

Molecular Modeling, Structural Analysis and Evaluation of 1-Deoxy-D-Xylulose-5-Phosphate Reductoisomerase (DXR) as a Putative Drug Target For *Theileria Parva*

Irmak Icen-Taskin^{1*}, Omer Munzuroglu².

¹Firat University - Department of Biology, Faculty of Sciences, Elazig, Turkey; ²Department of Bioengineering, Firat University, Elazig, Turkey.

ABSTRACT

The apicomplexan parasite *Theileria parva*, the causative agent of ECF, is an important pathogen affecting both domestic and wild animals, causing major economic losses in the world. Problems such as high cost of drugs, development of resistance, and absence of effective vaccines prevent effective combating of the pathogen. Thus, it is necessary to explore new targets for affordable and higher therapeutic value drugs. 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) in the non-mevalonate isoprene biosynthesis pathway is vital to the organism and therefore has been selected as a target for developing antitheilerial drugs. In this study, the 3D structure of TpDXR was identified by template-based in silico homology modelling method, the constructed model was validated and structurally analysed, and possible ligand binding pockets were identified for the first time in the literature. A reliable 3D model for TpLDH was modelled by using 3AU9 chain 'A' *Plasmodium falciparum* as a template. The obtained result showed that the model has a good resolution structure with 86.768 overall quality factor and a -9.15 z-score for TpDXR. The present study promises the possibility of exploiting new and safe inhibitors using the structure-based drug design that is effective against ECF through docking studies.

Key words: Homology modelling; *Theileria parva*; 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (DXR); East Coast Fever

* Author for correspondence: irmak.icen@hotmail.com, iicen@firat.edu.tr

INTRODUCTION

Theileria parva is one of the two economically important protozoa of the *Theileria* genus belonging to the apicomplexan phylum which includes *Plasmodium spp.* and *Toxoplasma gondii*, causative agents of malaria and toxoplasmosis, respectively (1). *Theileria parva* is the disease agent of tick-borne East Coast Fever (ECF), which ranks first among the tick-borne diseases affecting cattle in sub-Saharan Africa (2). The disease caused by the parasite affects a large proportion of domestic and wild animals in the world. This parasite is highly pathogenic for cattle as the vertebrate host, leading to serious economic losses (3).

In general, the ECF therapy is based on controlling strategies that utilize acaricides for tick vector (4) immunization via the live attenuated cell-line vaccine (5) and the medical consensus that combines drugs such as parvaquone and buparvaquone (6, 7) for *Theileria parva*. On the other hand, the factors limiting the wide use of this treatment are the high costs of the acaricides that are utilized for tick control (8) and the rapid resistance development against a wide variety of these (9, 10), as well as the absence of an efficient vaccine against *T. parva* that is proven to be safe (4). Moreover, in 2010, resistance to buparvaquone has been observed as well (11); this was followed by a new report in the year 2012 caused by point mutation mitochondrial cytochrome *b* gene in the parasite (12, 13). Considering these factors, the development of an efficient and affordable anti-theilerial agent in a different mode of action that can be used safely is vital.

With the complementation of the genome sequence of *T. parva* (14), improved knowledge about the biochemical pathways utilized by this organism has opened novel approaches for development of a chemotherapeutic agent. The 1-deoxy-D-xylulose 5-phosphate/2-C-methyl-D-erythritol-4-phosphate (DXP/MEP) pathway is one of the essential metabolic pathways targeted for rational drug discovery (15, 16). The MEP pathway is utilized by most bacteria and apicomplexan parasites, including *Mycobacterium tuberculosis*, *Helicobacter pylori*, *T. gondii* and *Plasmodium spp.* (malaria parasites), to synthesize isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) for the synthesis of isoprenoid compounds. There is no functional equivalent of this pathway in mammals and since the DXP/MEP pathway is necessary in these organisms (15), the enzymes involved in this pathway become a promising target for novel therapeutic intervention, without harming host vertebrates. 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (DXR) is the second enzyme of the pathway, catalysing the reduction and isomerization of 1-deoxy-D-xylulose-5-phosphate (DXP) to 2-C-methyl-D-erythritol-4-phosphate (MEP) by utilizing Mg^{2+} and NADPH as enzyme cofactors. Potential inhibitor, fosmidomycin, a naturally occurring antibiotic against DXR, has already been identified and has demonstrated antimalarial activities in preclinical studies and clinical tribulations (17-19). These findings insinuate that DXR is a valid target, and inhibitors of this enzyme could serve as potential guides for apicomplexan's chemotherapy.

Even though DXR of *Plasmodium spp.* has been considered to identify a novel drug against malaria (17), unavailability of information about *Theileria parva* 1-Deoxy-D-xylulose-5-phosphate reductoisomerase (*TpDXR*) restricts rapid improvement of drug development against this protozoan pointed *TpDXR*. For this reason, a three-dimensional model (3D) of *TpDXR* was created through comparative modelling for the first time ever, after intensive optimization, minimization, validation and identification of possible drug target pockets to open a new route to design specific inhibitors.

MATERIALS & METHODS

Template Selection, Physicochemical And Functional Properties And Secondary Structure Prediction

The amino acid sequence of the DXR from *T. parva* (*TpDXR*) (accession number XP_764642.1) was retrieved from the National Centre for Biotechnology Information database. The physicochemical features of *TpDXR*, such as theoretical isoelectric point (pI), molecular weight, the total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average hydropathy (GRAVY), were determined with the Expasy's ProtParam server (20). The secondary structure features of the *TpDXR* were revealed with the web-based program SOPMA (21).

Homology Model Development And Model Validation

Initially, an automatic 3D model of *TpDXR* was constructed based on structural homologies with a template protein using the programme Protein Homology/analogy Recognition engine 2 (PHYRE2) (22), a free online homology modelling server. This program utilizes the alignment of hidden Markov models through HHsearch to remarkably increase the efficiency of alignment and recognition rate. Energy minimisation and root mean square deviation (RMSD) were performed using 3Drefine (23) and SuperPose servers (23), respectively. To ensure the quality of modelling, stereochemistry, energy profile and residue environment of modelled structure were checked by different servers. RAMPAGE (24) was used to test the stereochemistry of the model. The model was further examined by ProSA (25) and ERRAT servers (26). Protein Structure Validation Suite-PSVS 1.3 (<http://psvs-1.4-dev.nesg.org>) was used to analyse the quality of the model.

Ligand Binding Site Identification

The MetaPocket 2.0 server (27) was used to distinguish ligand-binding sites on the protein surface. This server is employed as an agreement strategy to predicted binding sites from PASS11 (PAS), LigsiteCS (LCS), Q_SiteFinder (QSF), GHECOM (GHE), POCASA (PCS), Fpocket (FPK), SURFNET (SFN), and ConCavity (CON) methods.

RESULTS AND DISCUSSION

Template Selection, Physicochemical And Functional Properties And Secondary Structure Prediction

Theileria parva 1-deoxy-D-xylulose-5-phosphate reductoisomerase (*TpDXR*) (EC:1.1.1.267) has been selected in this study as a drug target, having previously been analysed extensively in other apicomplexa (28, 29) but not in *T. parva*. *TpDXR* is one of the essential enzymes for the survival of the parasite, and therefore could be a putative drug target for the design and evaluation of a new class of antitheilerials. Expasy's ProtParam server was used to analyse the stereochemistry, energy profiles, potential errors, nonbonding interactions and some chemical and physical traits of *TpDXR*.



Fig. 1 : SOPMA server results for the secondary structure of the modelled *TpDXR*: Alpha helix (Hh), Extended strand (Ee), Beta-turn (Tt), Random coil (Cc)

The protein model was about 44617.2 kDa and calculated pI (isoelectric point) of the *TpDXP* was 7.57 (pI>7). The total number of negatively charged residues (Asp + Glu) was % 46 and % 47 positively charged residues (Arg + Lys). The extinction coefficient of protein was 37,360 and the instability index of this enzyme was measured as 37.42 which indicated the protein was stable. The value for the aliphatic index was found to be 93.34. The grand average of hydropathicity (GRAVY) value was -0.216. The secondary structure of *TpDXR* was forecasted by the SOPMA server. According to the result, Alpha helix (46.88%) and random coil (29.68%) composed the large proportion of the physical feature. Extended strands and beta turns were determined to be 16.46% and 6.98%, respectively (**Fig. 1**).

Homology Model Development And Model Validation

The template used for 3D structure modelling was 3AU9 chain 'A' Plasmodium falciparum (30), the resolution was 1.90 Å, which was analysed by X-ray diffraction and chosen in light of heuristics to expand certainty, percentage identity and alignment scope (**Fig. 2**).

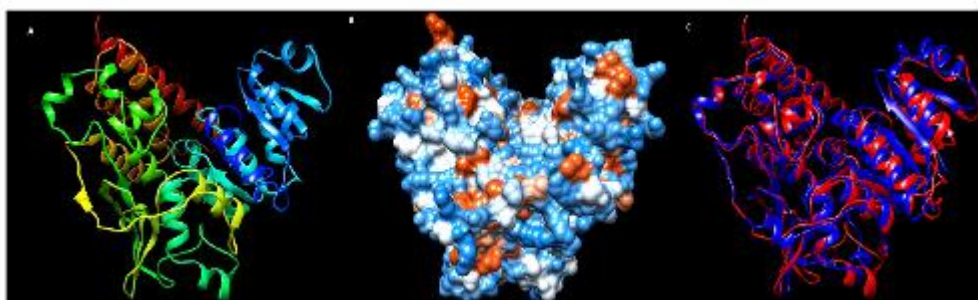


Fig. 2: Predicted 3D structure of *TpDXR*, A: Representation of *TpDXR* in ribbon style B: Surface representation of *TpDXR*. C Superposition of the predicted structure *TpDXR* with template (PDB access code: 3AU9)

The modelled structure refinement was achieved by local energy minimization and the final value was -8102.2902 kJ/mol. The $C\alpha$ RMSD and the backbone RMSD deviations for the model and the template crystal structure were observed to be 0.85 and 0.86 Å, respectively. The Ramachandran plot for the anticipated model by RAMPAGE revealed that 95 % (379) of residues were found in the most favourable region, 4 % (16) of residues were in the allowed regions and 1 % (4) of residues were in the outlier region (**Fig. 3**).

A new drug target against ECF

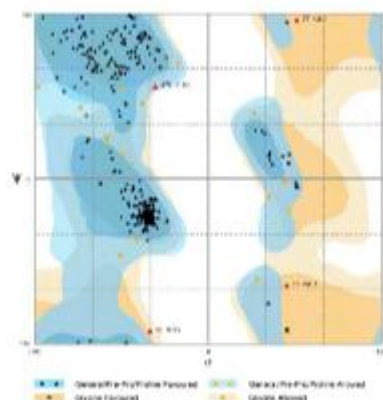


Fig. 3: Ramachandran plot of 1-deoxy-D-xylulose-5-phosphate Reductoisomerase from *Theileria parva* obtained by RAMPAGE: 95% residues in favourable regions; 4 % residues in allowed regions; 1 % residues in outlier region.

The ERRAT server was used to predict non-bonding interactions. The obtained result showed that the model had a good resolution structure with 86.768 overall quality factor (26). ProSA server was used for assessment of the overall quality of the modelled protein by means of the scores of all experimentally determined protein chains as of now accessible in the Protein Data Bank (PDB) (25). The program gave a -9.15 z-score and a plot of the residue energies for *TpDXR* demonstrated that the modelled quality was acceptable (**Fig. 4**). The overall G factor ascertained on the premise of the primary chain parameter construed that the modelled structure was satisfactory for virtual screening.

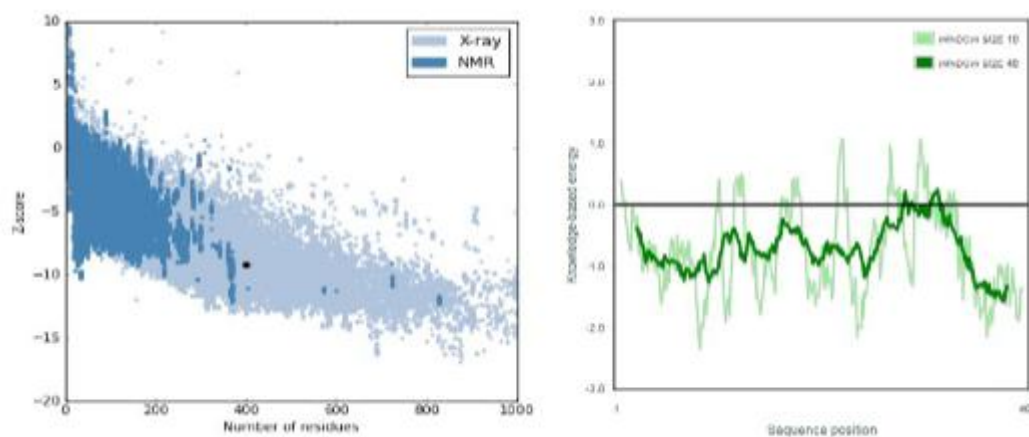


Fig. 4: ProSA web service analysis of *TpDXR* protein model

Ligand Binding Sites

The potential binding sites (PBS) of proteins are those residues or atoms which tie to ligands directly on the protein surface. They are predicted by the MetaPocket 2.0 with distinctive strategies, such as PAS, QSF, FPK, SFN, GHE, CON, and LCS. We have considered the top three sites (**Fig. 5**).

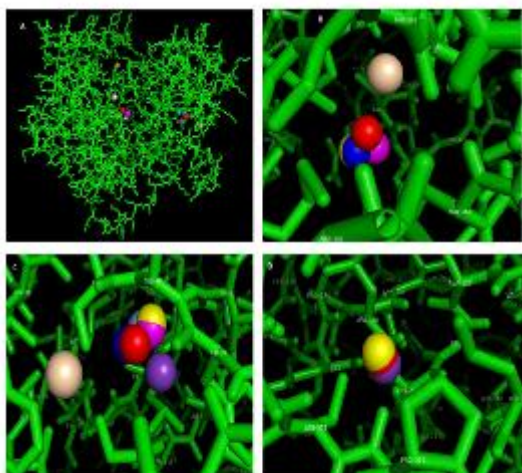


Fig. 5: The predicted potential top 3 binding sites in *TpDXR(A)* : MPK1(B), MPK2(C), MPK3(D); Pocket color description are indicated as: red - MPK, actinium - PAS, magenta - QSF, potassium - FPK, wheat - SFN, yellow - GHE, blue – CON and raspberry - PCS. The exact residue location information is given in Table 1.

After clustering the top three sites from PAS, LCS, FPK, SFN, GHE, and CON, six MetaPocket clusters were obtained. The initial MetaPocket site (MPK1) contained four pocket sites, namely, the first pocket from SURFNET(SFN-1), the first pocket from LigisiteCS (LCS-1), the first pocket from GHECOM (GHE-1), and the first pocket from Concavity (CON-1), with a total 19.16 z-score. The second MetaPocket site (MPK2) consisted of six pockets, namely, GHE-2, FPK-2 (Fpocket), LCS-2, SFN-2, PAS-2(PASS), and CON-2, with a total z-score of 11.19. The third MetaPocket site (MPK3) contained two pocket sites, namely, FPK-1 and GHE-3, with a total 4.18 z-score. The fourth MetaPocket site contained four pocket sites, namely, SFN-3, LCS-3, CON-3 and PAS-1, with a total 3.95 z-score. The fifth MetaPocket site contained one pocket site, FPK-3, with a total 1.66 z-score. The last MetaPocket site contained one pocket site, PAS-3, with a total z-score of 0.55. Table 1 shows the potential binding sites from the modelled *TpDXR* residue.

Table 1 : Identified possible ligand binding pockets:

Header

Binding Site 1

RESI	GLN_9^10^	TRP_9^214^	PRO_9^276^	ASP_9^277^	MET_9^278^
RESI	GLN_9^215^	MET_9^216^	SER_9^6^	ILE_9^220^	GLY_9^217^
RESI	VAL_9^7^	ASP_9^146^	SER_9^147^	LYS_9^121^	ASN_9^229^
RESI	GLU_9^148^	SER_9^119^	SER_9^98^	VAL_9^145^	ALA_9^96^
RESI	SER_9^224^	GLU_9^233^	LYS_9^230^	SER_9^188^	ASN_9^120^
RESI	ILE_9^97^	ASP_9^223^	GLY_9^99^	HIS_9^211^	SER_9^187^
RESI	GLY_9^189^	LYS_9^219^	GLU_9^122^	GLY_9^2^	THR_9^4^
RESI	GLY_9^5^	LEU_9^232^	ASP_9^341^	ASN_9^337^	GLY_9^190^
RESI	THR_9^186^	CYS_9^256^	MET_9^227^	ASN_9^340^	ASN_9^37^
RESI	ALA_9^36^	LEU_9^58^	HIS_9^259^	ASP_9^101^	VAL_9^213^
RESI	GLU_9^255^	VAL_9^257^	HIS_9^253^	GLY_9^8^	SER_9^38^
RESI	MET_9^1^	THR_9^35^	LEU_9^81^	MET_9^95^	LEU_9^118^
RESI	ASN_9^344^	GLY_9^102^	GLU_9^236^	CYS_9^100^	MET_9^228^
RESI	VAL_9^125^	LYS_9^389^	ASP_9^385^	ALA_9^338^	

Header

Binding Site 2

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RESI	SER_9^3^	GLY_9^8^	THR_9^9^	ALA_9^11^	LEU_9^12^
RESI	LEU_9^34^	GLN_9^46^	ILE_9^15^	VAL_9^31^	LEU_9^94^
RESI	PHE_9^50^	PHE_9^29^	GLU_9^30^	GLY_9^2^	LEU_9^286^
RESI	VAL_9^32^	ALA_9^33^	MET_9^1^	VAL_9^7^	ALA_9^96^
RESI	LYS_9^28^	ILE_9^92^	TYR_9^90^	ASP_9^91^	ASP_9^89^
RESI	MET_9^95^	ILE_9^93^	LEU_9^84^	ILE_9^97^	PRO_9^27^
RESI	GLY_9^26^	VAL_9^14^	SER_9^119^	VAL_9^145^	SER_9^283^
RESI	ALA_9^117^				

Header Binding Site 3

RESI	ASP_9^376^	ILE_9^377^	LEU_9^378^	SER_9^129^	LEU_9^130^
RESI	LEU_9^373^	ASP_9^375^	ILE_9^381^	LEU_9^331^	ASP_9^380^
RESI	LYS_9^133^	GLY_9^330^	ASP_9^372^	GLY_9^329^	VAL_9^125^
RESI	SER_9^126^	ALA_9^127^	GLY_9^128^	HIS_9^239^	PRO_9^333^
RESI	PRO_9^371^	SER_9^374^	LYS_9^111^	LYS_9^137^	VAL_9^134^
RESI	SER_9^138^	LEU_9^235^	HIS_9^238^	LEU_9^245^	GLY_9^326^
RESI	ARG_9^327^	ALA_9^334^	LEU_9^107^	MET_9^328^	GLN_9^379^

The potential binding sites of three MetaPockets are shown and they are present in residue format with each line starting with 'RESI'. The residue specified in the table is constructed of three parts: residue name, chain indicator and residue sequence number.

CONCLUSIONS

The DXR has been proposed as a drug target (e.g. fosmidomycin, Fos) against apicomplexan parasites (17). Fosmidomycin is used widely as a DXR inhibitor. In a clinical study, fosmidomycin-clindamycin was found to be efficient in the treatment of 105 Gabonese patients representing children aged 3 to 14 years with uncomplicated malaria, demonstrating its application in clinical trials (19). Moreover, eleven mixes with expansive basic assorted qualities were tried against *Toxoplasma gondii* DXR and a few strong inhibitors were identified with very low 48 nM K_i values (29). These outcomes show DXR is an important target, and conceivable inhibitors of this compound may serve as a potential route for ECF chemotherapy. However, even though significant data were obtained about the metabolism of the parasite through the complementation genome project of *T. parva* in 2005, no additional study about *TpDXR* was conducted. In the present work, a computational molecular modelling method was applied to resolve the three-dimensional structure of *TpDXR*, to overcome the lack of its crystallographic structure. After a few stages of energy minimization and refinement, possible binding pockets were identified. These results have critical ramifications for future screens aimed to discover new and safe molecular entities with activity against *TpDXR*.

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