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In silico screening against wild-type and mutant *Plasmodium falciparum* dihydrofolate reductase

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Abstract

Modeling studies were performed on known inhibitors of wild-type as well as quadruple mutant *Plasmodium falciparum* dihydrofolate reductase (DHFR). GOLD was used to dock 31 pyrimethamine derivatives into the active site of DHFR obtained from the X-ray crystal structures 1J3I.pdb and 1J3K.pdb. Predicted binding affinities from a scoring function were analyzed and evaluated in order to develop criteria for selecting compounds having a greater chance of activity versus wild-type and resistant strains of *P. falciparum* for future high-throughput screening experiments.

Keywords: Dihydrofolate reductase; Malaria; Molecular docking; Evolutionary computation

1. Introduction

In recent years, there has been an alarming emergence of drug-resistant strains of bacteria, viruses, and parasites. These include: methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin/glycopeptide-intermediate *S. aureus* (VISA/GISA), *Streptococcus pneumoniae*, vancomycin-resistant enterococci (VRE), human immunodeficiency virus (HIV), and *Plasmodium* [1]. Even as new therapeutics are developed, these organisms have the potential to rapidly evolve and regain resistance. The evolution of multiple resistant strains of pathogens demands not only immediate proper diagnosis and treatment but requires a more effective drug development strategy.

Traditional drug discovery follows a common dogma: one protein per target, one target per drug. Recent advances in systems biology arising from our increased understanding of gene expression profiles, pathways, protein translation, and postprocessing, have caused some researchers to question this approach in support of a method to design and develop drugs that are effective simultaneously against multiple targets [2–4]. Some successful applications of this approach include: anti-apoptotic pan-caspase inhibitors for HBV and HCV [5], cancer [6], Alzheimer's disease [7], as well as the development of promiscuous tyrosine kinase inhibitors and G-protein receptor antagonists for cardiovascular disease [8].

This approach shows promise for addressing drug resistance that arises through the evolution and diversification of targets. For example, as a protein target adapts in response to drug therapy, a multi-targeted drug may still retain the ability to inhibit activity of mutant protein targets. The key to this strategy is to identify and develop compounds that bind with high affinity to different active sites and/or binding sites simultaneously.

Malaria provides an ideal model system to evaluate this strategy. In the case of two of the most prevalent malaria strains, *Plasmodium falciparum* (*Pf*) and *P. vivax* (*Pv*), clinical resistance arises from key amino acid substitutions in the target protein. These strains of malaria have developed resistance to anti-folate compounds such as pyrimethamine and cycloguanil that target dihydrofolate reductase (DHFR)–thymidylate synthetase [9–11]. Resistance arises from amino acid substitutions in *Pf*-DHFR at residues 51, 59, 108 and 164 and *Pv*-DHFR at residues 58, 117, and 173 [12–14].

Additionally, there is a wealth of structure–activity relationship (SAR) data in malaria in the literature. Structure-based

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drug design and discovery has been employed to improve the efficiency and productivity of new-leads discovery efforts for anti-malarials [15,16]. These efforts include X-ray crystallography [12,14], molecular modeling [17], and quantitative structure–activity relationships (QSAR) [18–27]. From these efforts, a third generation anti-folate active compound (WR99210) in resistant strains of both *Pf* and *Pv*, was discovered [28].

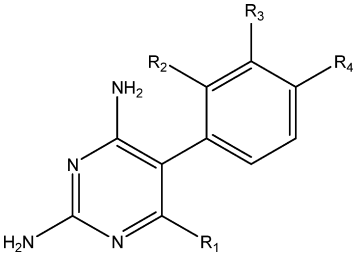
In this study, we present a methodology employing *in silico* protein–ligand docking and scoring algorithms for identifying inhibitors active simultaneously against wild-type (WT) and mutant forms of *Pf*-DHFR. This methodology is designed to filter small molecule libraries based on predicted relative binding affinity and to generate a small, "focused," compound screening library enriched with dual inhibitors. Additionally, this approach provides models of the predicted binding mode(s) and conformation(s) in an active site that can be useful in the generation and evaluation of SAR hypotheses.

2. Materials and methods

2.1. Docking protocols

The X-ray crystal structures of both *Pf* WT DHFR–TS (1J3I.pdb) [12] and quadruple mutant DHFR–TS (1J3K.pdb) [12] were identified for these studies. Both of these structures contain the third-generation *Pf*-DHFR inhibitor WR99210

Table 1
Structures, experimental pK_i values and docking scores are listed [18,19]



ID	R_1	R_2	R_3	R_4	p$K_{i\mathrm{WT}}$(nM) [18,19]	p$K_{i\mathrm{Mut}}$ (nM) [18,19]	Protein–ligand score (WT)	Protein–ligand score (Mut)
P39	**–C_6H_{13}**	**H**	**H**	**H**	9.52	8.85	−97.57	−105.21
P40	**–$(CH_2)_2O(CH_2)_3OC_6H_5$**	**H**	**Cl**	**H**	9.40	8.77	***−136.08***	***−130.61***
P32	**–$(CH_2)_3C_6H_4$–(*p*-OCH_3)**	**H**	**Cl**	**H**	8.66	8.70	***−115.14***	***−107.94***
P31	**–$(CH_2)_3C_6H_5$**	**H**	**Cl**	**H**	8.92	8.70	***−124.93***	***−108.72***
P29	**–$(CH_2)_3COOCH_3$**	**H**	**Cl**	**H**	9.30	8.57	***−110.73***	***−103.00***
P30	**–CH_2CH_3**	**H**	**Cl**	**H**	9.10	8.48	−96.77	−90.93
P43	**–$(CH_2)_3OCOC_6H_5$**	**H**	**Cl**	**H**	8.82	8.44	***−127.66***	***−117.77***
P44	**–$(CH_2)_3OCOOCH_2C_6H_5$**	**H**	**Cl**	**H**	8.92	8.44	***−144.47***	***−126.37***
P33	**–$(CH_2)_3C_6H_5$**	**H**	**H**	**H**	9.30	8.33	***−109.87***	***−113.89***
P47	**–$(CH_2)_3OCOC_6H_5$**	**H**	**H**	**H**	8.47	7.85	***−130.95***	***−119.05***
P38	**–CH_3**	**H**	**Cl**	**H**	8.72	7.85	−91.81	−84.39
P26	**–$(CH_2)_3COOCH_3$**	**H**	**H**	**H**	9.22	7.62	***−103.39***	***−105.85***
P42	**–$(CH_2)_3OCOCH_3$**	**H**	**Cl**	**H**	8.51	7.50	***−110.62***	***−103.05***
P20	**–CH_2CH_3**	**H**	**H**	**H**	8.64	7.50	−93.09	−88.75
P13	**–CH_2CH_3**	**H**	**Cl**	**Cl**	9.00	7.27	−96.22	−83.82
P41	**–$(CH_2)_3OH$**	**H**	**Cl**	**H**	8.04	7.24	−113.73	−91.47
P12	**–$(CH_2)_3C_6H_5$**	**H**	**H**	**Cl**	9.15	6.77	−118.58	−87.58
P46	**–$(CH_2)_3OCOCH_3$**	**H**	**H**	**H**	7.97	6.63	−102.20	−98.12
P15	**–CH_2CH_3**	**H**	**R_3-OCH_2O-R_4**		8.96	6.57	−97.93	−87.31
P17	**–CH_2CH_3**	**H**	**H**	**–CH_3**	9.40	6.55	−92.72	−85.35
P21	**–CH_2CH_3**	**H**	**H**	**Br**	9.52	6.52	−92.25	−80.45
P7	**–$CH_2CH_2CH_3$**	**H**	**H**	**Cl**	9.30	6.44	−100.75	−90.32
P16	**–$(CH_2)_3COOCH_3$**	**H**	**H**	**Cl**	9.52	6.44	−103.77	−93.83
Pyrimidine	**–CH_2CH_3**	**H**	**H**	**Cl**	9.52	6.41	−95.77	−81.57
P2	**–$CH(CH_3)_2$**	**H**	**H**	**Cl**	9.52	6.27	−97.27	−84.65
P45	**–CH_2OH**	**H**	**H**	**H**	7.07	6.26	−111.26	−85.62
P18	**–CH_2CH_3**	**H**	**H**	**OCH_3**	9.05	6.21	−92.23	−87.31
P3	**–$CH_2CH(CH_3)_2$**	**H**	**H**	**Cl**	9.52	6.10	−95.07	NA*
P5	**–C_6H_4–(*p*-OCH_3)**	**H**	**H**	**Cl**	8.37	5.80	−100.12	NA*
P14	**–CH_2CH_3**	**Cl**	**H**	**Cl**	7.90	5.73	−67.73	−68.04
P4	**–C_6H_5**	**H**	**H**	**Cl**	8.54	5.46	−88.76	NA*

Bold and italicized values in the ID column represent compounds identified as active against both WT and mutant forms of *Pf*-DHFR. Compounds with unrealistic poses were considered inactive and are indicated with an asterisk in the protein–ligand score column.