly attractive. Novel candidates from the R-Table Explosion for that class were further elaborated, seven of which were then synthesized by Kalexsyn, Inc., of Kalamazoo, MI.

Results

Metabolic lability due to cytochrome P450s (CYPs) was reasonably close to predictions in most cases, but dealkylation by CYP 3A4 proceeded much faster than was expected. Metabolites observed in human liver microsomes were broadly consistent with what we had predicted, however.

Biological activity was determined by K.G. Le Roch's group at the University of California, Riverside, under conditions essentially identical to those used by GSK [2]. Table 1 shows the experimental results obtained in asynchronous (mixed) cultures; the XC50s found for synchronized cultures were about 30% lower.

Table 1: Predicted K_i's and observed biological activities of candidates and their closest GSK analogs in AID 2306.

SLP ID	Structure	Pred. DHODH K_i (μM)	XC50 (μM)[a,b] 3d7(-)	Dd2(+)	Resistance 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 blood culture by 50%.
[b] (-) and (+) denote chloroquine-susceptible and -resistant strains, resp.
[c] Closest structural analogs to SLP-0006 in the GSK data set.

Selected ADMET properties for four of the candidates were measured by Absorption Systems (Exton, PA). The results obtained (Table 2) agree well with our *in silico* predictions, given the reported RMSEs of about 0.3-0.6 log units (2-4x) for the respective ADMET Predictor models.

Table 2: Predicted and observed properties of some candidates.

Property	SLP-0003	SLP-0004	SLP-0005	SLP-0006
S+Sw (mg/mL)	1.4	13	4.9	5.3
obsd. solubility	0.76	32	33	22
S+logP	5.5	4.2	4.7	5.1
obsd. logP	4.4	3.5	4.2	5.6
S+pKa1	5.4	5.4	5.4	5.1
obsd. pKa1	4.8	4.2	5.0	4.8
S+pKa2	7.8	8.1	8.6	8.0
obsd. pKa2	7.4	7.8	8.3	7.6
S+log D6.8	4.4	2.9	3.0	3.9
obsd. log D6.8	3.8	2.5	2.7	4.7

Conclusions

The results indicate that the most potent NCEs have half-lives that are too short for them to be clinical candidates. The *in silico* design methods were remarkably successful, however, in identifying and advancing a promising area of antimalarial chemistry with a very limited expenditure of time and money. Further work remains before compounds from this structural class will make it to the clinic, including synthesis of additional analogs designed to address the shortcomings of the compounds synthesized and tested to date.

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