NOVEL ANTIMALARIAL DRUG CANDIDATES GENERATED *IN SILICO* BY ANALYSIS OF PUBLIC HTS DATA

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Introduction

The World Health Organization has estimated that over 200 million people suffered from malaria in 2010 and that over 600,000 people died from it that year [1]. Growing problems with resistance to existing anti-malarial drugs makes identification of new drugs a high priority. We applied a series of state-of-the-art *in silico* tools to publicly available activity data from screens carried out on intact *Plasmodium falciparum* parasites to yield a handful of candidate molecules predicted to combine potency with good absorption, distribution, metabolism, excretion and toxicity (ADMET) properties.

Here we describe the overall process used to design these molecules and report encouraging results for their activity against the parasite in culture. We also show that the ADMET properties predicted for them generally compare well to the experimentally determined values.

Plan of Attack

1. **Find a large data set of *in vivo* activities**
2. **Build predictive models for selected molecular targets using ADMET Modeler™**
3. **Use the *in vivo* activities with the predicted potencies and ADMET properties to identify attractive classes in MedChem Studio™**
4. **Generate analogs by recombining substituents from the best compounds in the selected classes**
5. **Select a set of novel analogs predicted to have good potency and minimal ADMET Risk™ and elaborate them**
6. **Predict expected dosing requirements by doing simulations in GastroPlus™**
7. **Synthesize selected candidates & determine their activities and ADMET properties experimentally**

Screening Data

PubChem AID 2306 is a screen for growth inhibition of a chloroquine-susceptible ("wild type") malaria strain (3d7) cultured in parasitized red blood cells. Data and structures for 13456 compounds that were active when tested at 2 µM were donated to the public domain by GlaxoSmithKline [2].

AID 2303 is a companion counter-screen for human cell (HepG2) viability. Of the antimalarial actives from AID 2306, 2006 (15%) were identified as toxic when tested at 10 µM.

AID 2302 is a counter-screen against a chloroquine-resistant strain (Dd2) carried out at 2 µM.

References

1. World Malaria Report 2011 (see http://www.who.int/malaria/world_malaria_report_2011/wmr2011_summary_keypoints.pdf)
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Building Predictive Models

Two parasite enzymes were chosen as targets: dihydroorotate dehydrogenase (DHODH), which is central to the *de novo* pyrimidine synthesis pathway critical to parasite survival [3,4]; and (Pfmrk), which is 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
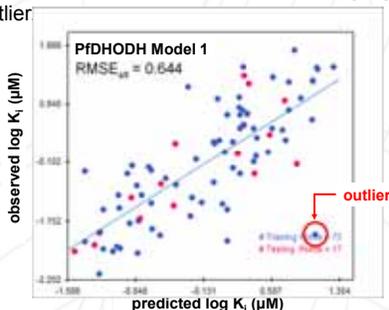

Figure 1: Predictive performance of PfDHODH Model 1. **Blue points** represent compounds from the training set **Red points** represent compounds in the external test set.

Identifying Attractive Classes

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.
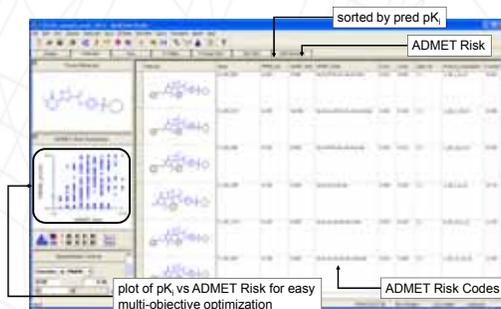
Figure 2: Survey of candidate Pfmrk inhibitors

Synthesis

One PfDHODH inhibitor scaffold comprised of three substructure 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.

Test Results

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.

Select 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 RMSEs of about 0.6 log units (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

Metabolic labilty due to most cytochrome P450s (CYPs) was reasonably close to predictions, 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.

Conclusions

Our goal was to provide proof-in-principle that *in silico* tools could be applied to public data so as to efficiently identify active chemistry with good ADMET properties, which we have done. The candidates produced exhibit good to excellent potencies in culture and hit all tested ADMET targets other than CYP 3A4 stability.

Future Plans

- Determine the *in vitro* potency of the class in general and our compounds in particular against PfDHODH.
- Find a partner with whom we can continue to develop this class of chemistry for increased metabolic stability and better activity against drug resistant strains.
- Obtain funding to apply these tools to other disease targets, either within the antimalarial arena or in other disease areas of interest

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