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RESEARCH ARTICLE

Designing synthetic drugs against *Plasmodium falciparum*: a computational study of histone-lysine *N*-methyltransferase (PfHKMT)

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Abstract Histone lysine methyltransferase (HKMT) are histone-modifying enzymes that catalyze the transfer of methyl groups to lysine and arginine residues of histone protein. HKMTs have been involved in transcriptional regulation of various proteins in organisms. Malaria parasite also has HKMT, which plays a major role in parasite development and pathogenesis and also in regulation of various biological process and pathways. Our aim is to study fundamental biology of key molecules involved in the survival of *Plasmodium falciparum* and use these to develop efficient synthetic peptides and chemical compounds. As a first step in this direction, we computationally predicted the three-dimensional structure of HKMT of *P. falciparum* (PfHKMT) by using iterative threading assembly refinement. The PfHKMT three-dimensional model was validated using PROCHECK and docked with known HKMT inhibitor Bix01294 using Autodock. Our initial results are encouraging and indicate that structural analysis of PfHKMT could be important in developing novel synthetic molecules against malaria.

Keywords HMKT · Transcriptional regulation · 3D structure · Plasmodium · Malaria

Introduction

Over the last decade, epigenetics has been intensely investigated by researchers as it plays a major role in area of human health. Histone proteins are the major part of chromatin (Bernstein et al. 2007). The state of chromatin is mainly regulated by post translational modifications to histones and DNA. Post translational modifications include histone lysine methylation, arginine methylation, lysine acetylation and phosphorylation as well as DNA methylation (Gelato and Fischle 2008). Histone lysine methylation is important in transcriptional activation and silencing (Sawan and Herceg 2010). Histone methylation is regulated by actions of two enzymes namely histone methyltransferases (HKMTs) and histone demethylases. Two types of HKMTs are present in eukaryotic cells; lysine specific and arginine specific. Lysine specific can be Su(var)3-9, Enhancer of Zeste, Trithorax (SET) domain containing or non-SET domain containing disruptor of telomeric silencing-1 (DOT1) (Feng et al. 2002; Ng et al. 2002). Current studies in apicomplexan parasites have demonstrated the significance of histone lysine methylation in parasite development and pathogenesis similar to as reported in regulation of various biological process and pathways in other organisms. Inhibition studies of HKMTs activity have been established as a proven treatment for many diseases (Chookajorn et al. 2007; Cui et al. 2007; Lopez-Rubio et al. 2007; Sautel et al. 2007).

Malaria is an infectious disease caused by Plasmodium and led to enormous amount of morbidity and mortality. Plasmodium completes its life cycle with two alternate hosts a vertebrate and an invertebrate. The parasite transcription mechanism is firmly regulated in both hosts (Cowman et al. 2012) and reported to be regulated by transcription factors and chromatin modifying proteins. Malaria parasite has a nucleosome organization like other eukaryotes and lysine and arginine methylation are also reported in *Plasmodium falciparum* histones. These modifications in histone are shown to play an important role in transcription regulation

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(Miao et al. 2006). Moreover, in *P. falciparum*, epigenetic mechanisms have been implicated in the control of antigenic variation in parasite, a mechanism of immune evasion that contributes to its pathogenicity (Chookajorn et al. 2007; Duraisingh et al. 2005; Frank et al. 2006; Freitas-Junior et al. 2005; Voss et al. 2006; Lopez-Rubio et al. 2009). The methylation by *N*-methyltransferase 1 (PfMT1) activity and its inhibition has been described (Fan et al. 2009) in plasmodium. Similarly the drug discovery efforts for targeting the parasite HKMTs have also been reported (Malmquist et al. 2012). The Plasmodium genome contains five HKMTs in which four of them are SET-domain-containing methyltransferases with predicted H3K4 specificity and one is with predicted H3K9 activity (Cui et al. 2008; Volz et al. 2010, 2012). These studies revealed the importance of epigenetic mechanism in *Plasmodium*.

In the present study, we performed in silico analysis of HKMT of *P. falciparum* and computed effect on its enzymatic activity by docking with one of the known inhibitor Bix01294 (Malmquist et al. 2012). Our study opens up a new direction for histone lysine methylation role in parasite and explore the possibility of using it to develop synthetic molecules.

Methods and materials

The sequence of PfHKMT protein was obtained from NCBI (XM_001350229.1) and the chemical structure of the Bix01294 inhibitor was obtained from the existing literature (Malmquist et al. 2012).

Bioinformatics studies

PSIPRED server (Johns 1999) was used for the prediction of secondary structure of PfHKMT. Conserved domain of PfHKMT was denoted using structure conserved domain database (CDD) available at NCBI (www.ncbi.nlm.nih.gov/structure/cdd/wrpsb.cgi) (Geer et al. 2002).

3D structure of *P. falciparum* histone lysine *N*-methyltransferase

3D structure of PfHKMT was generated using iterative threading assembly refinement (I-TASSER) program server (Roy et al. 2010). I-TASSER server is online service for generating protein structure and for function predictions. It generates 3D structure of protein molecules from amino acid sequences. Five models were generated and final model was selected following validation through PROCHECK (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK) validation tools available online (Laskowski et al. 2001).

Docking study

All the docking experiments were performed using automated docking program Auto-Dock (v.4.0) (Morris et al. 1998). Before the actual docking process, the ligand and receptor preparation was performed involving addition of hydrogen atoms and computing charges. The rotatable torsion bond information for ligand was acquired. This defines the bond flexibility parameters. Following the ligand and receptor preparation Auto-Grid module of Auto-Dock was used for grid map calculations. Grid construction involved identification of atom types of the ligand which acted as probes. The grid with a volume 68 × 84 × 70 Å with a spacing of 0.375 Å centered on the receptor binding site. Then the docking calculations were performed using the genetic algorithm (GA) procedure with default parameters.

Results and discussion

Domain organization

In silico studies exposed two putative conserved domains in the PfHKMT namely as SET and myeloid, Nervy, and DEAF-1 (MYND) zinc finger domain, shown in Fig. 1. SET domains catalyze site- and state-specific lysine methylation of histones and play an important role in epigenetic regulation of gene activation and silencing of all eukaryotes. Recent studies revealed that SET domain proteins act as lysine methyltransferases. It catalyzes lysine methylation of cellular proteins like cytochrome c, Rubisco, p53 and Taf 10 (Qian et al. 2006). MYND domain is zinc finger motif that mediates protein–protein interactions. It is reported that SMYD2 interacts with proline-rich proteins by using MYND domain (Abu-Farha et al. 2008).

Secondary structure prediction

The secondary structure indicates confirmation of amino acids in which they are present. Secondary structure of PfHKMT indicated that protein has occurrence of 42.6 % helical and 6.1 % of sheets suggesting helical has dominancy as shown in Fig. 2.

Molecular modelling of PfHKMT

Tertiary structure of PfHKMT was generated using the I-TASSER program (Roy et al. 2010). Total five models were generated and model one was chosen for further analysis as it showed −0.17 confidence score (Cscore), which was least in all. TM score and root mean square deviation (RMSD) values for this model were 0.69 ± 0.12,

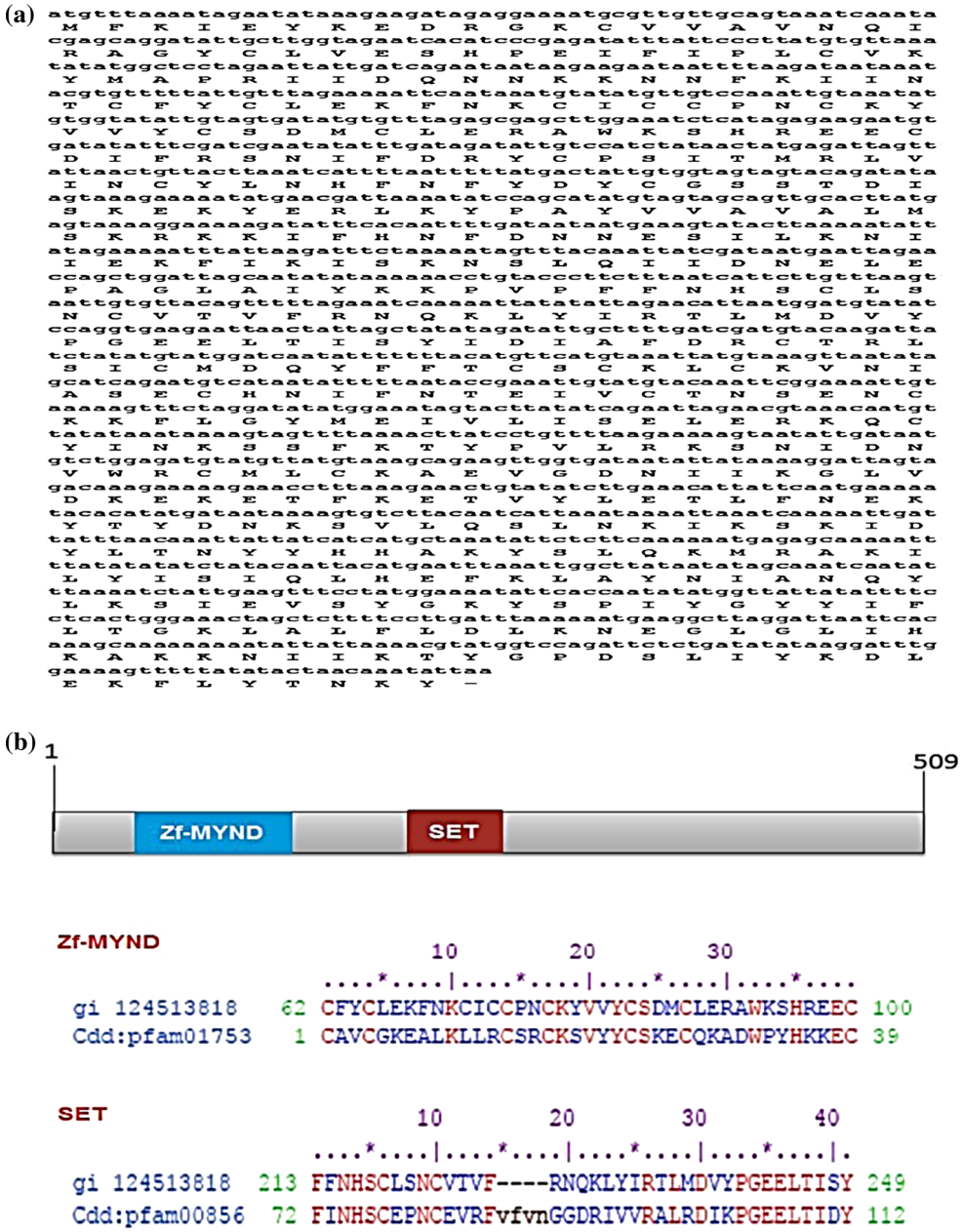

Fig. 1 **a** Deduced amino acid sequence of PfHKMT (XM_001350229.1). **b** Domain organization of PfHKMT: schematic representation of two conserved domains presents in PfHKMT namely SET and MYND domain

7.7 ± 3 Å respectively. Finally this model of PfHKMT was taken for further validation analysis.

Structure validation

PROCHECK were used for the stereo chemical validation of the model. Ramachandran plot was depicted in Fig. 3b, indicating that 81.3 % residues are present in most favorable region, 10.6 % residues are in additional allowed region, 4.4 % residues are in generously allowed regions and only 1.9 % residues are in disallowed region of plot. Totally 93.9 % residues are in most favored and allowed regions. The statistic data confirmed that quality of model is acceptable and reliable (Fig. 4).

Active site prediction

Once the final model was built, the possible binding sites of PfHKMT were revealed using COACH server for protein and ligand binding site prediction (Roy et al. 2010). The

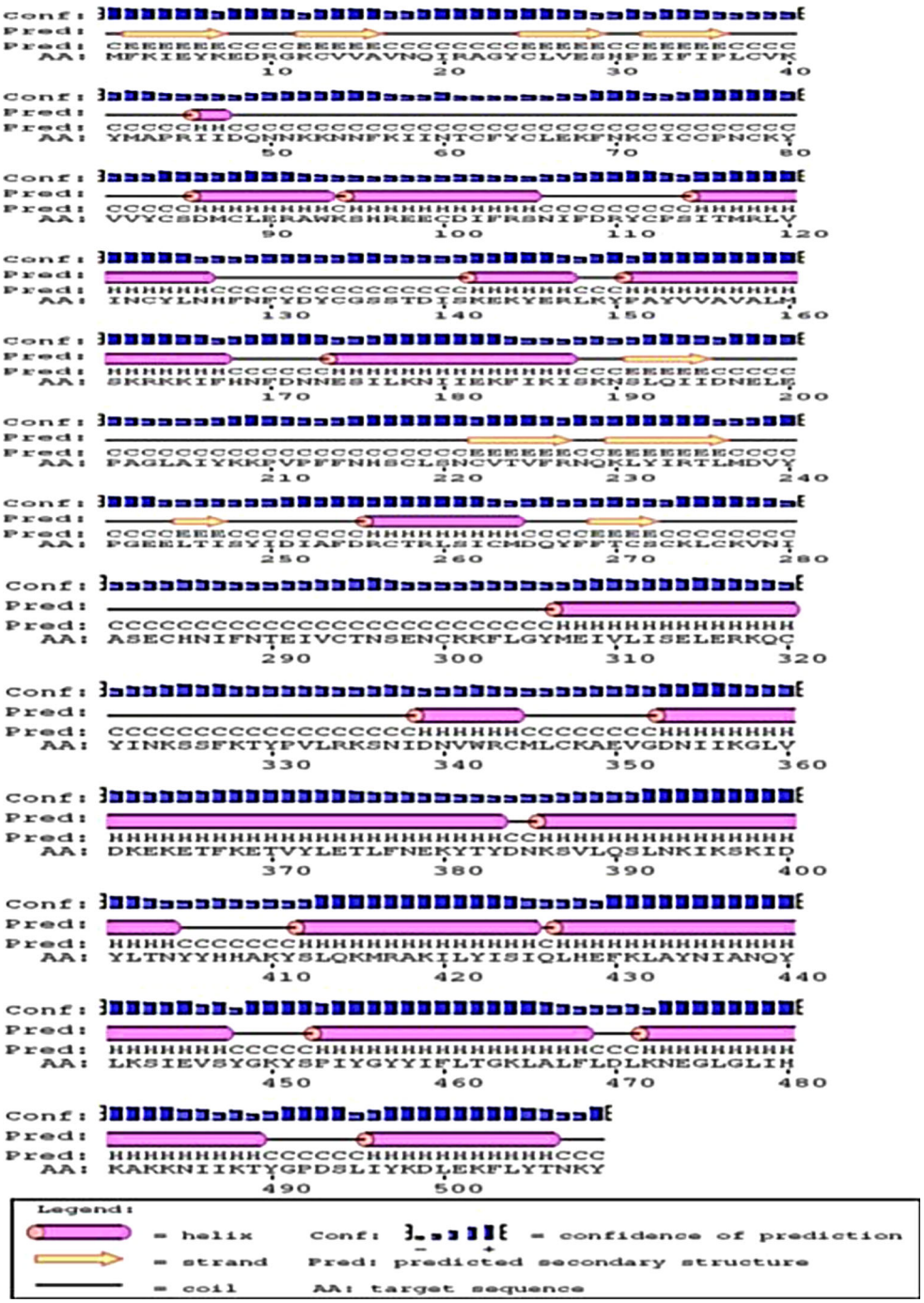

Fig. 2 Secondary structure of PfHKMT: PSIPERD was used to predict PfHKMT helix, sheets and coils. *Pink*, *yellow* and *black color* represents helix, sheets and coils respectively. (Color figure online)

active site was predicted to have Cscore and BS-score 0.44, 1.41 respectively that indicate the more reliable ligand binding site prediction. The residues involved in pocket formation were Arg10, Lys12, Ser137, Tyr145, Lys189, Asn190, Pro212, Phe213, Phe214, Asn215, His216, Tyr249, Tyr267 and Phe269.

Docking with Bix01294

We performed docking studies by using flexible docking program Auto Dock to understand how Bix01294 inhibitor binds to the PfHKMT and found that three residues that are important for binding of this inhibitor are Arg10, Tyr145

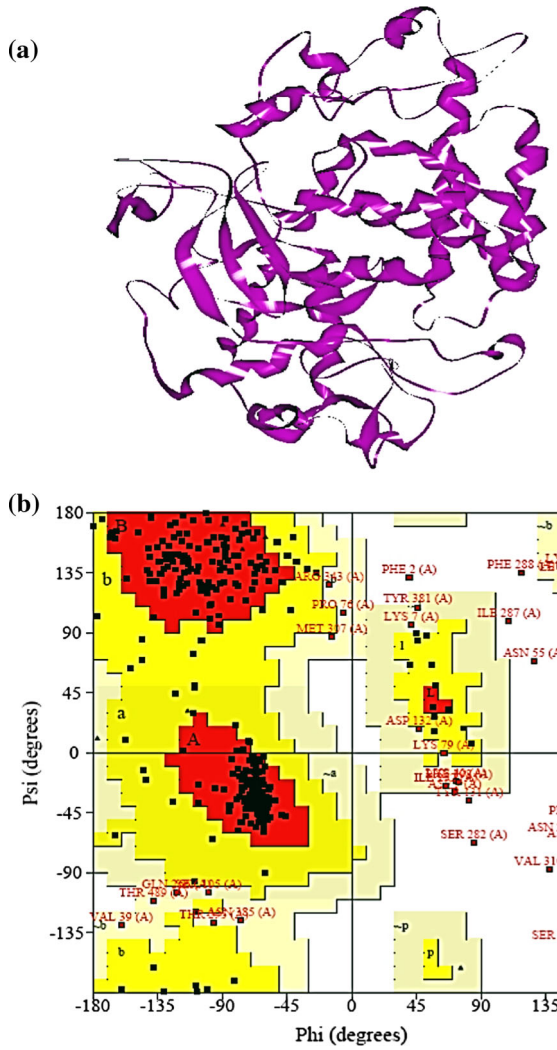

Fig. 3 **a** Ribbon representation of 3D structure of PfHKMT: molecular model has been generated by providing inputs of amino acid sequence of HKMT (XM_001350229.1) of *P. falciparum* in I-TASSER program. **b** Ramachandran plot analysis: The plot statistics of 3D model of PfHKMT was calculated with the PROCHECK program

and Pro212. In the follow up study, we shall be using these sites to develop novel synthetic molecules.

Conclusion

Biochemical characterization of HKMT of *P. falciparum* has been performed in the past but a detailed structural study of this enzyme was missing. This work provides in silico evidence in support of PfHKMT as a promising drug target in malaria leading to the possibility of development of synthetic molecules. The 3D structure of PfHKMT provides a reasonable template for further in silico drug screening. Furthermore, prediction of active site, in the present study, is useful in understanding enzymatic activity

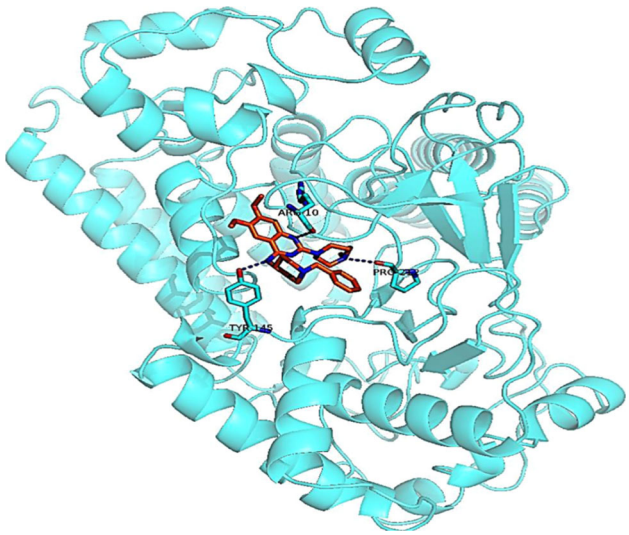

Fig. 4 Docking of PfHKMT with its inhibitor Bix01294

of PfHKMT that is crucial in deciphering the regulation of transcriptional control. More work is needed in future to develop synthetic molecules against PfHKMT in future.

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Conflict of interest The authors declare no conflict of interest.

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