August 30, 2018

PharmacologyOnLine

Archives • 2018 • vol.2 • 243-254

CHEMOMETRIC MODELING, MOLECULAR DOCKING AND IN SILICO DESIGN OF PROGUANIL ANALOGUES AS DHFR-TS INHIBITORS TO TREAT MALARIA EFFECTIVELY

Arifur Rahman[1,3*], Md. Fakhrul Islam[1], Ajoy Kumar Bongshal[1], Sabreena Chowdhury Raka[2], and AZM Ruhul Momen[1,3]

[1]Computational Biology Research Lab, Department of Pharmacy, Jagannath University, Dhaka-1100, Banglagesh.
[2]Department of Pharmacy, Faculty of Allied Health Sciences, Daffodil International University, Dhaka-1207, Bangladesh.
[3]FDCC Testing & Research Lab, Department of Pharmacy, Jagannath University, Dhaka-1100, Bangladesh.

*arifur@pharm.jnu.ac.bd; rahman.arifpharma@gmail.com

Abstract

Malaria is a prevalent disease threatening worldwide which is affected by Plasmodium parasites. Plasmodium parasites are making resistance gradually which accelerate the innovation of new anti-malarial drugs concernment to stand against this disaster extremely. DHFR-TS enzymes are expected target protein which help reproduction of the parasites. Proguanil is a biguanide derivative, most commonly used against of the parasites. According to recent scientific report, malaria parasites are growing strong resistance against proguanil and other anti-malarial drugs constantly. Current study executes to find out the successor bioactive analogues of proguanil on a basis of molecular docking score through in silico analysis which will help to halt the parasites life cycle. AutoDock Vina and Chimera docking tools were used to elucidate the ligand-protein docking and binding interactions. In docking analysis, analogue ZINC16343331 and analogue PubChem CID 10684194 were found to interact with target receptor sites. Using AutoDock Vina and Chimera, binding affinity for analogue ZINC16343331 and PubChem CID: 10684194 were found -7.5 Kcal/mol and 7.3 Kcal/mol respectively, whereas obtained binding energy of proguanil was -6.6 Kcal/mol. Moreover, ZINC16343331 was showing best positive AMES test than proguanil. So, further studies on this analogue could gift the humanity a major breakthrough against the world deadliest disease.

Keywords: *Malaria; Dihydrofolate Reductase-Thymidylate Synthase; Proguanil; Docking; AutoDock Vina; Analogues.*

Acronyms and Abbreviations:

DHFR	Dihydrofolate Reductase
DHFR-TS	Dihydrofolate Reductase-Thymidylate Synthase
Pf	*Plasmodium falciparum*
P.	*Plasmodium*
PDB	Protein Data Bank
SMILES	Simplified Molecular Input Line Entry Specification
SDF	Standard Data Format
FASTA	FAST-All

Introduction

Malaria, the deadliest disease in the human-earth, reigned all over the world predominantly in the developing country by killing millions of people every year. Especially in Africa malaria costs many lives. According to WHO, 2008 each year one to two million deaths happen which is equal to 150 to 300 deaths each hour. To carry out its deadly life cycle the malarial parasite closely depends on both humans and mosquitoes.

The protozoan parasites Plasmodium is responsible for this, and anopheles mosquito's vector transmits it one person to another. In the gut of the mosquitos, plasmodium develops and when it takes blood into the new host it passes to the host [3]. This parasite goes rapidly into the liver of the host and rapidly reproduces in the liver. Then the parasite enters into the red blood cell and spread into the host blood. After that, it is injected into another mosquito and the life cycle is continuing [3]. There are five types of Plasmodium species which are affecting humans [4]. Those are *P. falciparum, P. vivax, P. ovale, P. malariae* and *P. knowlesi*, out focusing them *P. falciparum* is the most prevalent malaria species in worldwide [5]. Subsequently, anti-malarial drugs have been developed to target specific sites in the pathway of P. falciparum to pause its life cycle. Patients suffering from malaria are treated by a combination of drugs. As a prophylactic anti-malarial drug, biguanide derivative proguanil stops the reproduction of the malaria parasite if once it enters into the red blood cells, inhibits the enzyme dihydrofolate reductase (DHFR) [7]. But the concern is, at an alarming rate, anti-malarial drugs have been developed resistance to the target. Even though in the 1940/1950s proguanil and pyrimethamine were initially used alone after their introduction, resistance arose rapidly and it was only in strongly synergistic combinations with sulfa drugs that formulations with longer-term utility were produced [1]. The genetic basis of antifolate drugs resistance is a small number of point mutations in target genes appear to be accountable for the major part of resistance [1]. To be apprehended, malarial drugs resistance growing throughout the world has provoked the problem of determining which antimalarial drugs to use, particularly where Plasmodium falciparum has developed resistance to chloroquine, sulfadoxine/pyrimethamine combinations and, to some extent, quinine which was effective in the treatment of severe and complicated disease in previously. As a result, new anti-malarial drug development and enhancement of existing ones are therefore crucial to the reduction of the increasing disease burden and economic loss due to malaria.

It is essential to identify potential malarial drug target sites to which *Plasmodium falciparum* would have low or no resistance [2]. In parallel, it is also indispensable to search new and more effective therapeutic agents based on understanding their anti-malaria mechanism for treating infected patients eliminating the problem of drug resistance [6-8]. The life cycle of *P. falciparum* can be stopped if any specific agent may develop that can bind to the specific target site [8-9]. For this, the receptor is the ultimate site of drug action prevalently responsible for the pharmaceutical effect [9]. By determining the binding site of the protein which makes a complex form with a ligand should be determined first. This helps to select correct ligands which show a good binding affinity with the protein [6-10]. Other than DHFR, *Pf* DHFR-TS is involved in the reproduction of the parasite which blocks the biosynthesis of purines and pyrimidines, essential for DNA synthesis and cell multiplication [8-11].

With the advancement of computational simulation, potential malarial drug target prediction and *in Silico* analysis approaches have helped immensely in drug design. Through modeling of protein structures, the inner structure of the protein has realized closer to understanding and its' function. Computational approach obtained results accuracy level still has an extensive way to becoming hundred percent but contributes an insight into results that would take biologist months to get a result and in some cases years [2].

In this study, *In silco* approach and molecular docking studies carried out to predict the binding affinity with preferred orientation of selected bioactive analogues of proguanil drug with the most desired target protein *Pf* DHFR-TS whereas studies will help to screen out the potential complementary and therapeutically active analogues of proguanil for halting the life cycle of malaria parasites, the

core goal of this docking studies, which may flourish the way of new anti-malaria drug discovery.

Methods

Retrieval of target protein DHFR-TS of malaria and potential Proguanil:

For *Insilco* analysis and Molecular docking studies, authentic dynamic information on extensively used anti-malarial drug proguanil and its prevalent receptor or target protein *Pf*DHFR-TS were procured from PDB, NCBI PubChem database, UniProt database, Drug bank and many others literature surveys [12-14] **(Figure 1)**.

Proguanil 3D structure was retrieved from NCBI PubChem database as SDF along with its PCID: 6178111, Molecular formula (C11H16CIN5) and Molecular weight (253.731 G/MOL) **(Figure 2)**. This SDF format was then converted into PDB format using Open Babel GUI followed by 3D structure visualized through PyMOL (academic version) tool, Discovery Studio v3.5 visualizer tools as per requirement [15]. Reported target protein *Pf* DHFR-TS took from UniProt database containing UniProt ID A7UD81 as FASTA format.

Homology modeling of target:

The 3D structure of the noted target protein *Pf*DHFR-TS was predicted by homology modeling tool SWISS-MODEL services and Modeler 9.15 both of which contain Amino Acids (AA) sequence from 1-608; Templates- 4dpd.1.B and X-RAY DIFFRACTION 2.50 Å [16]. Discovery Studio Visualizer was used to visualize the model target protein.

Purification and validation of target model:

As a part of structure validation, different web servers were used to validate the modelled structure of Pf DHFR-TS (Table 1). PyMOL educational tools used to extract the pure protein [15]. 3D structure refinement was done by using Sysbio 3D refine tools. Structure validation scores were analyzed by MolProbity [17]. Discovery Studio Visualizer and PROCHECK were used to evaluate the quality of generated models by Ramachandran plot analysis [18]. The accuracy of the selected target model with stereochemical quality was further accelerated by PyRx plugin tools subjecting it to auto energy minimization process parameters. Further validation steps were performed by DeepView-Swiss PDB Viewer, Verify 3D and ERRAT programs [19]. ProSA server uses to analyze the energy plots and Z-scores [20].

Approach to binding site prediction:

GHECOM 1.0 and *f*pocket were run to structural and active site prediction studies for the protein *Pf*DHFR-TS where both binding site studies performed as a comparative analysis of binding pocket [21-22] **(Figure 4)**.

Proguanil analogues preparation:

Bioactive analogues of ligand proguanil drug were procured from Zinc15 database and NCBI PubChem as SDF/SMILES and then modified as required [14] [23] **(Figure 4)**.

Molecular Docking:

AutoDock Vina 2018 and UCSF Chimera, two most popular docking tools utilized to run molecular docking studies between proguanil and *Pf*DHFR-TS [15-24]. Followed by docking studies carried out for analogues of proguanil ZINC16343331 (Analogue 1), ZINC96068488 (Analogue 2), ZINC00001127 (Analogue 3), PubChem CID: 2802593 (Analogue 4),

PubChem CID: 19689601 (Analogue 5), PubChem CID: 10684194 (Analogue 6), PubChem CID: 44523220 (Analogue 7). A comparative study, based on the highest docking scores with energy minimization values between proguanil & *Pf*DHFR-TS and Analogues of proguanil with *Pf* DHFR-TS were carried out. PyMOL, Discovery Studio Visualizer were executed to study of visualizing the interaction between a ligand (proguanil and its analogues) and protein (*Pf*DHFR-TS) along with different bonding interactions.

Results and Discussion

(Table 1) values represent the structure validation of the *Pf* DHFR-TS modelled structure validated using different web servers and software. The PROCHECK and Discovery Studio Visualizer result of *Pf*DHFR-TS reported 89.3% of residues in the core region with no residues in the disallowed region. ProSA represented the Z-Score as -4.71 which is acceptable and considered to be a good structure. MolProbity reported all the residues in the favoured region with 0.24% Ramachandran outliers. Verify 3D

represented 82.45% residues in the core region. The active/Binding site of the reported *Pf*DHFR-TS target was predicted by *f*pocket represented in **(Table 2)**.

AutoDock Vina and UCSF Chimera docking studies revealed the respective ligand-protein docking scores depicted in **(Table 3)** and **(Table 4)**. Docking results of the drug and its derivatives via AutoDock Vina (prioritize) docking software reveals that the binding energy of analogue 1 (-7.5 Kcal/mol), analogue 2 (-7.2 Kcal/mol), analogue 3 (-6.8 Kcal/mol), analogue 6 (-7.4 Kcal/mol) and analogue 7 (-6.4 Kcal/mol) are better as compared to that of original marketed drug proguanil (-6.6 Kcal/mol) (AutoDock vina) / (-7.2 Kcal/mol) (Chimera). A comprehensive list of docking data comparison between AutoDock Vina and UCSF Chimera are depicted in **(Table 4)**. According to the representative data, the analogue 1 (ZINC16343331) and analogue 6 (PubChem CID:10684194) dominate with highest docking score along with best energy minimization values in comparison to the drug proguanil as well as represent more compatibility with the target than their other competitor analogues.

Moreover, the binding site of the analogue 1 was similar to that of its nearest competitors which means that functional groups involved were the same but the steric compatibility was varied. This states that the analogue 1; 2; 6 of proguanil drug may be of therapeutic importance for malaria patients in a role for clearing parasites from the liver **(Figure 5, 6)**.

The positive AMES test indicates the probability of causing cancer [18]. But Analogue 1 having AMES test in decrease decimal point than proguanil and rest of the Analogues which interpret that possibility of cancer is very less. Receptor-ligand complex interaction studies were performed and visualized in PyMol which are depicted in the **(Figure 7)**.

Conclusion

In structure-based drug development and designing protein-ligand interaction plays a significant role. Current anti-malarial drugs adverse effects and resistance against potential target stick out the significance of new and improved anti-malarial drugs. Using molecular docking, the appropriate conformations of series of compounds interact with Pf DHFR-TS which has been taken as potential target protein and thereof, most potential proguanil analogues are screened out. In docking analysis, target protein Pf DHFR-TS was docked with drug proguanil with the same scoring value of (-6.6 Kcal/mol) using AutoDock Vina and Chimera independently. Whereas the prevailing docking scorers among analogues are of analogue 1 (ZINC16343331) -7.5 (AutoDock vina)/-7.3 Kcal/mol (Chimera) docking score and for analogue 6 (PubChem CID: 10684194) -7.4 Kcal/mol independently by using this two docking tools. The results outline that proguanil analogues can be considered for the future anti-malarial drugs for better therapeutic efficacy against epidemic malaria diseases outperforms the existing commercially available drugs in the market. Suggested that ADME/T and drug likeliness properties should undergo wet lab analysis and research can be proceeded through in-vitro and in-vivo studies to confirm vast evaluation of efficacy and potency.

Acknowledgments

This research work and its authors are cordially grateful to the lab "Computational Biology Research Lab", Department of Pharmacy, Jagannath University, Dhaka for their necessary research support.

References

[1] Hyde, J.E., 2007. Drug-resistant malaria– an insight. The FEBS journal, 274(18), pp.4688-4698.

[2] Ekenna, C., Fatumo, S. and Adebiyi, E., 2010. In-silico evaluation of malaria drug targets. International Journal of Engineering and Technology, 2(2), p.132.

[3] Delves, M., Plouffe, D., Scheurer, C., Meister, S., Wittlin, S., Winzeler, E.A., Sinden, R.E. and Leroy, D., 2012. The activities of current antimalarial drugs on the life cycle stages of Plasmodium: a comparative study with human and rodent parasites. PLoS medicine, 9(2), p. e1001169.

[4] Prakash, N., Patel, S., Faldu, N.J., Ranjan, R. and Sudheer, D.V.N., 2010. Molecular docking studies of antimalarial drugs for malaria. J Comput Sci Syst Biol, 3(3), pp.70-73.

[5] Petersen, I., Eastman, R. and Lanzer, M., 2011. Drug-resistant malaria: Molecular mechanisms and implications for public health. FEBS letters, 585(11), pp.1551-1562.

[6] Srivastava, M., Singh, H., and Naik, P.K., 2010. Molecular modeling evaluation of the antimalarial activity of artemisinin Analogues: molecular docking and rescoring using Prime/MM-GBSA Approach. Curr Res J Biol Sci, 2(2), pp.83-102.

[7] Srivastava, I.K. and Vaidya, A.B., 1999. A mechanism for the synergistic antimalarial action of atovaquone and proguanil. Antimicrobial agents and chemotherapy, 43(6), pp.1334-1339.

[8] Ekenna, C., Fatumo, S. and Adebiyi, E., 2010. In-silico evaluation of malaria drug targets. International Journal of Engineering and Technology, 2(2), p.132.

[9] Richon, A.B., 1994. An introduction to molecular modeling. Mathematics, 1, p.83.

[10] D.A. Fidock, P.J. Rosenthal, S.L. Croft, R. Brun, S. Nwaka, Antimalarial drug discovery: Efficacy models for compound screening, Nat. Rev. Drug. Discov. 3 (2004) 509-520.

[11] Behera, S.K., Panda, A., Rana, M., and Bisht, S.S., 2016. Molecular Docking Studies and In-silico Computational Analysis of Analogues of Anti-Malarial Drugs. Journal of, 3(11), pp.1085-1090.

[12] Wishart, D.S., Knox, C., Guo, A.C., Shrivastava, S., Hassanali, M., Stothard, P., Chang, Z. and Woolsey, J., 2006. DrugBank: a comprehensive resource for in silico drug discovery and exploration. Nucleic acids research, 34(suppl_1), pp. D668-D672.

[13] Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P.E., 2006. The protein data bank, 1999–. In International Tables for Crystallography Volume F: Crystallography of biological macromolecules (pp. 675-684). Springer Netherlands.

[14] Kim, S., Thiessen, P.A., Bolton, E.E., Chen, J., Fu, G., Gindulyte, A., Han, L., He, J., He, S., Shoemaker, B.A. and Wang, J., 2015. PubChem substance and compound databases. Nucleic acids research, 44(D1), pp. D1202-D1213.

[15] Seeliger, D. and de Groot, B.L., 2010. Ligand docking and binding site analysis with PyMOL and Autodock/Vina. Journal of computer-aided molecular design, 24(5), pp.417-422.

[16] Guex, N., Peitsch, M.C. and Schwede, T., 2009. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. Electrophoresis, 30(S1).

[17] Davis, I.W., Leaver-Fay, A., Chen, V.B., Block, J.N., Kapral, G.J., Wang, X., Murray, L.W., Arendall III, W.B., Snoeyink, J., Richardson, J.S. and Richardson, D.C., 2007. MolProbity: all-atom contacts and structure validation for proteins and nucleic acids. Nucleic acids research, 35(suppl_2), pp. W375-W383.

[18] Laskowski, R.A., MacArthur, M.W., Moss, D.S. and Thornton, J.M., 1993. PROCHECK: a program to check the stereochemical quality of protein structures. Journal of applied crystallography, 26(2), pp.283-291.

[19] Eisenberg, D., Lüthy, R. and Bowie, J.U., 1997. [20] VERIFY3D: Assessment of protein models with three-dimensional profiles. In Methods in Enzymology (Vol. 277, pp. 396-404). Academic Press.

[20] Wiederstein, M. and Sippl, M.J., 2007. ProSA-web: interactive web service for the recognition of errors in three-dimensional structures of proteins. Nucleic acids research, 35(suppl_2), pp. W407-W410.

[21] Kawabata, T., 2010. Detection of multiscale pockets on protein surfaces using mathematical morphology. Proteins: Structure, Function, and Bioinformatics, 78(5), pp.1195-1211.

[22] Guex, N., Peitsch, M.C. and Schwede, T., 2009. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: A historical perspective. Electrophoresis, 30(S1).

[23] Irwin, J.J., Sterling, T., Mysinger, M.M., Bolstad, E.S., and Coleman, R.G., 2012. ZINC: a free tool to discover chemistry for biology. Journal of chemical information and modeling, 52(7), pp.1757-1768.

[24] Pettersen, E.F., Goddard, T.D., Huang, C.C., Couch, G.S., Greenblatt, D.M., Meng, E.C., and Ferrin, T.E., 2004. UCSF Chimera-a visualization system for exploratory research and analysis.

Journal of computational chemistry, 25(13), pp.1605-1612.

[25] Olliaro, P.L., and Trigg, P.I., 1995. Status of antimalarial drugs under development. Bulletin of the World Health Organization, 73(5), p.565.

Table 1: The structure validation scores of *Pf*DHFR-TS

Server	DHFR-TS	
PROCHECK and DS Visualizer	Most favoured regions	10.3%
	Core	89.3%
	Generously allowed Regions	0.4%
	Disallowed regions	0.0%
	G-factor	Dihedrals:0.00
		Covalent: -0.19
		Overall: -0.07
Verify 3D	Averaged 3D-1D Score > 0.2	82.45%
ERRAT	Overall Quality	90.609%
ProSA	Z-Score	-4.71
MolProbity	Cβ deviations > 0.25Å	0.86%
	Residues with bad bonds	0.01%
	Residues with bad angles	0.53%
	Ramachandran outliers	0.24%
	Favoured rotamers	93.76%
	Poor rotamers	-1.67%
	Ramachandran favoured	92.45%
	Cis prolines	5.26%
	Twisted Peptides	0.17%
Q mean score		0.55%

Table 2: Active/binding site of the proteins predicted by using *f*pocket.

No. of Total Binding Pocket	Target	Amino acid residues in the binding pocket
22	*Pf* DHFR-TS	THR2, ASP2, THR3, LYS9, GLU7, ASN6, VAL5, TYR4, SER8, GLU11, LEU13, TYR10,
		ILE14, ALA16, ARG17, LYS12, LYS14, LYS16, LYS19, TYR15, GLY26, LEU40,
		LEU46, ASP54, PHE58, LYS72, ASN88, GLY166, TYR170, ILE276, TYR278, ASP 284, GLU285,

Table 3: Docking results of proguanil against *Pf*DHFR-TS protein using AutoDock Vina and Chimera tool.

Ligand	Target	Docking Result	
		Auto Dock	Chimera
Proguanil	*Pf* DHFR-TS	-6.6 Kcal/mol	-6.6 Kcal/mol

Table 4: Docking results of proguanil analogues against *Pf*DHFR-TS protein using AutoDock Vina and Chimera tools.

Target	Ligand	Docking Result	
		AutoDock Vi	Chimera
Pf DHFR-TS	Analogue 1	-7.5 Kcal/mol	-7.3 Kcal/mol
	Analogue 2	-7.2 Kcal/mol	-7.3 Kcal/mol
	Analogue3	-6.8 Kcal/mol	-6.6 Kcal/mol
	Analogue 4	-6.1 Kcal/mol	-6.4 Kcal/mol
	Analogue 5	-6.2 Kcal/mol	-5.6 Kcal/mol
	Analogue 6	-7.4 Kcal/mol	-7.4 Kcal/mol
	Analogue 7	-6.4 Kcal/mol	-8.5 Kcal/mol

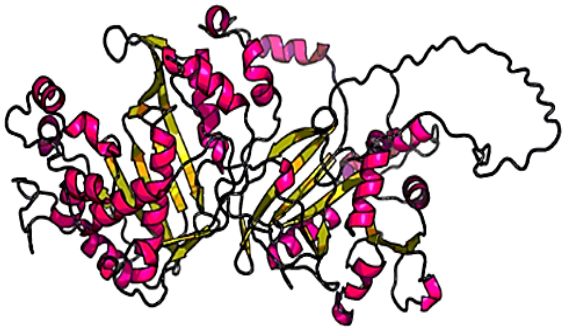


Figure 1: 3D structure of *Pf*DHFR-TS

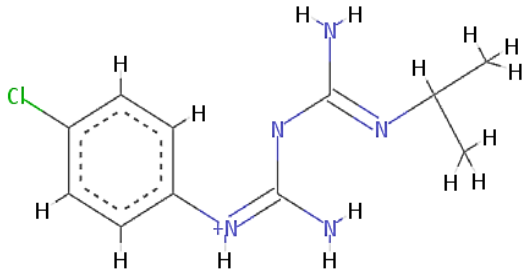
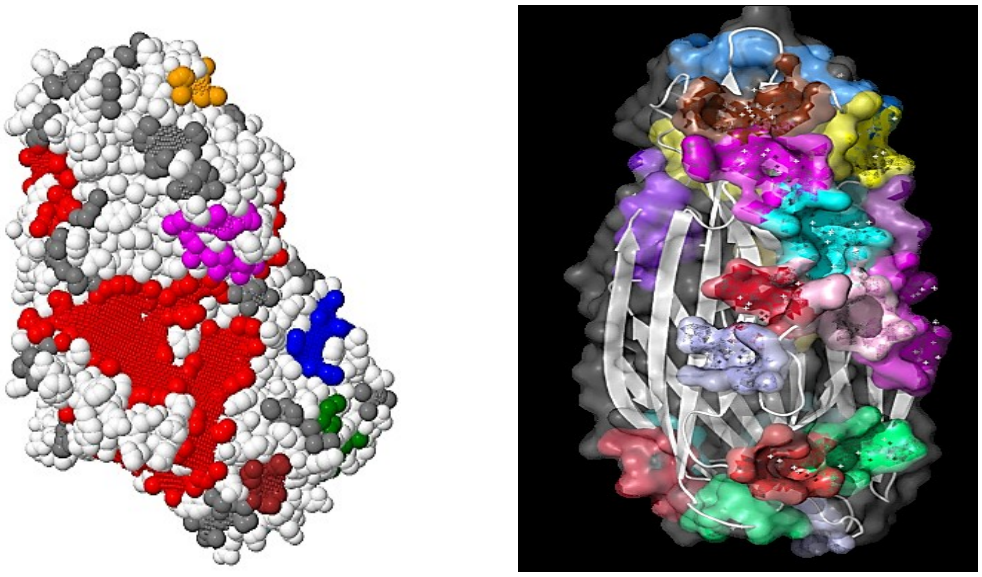
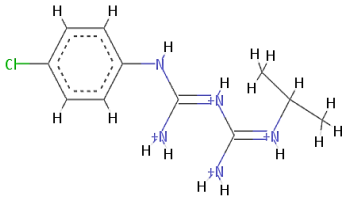


Figure 2: Proguanil

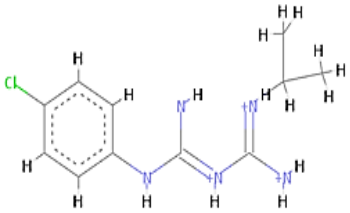


(a) (b)

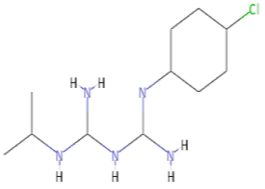
Figure 3: Visualization of *Pf*DHFR-TS active binding site GHECOM (a) *f*pocket (b)



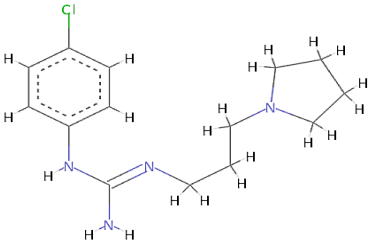
ZINC16343331



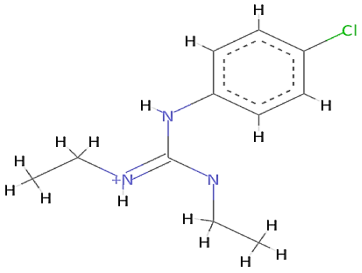
ZINC00001127



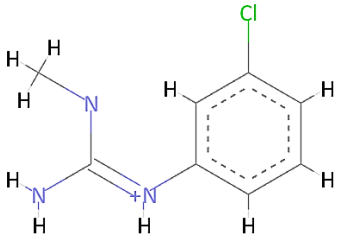
ZINC96068488



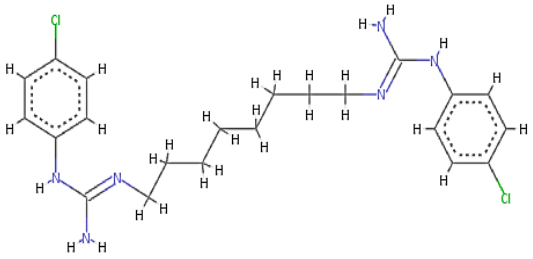
PubChem CID 11478363



1-(4-Chlorophenyl)-2,3-diethylguanidine



N-(3-Chlorophenyl)-N''-methylguanidine



PubChem CID 44523220

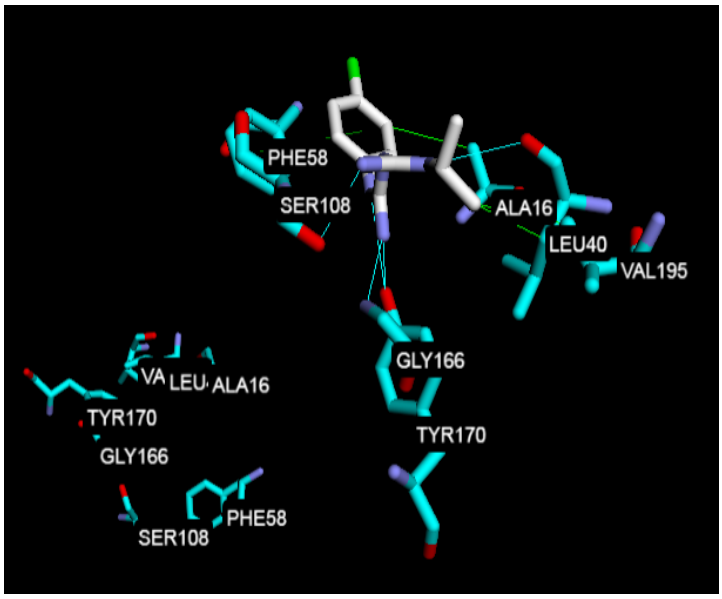
Figure 4: Analogues of proguanil

Excretion		
Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9701
	Non-inhibitor	0.9265
AMES Toxicity	Non AMES toxic	0.5887
Carcinogens	Non-carcinogens	0.6160
Fish Toxicity	High FHMT	0.8810
Tetrahymena Pyriformis Toxicity	High TPT	0.9925
Honey Bee Toxicity	Low HBT	0.7444
Biodegradation	Not ready biodegradable	0.9939
Acute Oral Toxicity	II	0.5608
Carcinogenicity (Three-class)	Non-required	0.6049

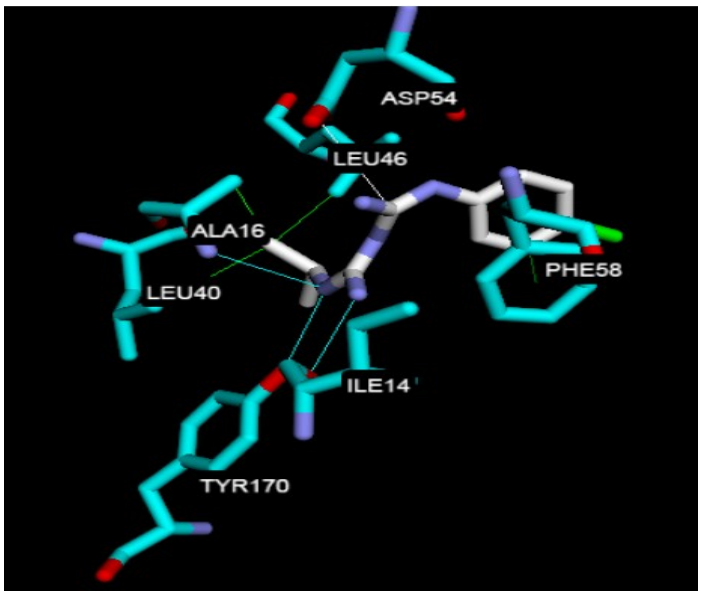
Figure 5: AMES test of Proguanil

Toxicity		
Human Ether-a-go-go-Related Gene Inhibition	Weak inhibitor	0.9644
	Non-inhibitor	0.9098
AMES Toxicity	Non AMES toxic	0.5515
Carcinogens	Non-carcinogens	0.6351
Fish Toxicity	High FHMT	0.8877
Tetrahymena Pyriformis Toxicity	High TPT	0.9901
Honey Bee Toxicity	Low HBT	0.7520
Biodegradation	Not ready biodegradable	0.9791
Acute Oral Toxicity	II	0.5243
Carcinogenicity (Three-class)	Non-required	0.6221

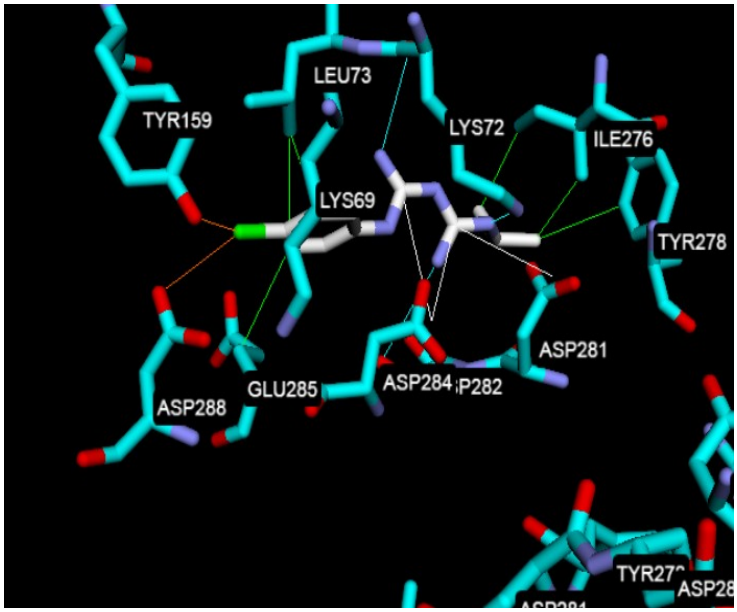
Figure 6: AMES test of ZINC1634333



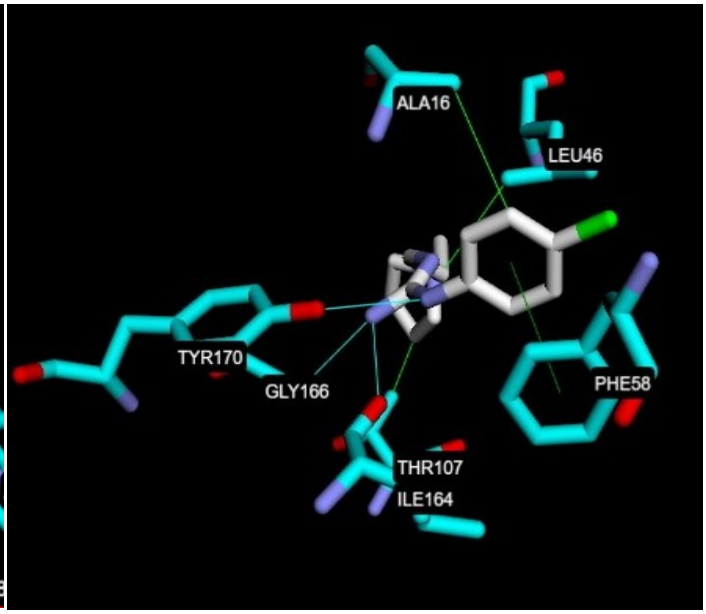
ZINC16343331-*Pf*DHFR-TS



Proguanil-*Pf*DHFR-TS



ZINC00001127-*Pf*DHFR-TS



CID: 10684194-*Pf*DHFR-TS

Figure 7: Receptor-ligand complex interaction in PyMol.