Journal of Natural Sciences Research
ISSN 2224-3186 (Paper) ISSN 2225-0921 (Online)
Vol.3, No.6, 2013

Homology Modeling, Docking and Comparative Study of the Selectivity of Some Hdac Inhibitors on Pfhdac-1 and hHdac 8 Models

Nnenna Winifred Odozi[1*] Moriam Dasola Adeoye[2] Peter Fristrup[3]

1. Department of Chemistry, University of Ibadan, Ibadan, Nigeria.

2. Department of Chemical Sciences, Fountain University, Osogbo, Nigeria.

3. Department of Chemistry, Denmark Technical University, Denmark

*E-mail of the corresponding author: nw.odozi@ui.edu.ng

Abstract

Malaria, one of the problems in many developing countries is caused by the protozoan parasite called Plasmodium and several cases of the disease are reported annually.

The emergence of multi-drug resistant malarial parasites necessitates the exploration of novel and promising antimalarial enzyme drug target called *Plasmodium falciparum* histone deacetylase 1 (PfHDAC-1). Thus, in this study, a ligand refined homology model of PfHDAC-1 was generated from the crystal structures of human HDAC8 and HDLP using a restraint guided optimization procedure involving the OPLS/GBSA potential setup. The model was validated using protein structure validation tools. A predictive docking study was carried out using nine sets of known HDAC inhibitors, which have been shown experimentally to have in vitro antimalarial activity against a strain of P. falciparum. Pose validation and score-based active and inactive separation studies provided independent validation of the geometric accuracy and the predictive ability of the generated model. Stereo chemical evaluation using Ramachandran plot revealed that 96.5% residues of the constructed model lie in the most favored and allowed regions, thus, suggesting a good quality model. Comparative analysis was carried out with the human HDAC 8 to ascertain the degree of selectivity of the inhibitors for both models. This revealed that YC-II-88 inhibitor was most selective for PfHDAC-1 model and showed no inhibitory activity for the human HDAC8 model.

Keywords Homology modeling; Plasmodium falciparum; Histone deacetylase; Docking; Inhibitor

1. Introduction

Malaria being a mosquito-borne infectious disease caused by protozoan parasites (Plasmodium) is widely spread in tropical and subtropical regions of the world. Approximately 500 million clinical cases of malaria are reported each year, of which 1-2 million result in death. 90% of the deaths occur in sub-Saharan Africa and most are children under the age of five. 50 million of the reported cases are pregnant women. Malaria in pregnancy contributes to nearly 20% low birth weight babies and also causes stillbirth and maternal deaths[1-3]. There are four known species of the Plasmodium that infect humans. These include *P. falciparum, P. vivax, P. malariae and P. oval*. Unfortunately, *P. falciparum* causes most of the severity and deaths attributed to the disease. They have become increasingly resistant to the currently used drugs; even the Artemisinin based Combination Therapies (ACTs) [4]. Due to this fact, development of drugs attacking crucial targets in the metabolism or mechanism of action of the malaria pathogen is imperative.

The histone deacetylases (HDACs) of *P. falciparum* are tipped as potential targets for new classes of antimalarial drugs. Histones are highly alkaline proteins found in eukaryotic cell nuclei and play a role in gene regulation. A number of HDAC are present in humans and can be classified into four categories[5] class I (HDAC 1,2,3,8), class II (HDAC 4,5,6,7,9,10), class III (Sirtuins), class IV (HDAC 11). The HDACS from classes I, II and IV exhibit zinc dependent mechanisms while the Sirtuins which are NAD^+ dependent demonstrates ADP-ribosyltransferase activity in addition to deacetylase activity[6].

HDAC inhibitors interfere with the functions of HDAC by arresting cell growth, inducing differentiation as well as apoptotic cell death. Generally, inhibitors show little selectivity for plasmodium cells compared with their selectivity for human cells[8]. For an outstanding success in the discovery of new and potent drugs, emphasis must be laid on the use of inhibitors that are selective towards the plasmodium cells even at very low concentrations. This paper therefore presents the homology model of PfHDAC1 and compares the results obtained from docking of selected inhibitors on PfHDAC 1 and human HDAC 8 models to that obtained experimentally (using the same inhibitors) as reported by Agbor-Enoh *et al*[8].

2. Materials And Methods

2.1 Computational Resources

All the computational studies were done on an Ubuntu 10.10 (Maverick Meerkat) Linux desktop. Sequence alignment, homology modeling, loop and side chain refinement were carried out using Prime version 2.1 (Schrodinger LLC, New York, NY, 2009). Molecular dynamics was carried out in Desmond version 2.2, while Epik and MacroModel versions 2.0 and 9.7 respectively were used for the preparation of the ligands before docking[9]. Glide version 5.5 was used for the receptor grid generation and for the docking calculations. Maestro version 9.0, which is a graphic unified interphase, was also used to generate figures[9].

2.2 Sequence Alignment and Model Building

The protein sequence of PfHDAC1 was downloaded from the Uniprot server[10]. The crystal structures 1C3R (PDB code) from HDLP; a HDAC homologue from *A. aeolicus* bacterium with 32% sequence identity and human HDAC8 (1T64 PDB code) 41% sequence identity were templates used for the building of the model. Within Prime, the target sequence was imported in the FASTA format while the templates were imported into Maestro and prepared with the Protein Preparation Wizard. Their sequences were extracted and aligned with the target sequence using the default alignment procedure of Prime applying BLOSUM62 similarity matrix. The HDLP template was used for modeling residues 5 – 26 of PfHDAC1 while residues 27 – 377 were built from HDAC8 template. This was possible through the Set Template Regions functionality. The ligand and the metal ions were incorporated in the model generation process and the coordinates of the TSA ligand and the Zn^{2+} ions were copied from the 1T64 structure. Model building was automatically terminated where no reference sequence existed for building the template.

2.3 Docking Calculations

Nine ligands for the docking study were built in maestro and minimized using Epik and MacroModel. Glide was then used to generate the receptor grid using the centroid of the TSA pose from the PfHDAC1 and the hHDAC8 model. The Zn^{2+} ion was identified for setting up a metal constraint while the docking calculations were carried out in the Glide XP mode. The metal constraint was used for docking and poses were collected and ranked using the scoring terms available in Glide.

3.0 Results And Discussion

Malaria treatment with new improved drugs is a high priority to addressing the global problem of parasite resistance to existing antimalarial drugs[11] HDAC enzymes in malaria parasites have been tipped to be promising target for the development of new antimalarial drugs[12] but most HDAC inhibitors have relatively poor selectivity for *P. falciparum* compared to normal mammalian cells hence the need to identify inhibitors that are more potent and more selective in killing *P. falciparum* than in killing normal cells. The homology model of PfHDAC1 (Ascension number Q9XYC7) was generated using hHDAC8 (PDB 1T64 chain A) and HDLP (PDB 1C3R chain A) as templates.

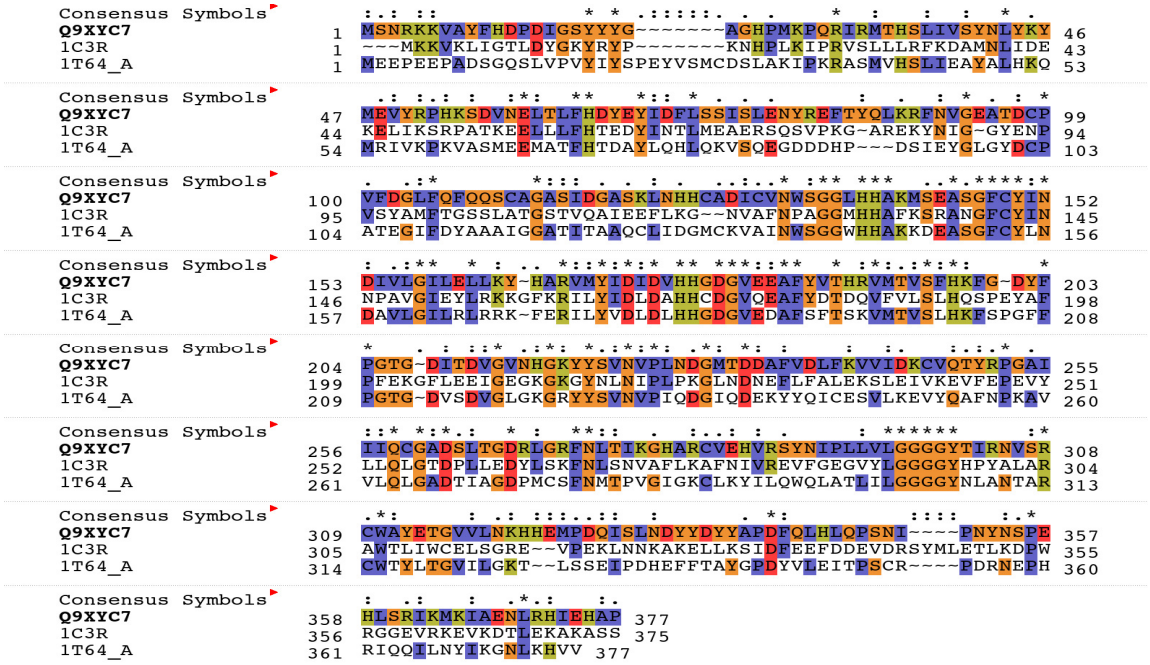


Fig. 1: Multiple sequence alignment of Q9XYC7, 1C3R (A) and 1T64 (A) used to generate PfHDAC 1 model. The structurally conserved regions are indicated by '*'.

The sequence alignment of the templates to the target sequence (Fig. 1) showed that the overall homology of the target sequence was greater for hHDAC8 by about 50%. On the bases of this, the HDLP structure was used to build the first loop while hHDAC8 was used for modeling the rest of the protein. The average sequence homology of the PfHDAC1 with the two homologs was 45%. Loop 1 region of PfHDAC1 (residues 15-30) and HDLP showed good residue conservation, whereas hHDAC8 has a large insert from P22 to C28. There were appreciable numbers of structurally conserved regions.

Fig. 2: Homology model of PfHDAC 1

The homology model (Fig. 2) resulted in a structure with RMSD of <1.0 for the heavy atoms and backbone. This may suggest a reasonable 3D model. The Ramachandran plot of the PfHDAC model from Rampage

showed a normal distribution of points with Phi (Φ) angles mostly restricted to negative values and Psi (Ψ) values clustered in a few distinct regions. It also showed that residue in favored region is 89%, residue in allowed region, 7.5% and the residue in outlier region is 3.5%.

3.1 Binding Site

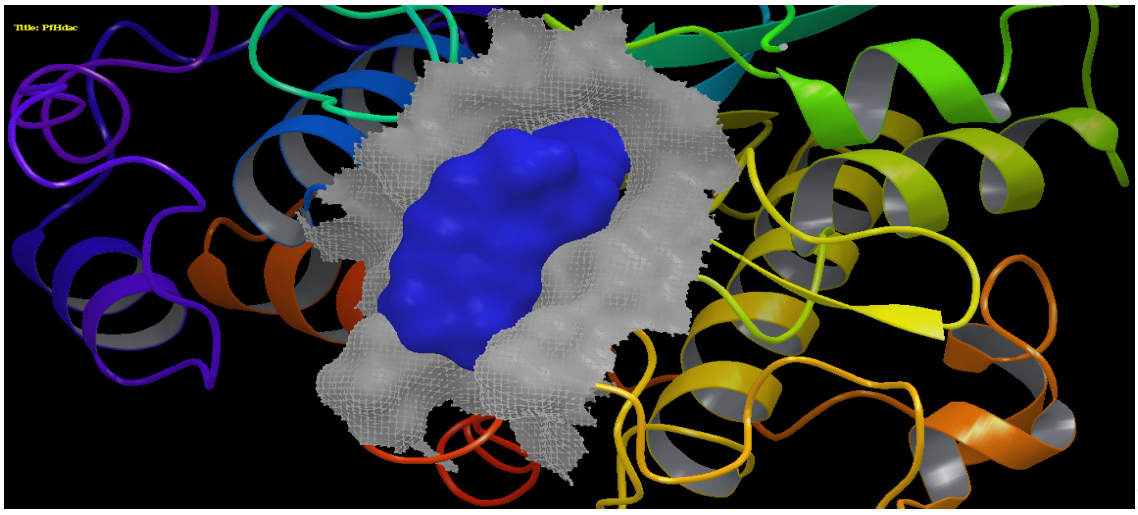


Fig. 3a: Receptor binding surface of PfHDAC1 model

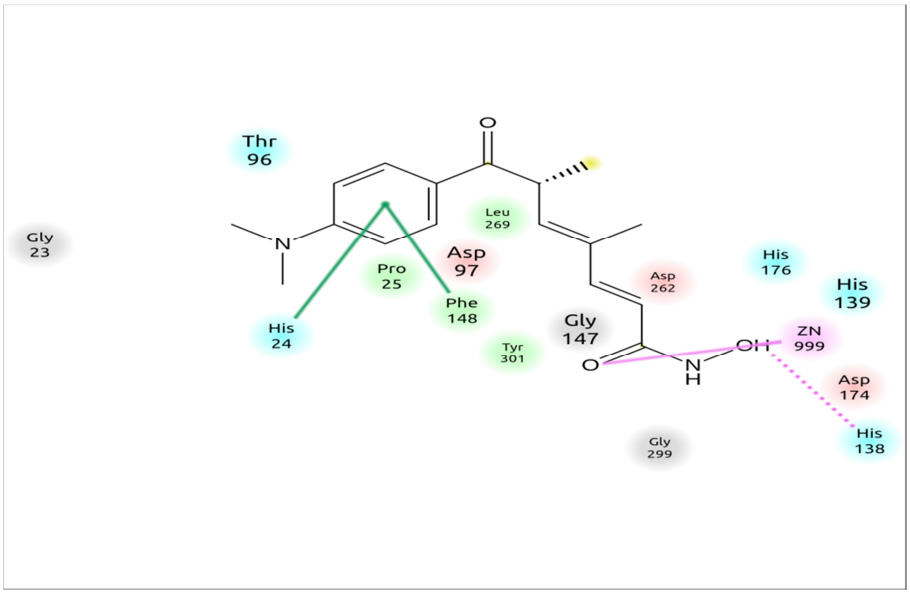
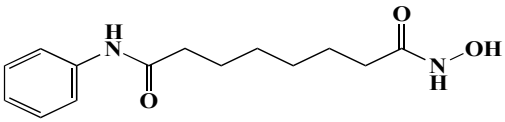
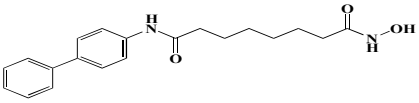
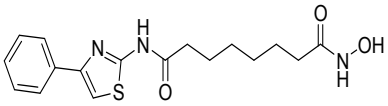
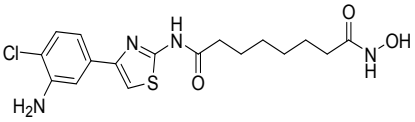
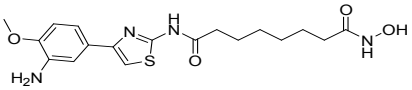
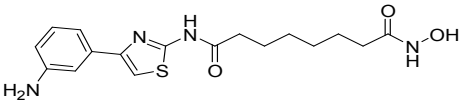
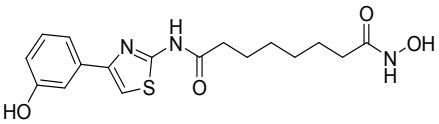
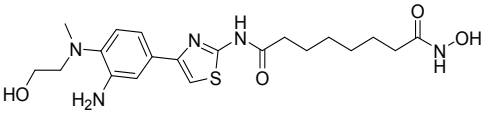


Fig. 3b: Ligand interaction diagram of PfHDAC 1 model

The binding site of the PfHDAC model (Fig. 3a & 3b) is made up of a narrow channel that leads to a deep cavity lined by the amino acid residues that are of great importance to the enzymes catalytic machinery. The aliphatic chain and dimethyl aniline group of TSA occupies this narrow channel and the terminal hydroxamate group enters into the deep cavity and forms several important interactions with the catalytic residues ranging from hydrophobic and polar interactions to coordination of the Zn^{2+} metal ion. The metal ion is coordinated at five points; three with the side chains of Asp 174, Asp 262, and His 176 while the other two are with the carbonyl and hydroxyl oxygen of the ligand's hydroxamate group as reported by Prasenjit[6].

3.2 Docking of Ligand

Table 1: Summary of the glide/docking score of PfHDAC 1 and hHDAC8 models

Ligand	Glide/Docking Score	
	PfHDAC 1 Model	hHDAC 8 Model
SAHA 	-3.348	-7.728
Apicidin	-5.448	-7.278
YC – II – 84 	-11.123	-
AG – Thai -01 	-11.504	-
WR308296 	-11.582	- 8.397
WR308291 	-11.702	- 6.205
YC – II – 88 	- 11.924	-
WR308294 	- 12.320	- 7.760
WR308298 	- 12.934	- 7.400

Note: The inhibitors are culled from Geoffrey *et al.*[7] and Agbor –Enoh *et al.*[8]

Table 1 shows the summary of the glide/docking scores of the PfHDAC1 and hHDAC8 models. The docked ligands have hydroxamate group as a zinc-binding group (ZBG) that coordinates with the Zn^{2+} ion through their carbonyl and hydroxyl oxygen. Table 1 shows that of the nine ligands docked, seven inhibited PfHdac 1

model (WR308298, WR308294, YC-II-88, WR308291, WR308296, AG-Thai-01, YC-II-84) and three were selective for PfHDAC1 (YC-II-88, YC-II-84 and AG-Thai-01) with YC-II-88 being the most selective for PfHDAC1 model showing no inhibitory activity for the hHDAC8 model. Experimental results reported by Dow *et. al*.[7], and Agbor-Enoh *et. al*.[8], for the action of these ligands on *P.falciparum cell* tipped YC-II-88 to have the greatest selectivity and more toxic against the parasite than on human cells in vitro and in vivo. This theoretical result is in agreement with the experimental results. Comparing the docking result of the PfHDAC1 model to that of the hHDAC8 model, it is clear that SAHA and Apicidin are more selective for the hHDAC8.

In terms of structure – activity relationships (SAR), YC-II-88 may have shown greater potency and selectivity for the PfHdac1 model due to the substitution of an amine at the Meta position of the terminal phenyl ring system while YC-II-84 have reduced potency when compared to YC-II-88 and AG-Thai-01 due to the substitution of a non thiazole ring system[7].

4.0 Conclusion

A 3D structure of PfHDAC1 was constructed through homology modeling using Prime (Schrodinger) soft ware. The model was then docked with nine ligands seven of which showed selectivity for PfHDAC1 than hHDAC8 model and three out of the seven showing greater selectivity for the PfHdac model than hHdac 8 model. These theoretical results were in agreement with the experimental result reported by Geoffrey et al[7]. and Agbor- Enoh et al.[8] in terms of selectivity. Results of this study after further investigation might be utilized to develop an antimalarial drug that incorporates YC-II-88 inhibitor.

Acknowledgements

We thank the MacArthur Foundation for sponsoring the overseas training in molecular modeling that led to this work. We thank specially Dr. Peter Fristrup of Denmark Technical University who offered a space in his laboratory and provided all the software and equipment necessary for the training.

References

WHO. World Malaria Report 2008; Geneva: World Health Organization, 2008.

WHO. World Malaria Report 2009; Geneva: World Health Organization, 2009.

Malaria and Children, Report on Progress in Intervention Coverage, 2010. UNICEF

Turschner, S. & Efferth, T. M. (2009). Drug Resistance in Plasmodium: Natural Products In The Fight Against Malaria. *Rev. Med. Chem.* 9, 206

Andrews, K.T., Tran, T.N., Lucke, A.J., Kahnberg, P., Le, G.T., Boyle, G.M., Gardina, D.L., Skinner-Adams, T.S. & Fairlie. D.P. (2008). Potent Antimalarial Activity of Histone Deacatylase Inhibitor Analogues, *Antimicrobial Agents and Chemotherapy* 52, 1454-1461

Prasenjit, M., Anupam, P., Falgun, S., Babu, L. T. & Mitchell, A. A. (2008). Structural Insights Into *Plasmodium falciparum* Histone Deacetylase 1 (PfHDAC-1): A Novel Target For The Development of Antimalarial Therapy, *Bioinorganic and Medicinal Chemistry* 16, 5254-5265

Dow, G.S., Chen, Y., Andrews, K.T., Caridha, D., Gerena, L., Gettayacamin, M., Johnson, J., Li, Q., Melendez, V., Obaldia, N., Tran, T.N. & Kozikowski, A.P. (2008). Antimalarial Activity of Phenylthiazolyl Bearing Hydroxamate-Based Histone Deacetylase Inhibitors, *Antimicrobial Agents And Chemotherapy*, 52(10), 3467–3477

Agbor-Enoh ,S., Seudieu, C., Davidson, E., Dritschilo, A. & Jung, M., (2009). A Novel Inhibitor of Plasmodium Histone Deacetylase That Cures P. berghei- Infected Mice, *Antimicrobial Agents and Chemotherapy* 53, 1727.

Schrodinger LLC, New York, NY, 2009

Joshi, M.B., Lin, D.T., Chiang, P.H., Goldman, N. D., Fujioka, H., Aikawa, M. & Syin, C. (1999). Molecular Cloning and Nuclear Localization of a Histone Deacetylase Homologue in *Plasmodium falciparum*, *Mol. Biochem. Parasitol*. 99, 11-19.

Fidock, D.A., Rosenthal, P.J., Croft, S. L., Brun, R. & Nwaka, S. (2004). Antimalarial drug discovery: efficacy models for compound screening, *Nat. Rev. Drug Discov*. 3(6), 509-520

Darkin-Rattray, S.J., Gurnett, A.M., Myers, R.W., Dulski, P.M., Crumley, T.M., Allocco, J.J., Cannova, C., Meinke, P.T., Colletti, S.L., Bednarek, M.A., Singh,S.B., Goetz, M.A., Dombrowski, A.W., Polishook, J.D. & Schmatz, D.M. (1996). Apicidin: A Novel Antiprotozoal Agent That Inhibits Parasite Histone Deacetylase. *Proc. Natl. Acad. Sci. USA* 12, 13143-13147