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In silico modeling of the type 2 IDI enzymes of *Bacillus licheniformis*, *Pseudomonas stutzeri*, *Streptococcus pyogenes*, and *Staphylococcus aureus* for virtual screening of potential inhibitors of this therapeutic target

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ABSTRACT

Isopentenyl diphosphate isomerase is an essential enzyme in those living organisms such as pathogenic strains of *Streptococcus* and *Staphylococcus* genera which rely on the Mevalonate pathway for the production of isoprenoids. The pathogens contain type 2 IDI in contrast to human that contains type 1 IDI. Therefore, the type 2 IDI may be a potential target for the therapy of some infectious diseases. In the current study, a virtual screening by docking was performed among 2000 chemicals from CoCoCo library to find a specific inhibitor for type 2 IDIs. To this end, the structures of the type 2 IDIs of *Bacillus licheniformis*, *Pseudomonas stutzeri*, *Streptococcus pyogenes*, and *Staphylococcus aureus* were molded using comparative modeling and Hidden Markov Model (HMM) based prediction. The predicted models were evaluated based on Q-mean and Prosa score. Molegro Virtual Docker with MolDock scoring function was used for measuring the binding affinity of the found inhibitor to the active site of the models. Also the inhibition effect of the compound was virtually tested on the crystallography-solved structures of the *Sulfolobus shibatae* and *Thermus thermophilus* type 2 IDIs as well as the *Escherichia coli* type 1 IDI. Finally, the inhibition effect of the found inhibitor was virtually tested on the human type 1 IDI. Interestingly, the results suggest that the inhibitor efficiently binds to and inhibits the bacterial IDIs especially the type 2 IDIs of pathogens while it is not inhibiting the human IDI.

1. Introduction

Isoprenoids are a large family of metabolite containing more than 23,000 different molecules with diverse functions in various living organisms. Any isoprenoid molecule is basically made up of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) molecules as building blocks [1]. Polymerization of the building blocks by prenyl diphosphate synthases and further modifications result in the formation of different isoprenoids. Isopentenyl disphosphate isomerase (IDI, EC 5.3.3.2) catalyzes the reversible interconversion of isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). It has been established that the biosynthesis of IPP and DMAPP may occurs through the mevalonate pathway and the 2-C-methyl-D-erythritol 4-phosphate (MEP) pathway [2]. The mevalonate pathway converts acetyl-CoA just to IPP; as such, mevalonate organisms require IDI for obtaining DMAPP from IPP. In contrast, the MEP pathway uses pyruvate and glyceraldehyde-3-phosphate to produce both IPP and DMAPP (Fig. 1). Therefore, IDI is not essential for the survival of non-mevalonate organisms [3,4]. There are two types of structurally unrelated IDI enzymes. Type 1 identified in eukaryotes and some bacteria is a metalloprotein requiring two divalent metal ions for activity [5–8]. Type 2 was discovered later in some bacteria including *Bacillus* and *Streptomyces* genera as well as archea [9–15]. This enzyme is a flavoprotein that requires FMN, NAD(P)H and one divalent metal ion for activity.

The expression of both type 1 and type 2 IDIs has been used in several metabolic engineering attempts for improving carotenoid biosynthesis in *Escherichia coli* [16,17]. On the other hand, some medically important pathogens such as multi-drug resistant strains of *Staphylococcus aureus*, *Streptococci* and *Enterococci* use type 2 IDI while human as the host of the pathogens uses type 1 IDI. Therefore, type 2 IDI of the pathogens was suggested as a candidate target for designing new antibacterial drugs [18]. The aim of this research is to use an in silico approach to find a potent inhibitor for IDI2. To this end, a homology-based approach was used to predict the structures of the IDI2 enzymes of *Bacillus licheniformis* (IDI_{Bl}), *Pseudomonas stutzeri* (IDI_{Ps}), *Streptococcus pyogenes* (IDI_{Sp}), and *Staphylococcus*

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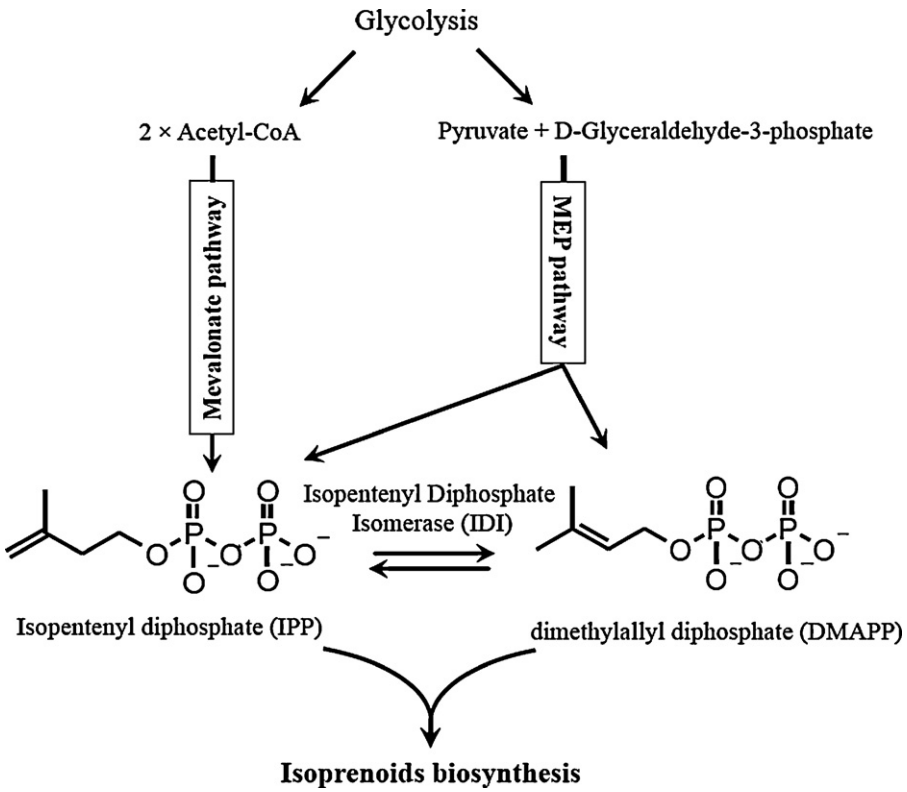


Fig. 1. Role of isopentenyl diphosphate isomerase (IDI) in isoprenoids biosynthesis. In mevalonate organisms, IDI catalyzes an essential reaction in isoprenoids biosynthesis; and it is considered as a potential target for chemotherapy.

aureus (IDI_{Sa}). Various molecules from public databases were analyzed for their likely inhibition effect on the predicted structures to find the best inhibitor candidate. The found inhibitor was further evaluated using several crystallography-solved IDI structures taken from Protein Database (PDB) including the IDIs of *E. coli* (IDI_{Ec}), *Bacillus subtilis* (IDI_{Bs}), *Sulfolobus shibatae* (IDI_{Ss}) and *Thermus thermophilus* (IDI_{Tt}). Finally the inhibition effect of the found molecule on the human (*Homo sapiens*) IDI (IDI_{Hs}) was studied.

2. Methodology

2.1. Homology modeling and structures

The structures of the IDI_{Bl}, IDI_{Ps}, IDI_{Sp} and IDI_{Sa} were predicted by homology modeling since to date there are no experimentally solved structures for these proteins. The FASTA format of amino acid sequences related to IDI_{Bl} (accession number: 302311024), IDI_{Ps} (accession number: 146284188), IDI_{Sp} (accession number: 378929506) and IDI_{Sa} (accession number: 151222459) were retrieved from NCBI database (http://www.ncbi.nlm.nih.gov). The sequences were submitted to swismodel [19], Phyre2 [20] and SAM-T08 [21] web servers to find a suitable template with sufficient query sequence coverage and sequence identity. As an alternative approach Modeller v9.8 [22] was used to predict the 3D structures of the queries. Primary predicted models were evaluated based on Q-mean [23] and Prosa [24] scores. Final complete models were generated by modeller v 9.8. The models were minimized regarding energy level using Chimera 1.5.3rc software. Crystallographic structures of IDI_{Ec}, IDI_{Ss}, IDI_{Tt}, IDI_{Bs}, and IDI_{Hs} were obtained from PDB database (http://www.pdb.org).

2.2. Predicting the active sites in the models

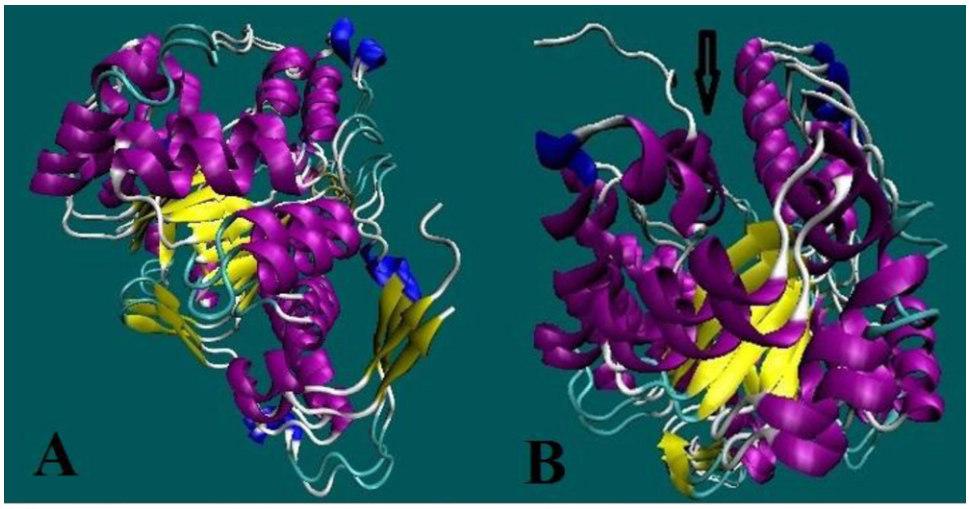
MetaPocket [25] and Molegro Virtual Docker (MVD) [26] were used to identifying the active sites in the predicted models. Regarding IDI_{Bl}, IDI_{Sp} and IDI_{Sa}, the biggest cavity that was found by MVD overlapped with the active site predicted by MetaPocket. As such, these cavities were taken as the active site in the models. Also, one cavity with the volume of 172.544 Å which was found by MVD in the predicted model of IDI_{Ps} was confirmed by MetaPocket as substrate binding site.

2.3. Ligands and inhibitors of IDIs

For in silico analysis, the structures of isopentenyl diphosphate and dimethylallyl diphosphate as ligands were obtained from KEGG compound database [27]. In a search to find potent inhibitors for prokaryotic IDIs, about 2000 shape complemented chemical compounds were retrieved from CoCoCo databases [28]. Shape complementation of the ligands' structures was performed using FindSite algorithm [29].

2.4. Molecular docking

The molecular docking was carried out by Molegro Virtual Docker (MVD) software. Before initiation of ducking operation, the molecular structures of the enzymes and ligands were prepared. To this end, charges were calculated by MVD and assigned to the models. Also explicit hydrogen bonds were assigned and flexible torsions in ligands were detected. In the first step of docking operation, the side chain flexibility was set for key amino acids predicted to be present in the active sites in the models. Grid-based MolDock score [30] with a grid resolution of 0.30 Å was used as scoring function for docking. Hydrogen bond formation between ligand and protein was permitted. MolDock SE was used as docking algorithm and ten runs for each ligand were defined. The energy threshold for pose generation was 100.00. Also similar poses were ignored. During all docking steps these parameters were held fixed. After docking, energy optimization of hydrogen bonds was performed.

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>P1;template1 structureX:template1.pdb:   9 : :+335 : :::-1.00:-1.00
--------RKNDHLDIVLDPTRAIAATGTGFGAFRFEHCALPELHLDQIDLQTALFGRRLRAPLLISSMTGGAAR
SAAINAHLAEAAQQLGIAMAVGSQRVALETAGDQGLTGQLRQLAPDILLLANFGAAQLVRGYGVDEARRAVEMIE
GDALIVHLNPLQEAVQTGGDRDWRGVLQAIEALAARLPVPVVIKEVGAGISAAVARRLVDAGVAAIDVAGAGGTS
WAAVEAARAADASQQAIAEAFADWGIPTAQALLAVRDACPNTPLIASGGIRDGVEAAKAICLGADLVGQAAGVLQ
AAMHSSEAVVSHFEVLIEQLRIACFCTGSADLAGLRQARLLQL---*

>P1;query sequence:query:     : :     : ::: 0.00: 0.00
MSKQSLVSRKNDHLDIVLDPTRAIAATGTGFGAFRFEHCALPELHLDQIDLQTALFGRRLRAPLLISSMTGGAAR
SAAINAHLAEAAQQLGIAMAVGSQRVALETAGDQGLTGQLRQLAPDILLLANFGAAQLVRGYGVDEARRAVEMIE
GDALIVHLNPLQEAVQTGGDRDWRGVLQAIEALAARLPVPVVIKEVGAGISAAVARRLVDAGVAAIDVAGAGGTS
WAAVEAARAADASQQAIAEAFADWGIPTAQALLAVRDACPNTPLIASGGIRDGVEAAKAICLGADLVGQAAGVLQ
AAMHSSEAVVSHFEVLIEQLRIACFCTGSADLAGLRQARLLQLSAC*
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Fig. 2. Loop modeling of the structure of the *Pseudomonas stutzeri* IDI by using Modeller v9.8 software. Orientations that show C-terminal (A) and N-terminal (B) loop modeling.

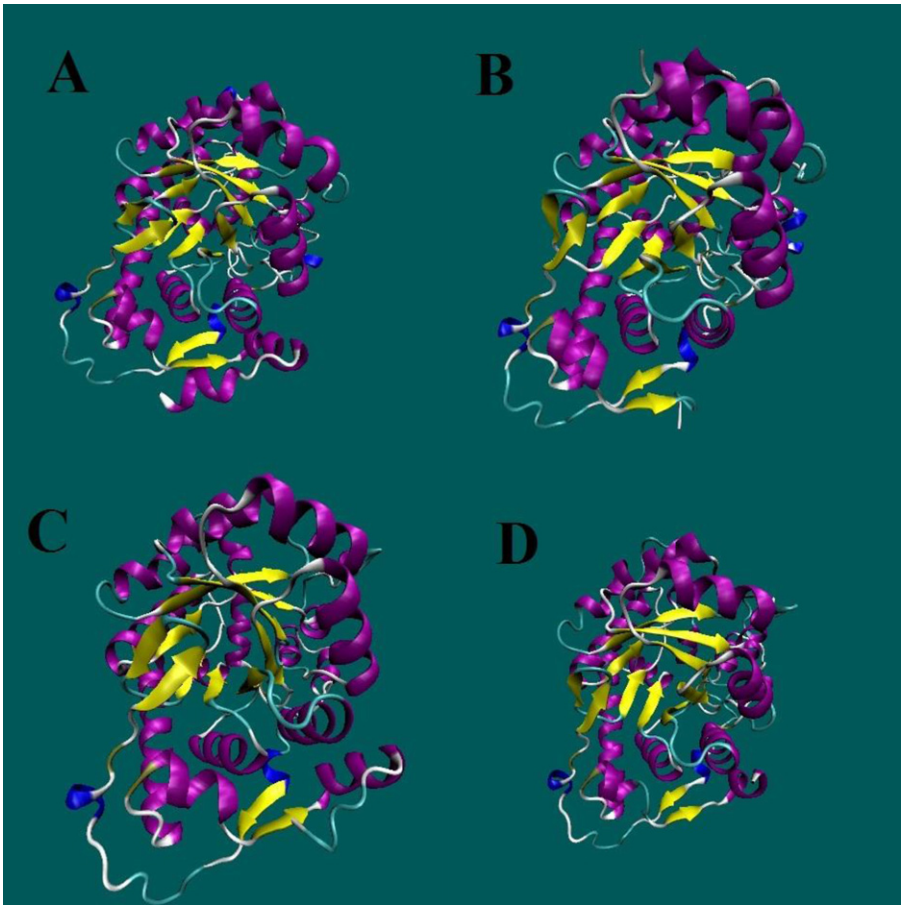

Fig. 3. Molded structures of the IDIs of (A) *Bacillus licheniformis* (IDI_{Bl}), (B) *Pseudomonas stutzeri* (IDI_{Ps}), (C) *Staphylococcus aureus* (IDI_{Sa}), and (D) *Streptococcus pyogenes* (IDI_{Sp}).

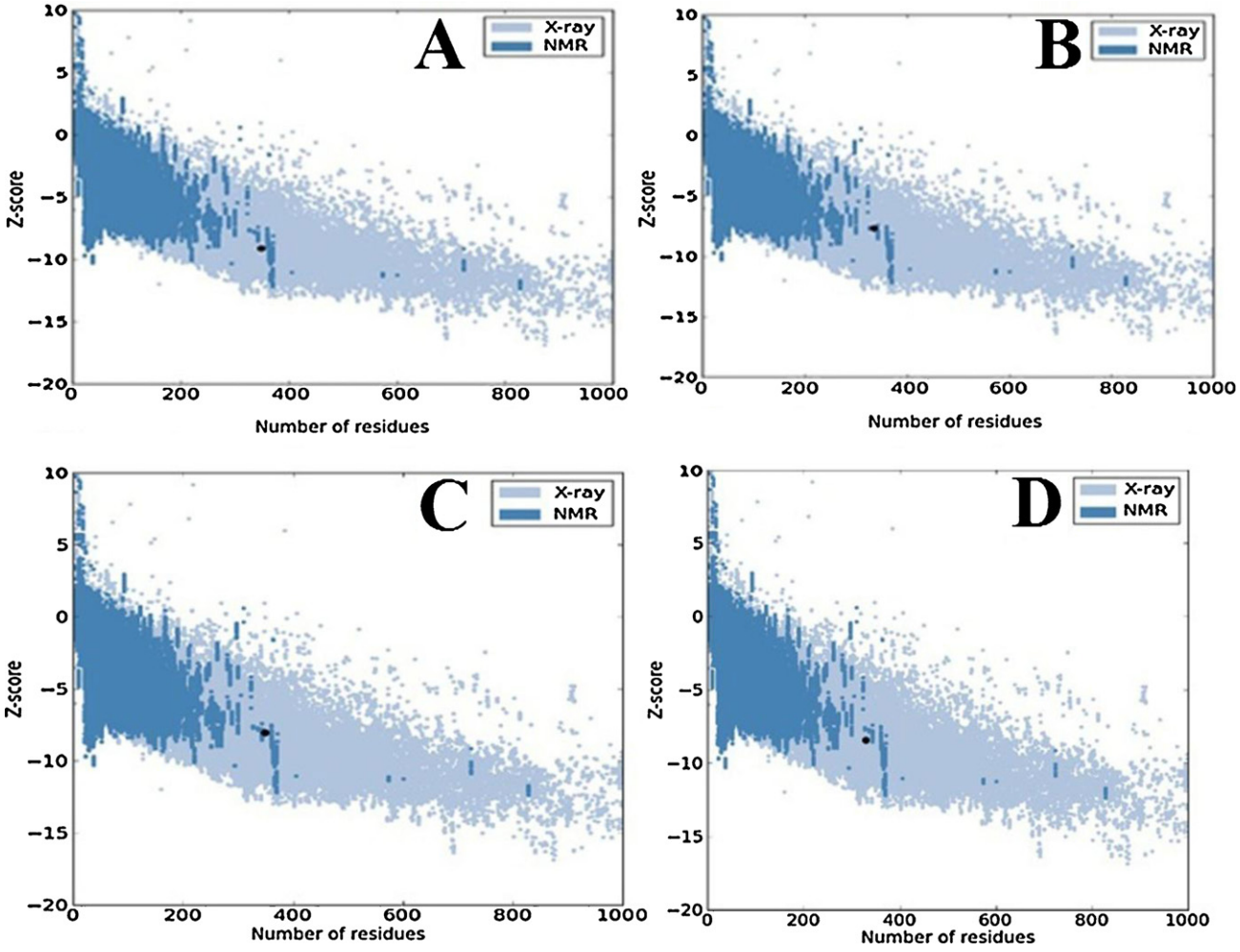

Fig. 4. Prosa scores obtained for the modeled structures of (A) IDI_{Bl}, (B) IDI_{Ps}, (C) IDI_{Sa}, and (D) IDI_{Sp}.

3. Results and discussion

3.1. The homology modeling of IDI_{Bl}, IDI_{Ps}, IDI_{Sp}, and IDI_{Sa}

Using automated mode of swissmodel, a final model was predicted for the IDI of *B. licheniformis* (IDI_{Bl}) based on the chain B of IDI type 2 apo structure of *B. subtilis* (PDB ID: 1p0k). Query coverage of the swissmodel-obtained model contained residues of 22–349. Because of low coverage, this model was not used for further study. In addition, Phyre2 predicted a query model using type 2 IDI of *S. shibatae* (IDI_{Ss}) as template (PDB ID: crzr). The Phyre2-predicted model had 100% confidence in alignment with 98% query coverage. SAM-T08 algorithm predicted 3D model of IDI_{Bl} using Hidden Markov Model algorithm. In this server the number of misaligned residues was decreased; and in output model of SAM-T08 all of residues were modeled. Also as an alternative approach, the crystallographic structures of type 2 IDI_{Ss} (PBD ID: crzr), IDI_{Tt} (PBD ID: 1VCG), and IDI_{Bs} (PBD ID: 1P0N) were used as inputs for Modeller to predict using multi template method the 3D models of type 2 IDI_{Bl}, IDI_{Sp}, and IDI_{Sa}. In order for predicting the 3D model of IDI_{Ps},

Table 1
Docking results of isopentenyl diphosphate and the found inhibitor molecule on the active site of the IDI enzymes of *Bacillus licheniformis*, *Pseudominas stutzeri*, *Sulfolobus shibatae*, *Thermus thermophiles*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Homo sapiens*.

Species	Isopentenyl diphosphate			Inhibitor molecule		
	ModDock score	Re-racking score	H bond	ModDock score	Re-racking score	H bond
B. licheniformis	−71.979	−51.089	−6.3102	−271.97	−51.089	−6.3102
P. stutzeri	−94.54	−60.181	−11.223	−179.16	66.2632	−11.285
S. shibatae	−93.464	−85.145	−11.379	−201.74	−49.568	−9.5681
T. thermophiles	−78.867	−68.264	−7.6782	−197.54	−8.6218	−19.218
B. subtilis	−102.56	−77.3	−8.992	−234.7	−89.611	−22.385
E. coli	−99.923	−88.147	−9.3371	−131.58	46.5692	−14.526
S. pyogenes	−97.908	−77.683	−1.8145	−168.16	291.277	−20.07
S. aureus	−93.23	−82.333	−6.1423	−224.13	161.926	−10.436
2ZRW	−89.767	−73.917	−15.86	−196.58	−51.33	−19.283
2ZRY	−87.373	−73.52	−16.345	−189.12	−48.699	−30.41
3B05	−82.499	−70.127	−13.675	−194.04	−40.487	−10.742
H. sapiens	−129.52	−109.48	−10.878	5.07711	1248.34	−8.7144

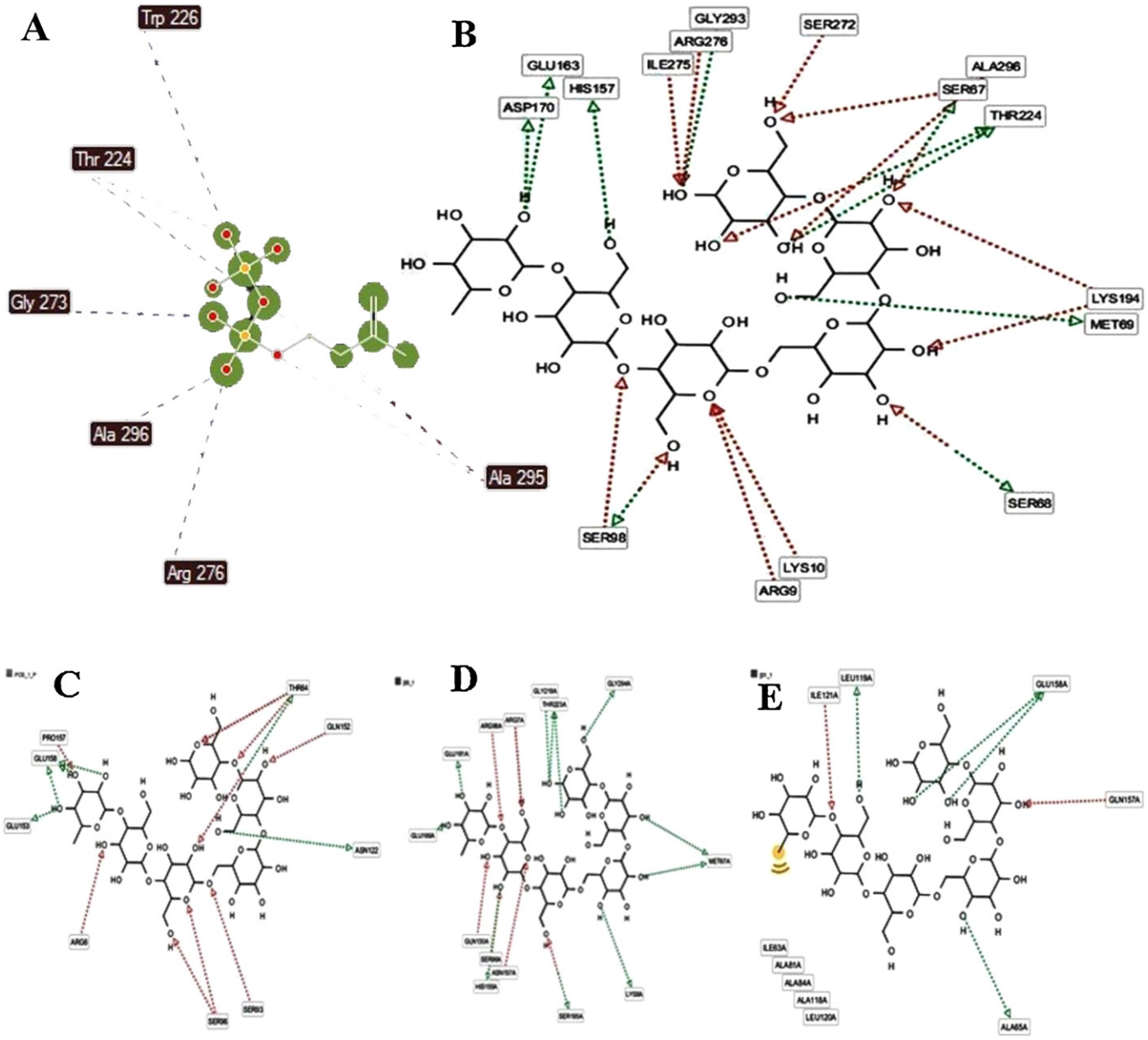

Fig. 5. Structures of isopentenyl diphosphate (natural substrate) and the inhibitor in relation with the contact residues in isopentenyl diphosphate isomerases. (A) Natural substrate in contact with the active site of IDI_{Ps}; (B–E) the inhibitor in contact with the active site of IDI_{Ps}, IDI_{Bl}, IDI_{Ss}, and IDI_{Tt} respectively.

Phyre2 used the crystallographic structure of IDI_{Ss} (PBD ID: crzr) with 38% identity as template. As a result, 97% of the query was modeled by this server and the three percent misaligned regions were completed by loopmodeling using modeller v 9.8 (Fig. 2).

3.2. Estimating the quality of predicted models

Qmean [23] assay was used for model quality estimation. Qmean is a parameter scaling from 0 to 1. The best of crystallography structures obtain a score near 1. For IDI_{Bl}, the Phyre2-modeled structure scored 0.76, the SAM-T08 predicted model scored 0.75, and the Modeler-predicted model scored 0.76 in Qmean assay. Also Qmean score of the Phyre2 predicted model of IDI_{Ps} was 0.815. Regarding IDI_{Sp} and IDI_{Sa}, the best Q-mean scores were respectively 0.710 and 0.750 obtained by the Modeller output models.

3.3. Generating final complete models

For all predicted models loopmodeling was performed using MODELLER v 9.8 [22]. The output models were optimized in 10 steps with default optimization options. Chimera 1.5.3rc was used for the structure minimization of the models. The minimization was performed with a step size of 0.02 in 100 stages. The final complete models of IDI_{Bl}, IDI_{Ps}, IDI_{Sp}, and IDI_{Sa} are depicted in Fig. 3. In order to estimate the quality of the final models, Prosa server [24] was used to calculate the *z*-score of each model. The output *z*-scores for IDI_{Bl}, IDI_{Ps}, IDI_{Sp}, and IDI_{Sa} models were −9.1, −7.65, −8.44, and −8.03 respectively. These scores indicate that the predicted models have NMR quality (Fig. 4) [24]

3.4. Molecular docking

Molegro Virtual Docker (MVD) was used for docking studies. MVD performs flexible ligand docking so that the optimal

geometry of the ligand is determined during the docking. MVD includes MolDock score [26] for evaluating docking solutions. In this study, docking results were evaluated on the basis of the MolDock score and re-ranking. MolDock software combines differential evolution with a cavity prediction algorithm [26]. The MolDock scoring function is based on a piecewise linear potential (PLP) [31,32] and takes into consideration the directionality and charges of hydrogen bonding. Docking scoring function used in this paper is defined as:

$$E_{\text{score}} = E_{\text{inter}} + E_{\text{intra}}$$

E_{inter} is ligand–protein interaction energy and is defined by

$$E_{\text{inter}} = \sum_{i \in \text{ligand}} \sum_{j \in \text{protein}} \left[E_{\text{PLP}}(r_{ij}) + 332.0 \frac{q_i q_j}{4r_{ij}^2} \right]$$

The summation encompasses all heavy atoms in the protein and the ligand as well as any cofactor atoms and water molecule atoms. The second term points up the electrostatic interactions between charged atoms. E_{intra} describes the internal energy of the ligand:

$$E_{\text{intra}} = \sum_{i \in \text{ligand}} \sum_{j \in \text{ligand}} E_{\text{PLP}}(r_{ij}) + \sum_{\text{flexible bonds}} A[1 - \cos(m, \theta - \theta_0)] + E_{\text{clash}}$$

The double summation contains all atom pairs in the ligand except those which are connected by two bonds or less. Second term is a torsional energy and θ is the torsional angle of the bond. E_{clash} term assigns penalty of 1000 provided that the distance between two heavy atoms is less than 2.0 Å. For finding chemical compounds with high affinity to the active site of IDI2, we selected 2000 shape-complemented chemicals from CoCoCo library. Virtual screening was performed using MVD software with MolDock as scoring function. Screening the data indicated that one chemical compound can bind to prokaryotic IDIs efficiently. In Fig. 5 structure of the inhibitor chemical and its contact bonds with the active site of IDI_{Bl}, IDI_{Ps}, IDI_{Ss}, and IDI_{Tt} is depicted.

The docking results indicated that the best pose of IPP in active site of IDI_{Bl} has a binding affinity of −71.9792 (in arbitrary unit) while the MolDock score for the found inhibitor was −251.972. Also this potential inhibitor can bind to the active site of IDI_{Ps} with a MolDock score of −179.159 while IPP as the natural ligand reached the score of −94.5398 in the best pose. Also the docking operation was performed with the same condition on the crystallography-solved structures of IDI_{Ss}, IDI_{Tt} and IDI_{Bs}. The obtained results confirmed the fact that the found potential inhibitor theoretically can bind to active site of type 2 IDIs efficiently. Also to predict the inhibitory effect of the found molecule on pathogenic bacteria such as species related to *Streptococcus* and *Staphylococcus* genera, we docked the chemical inhibitor against the active sites of IDI_{Sp} and IDI_{Sa}. The results suggest that the binding efficiency of the inhibitor in comparison with the natural substrate is −168.164 vs. −97.9077 for IDI_{Sp} and −224.138 vs. −93.2295 for IDI_{Sa}. Also the crystallographic structures of 2ZRW, 2ZRY and 3B05 related to IDI_{Ss} were used for docking analysis. Table 1 indicates the docking scores of inhibitor molecule and isopentenyl diphosphate for each separate model.

3.5. Simulated docking study with human IDI

In order to assay the effect of the found inhibitor on the human IDI (IDI_{Hs}), the docking operation was performed with the same condition on the crystallography-solved structure of the enzyme. The X-ray diffraction crystallographic structure of IDI_{Hs} was retrieved from PDB database (ID: 2PNY) with resolution of 1.81 Å. The results showed that the docking score of isopentenyl diphosphate for IDI_{Hs} is −129.517 while that of the inhibitor is +5.07711. The docking results indicate that the inhibitor is potent with high affinity specifically for the prokaryotic IDIs.

3.6. Determining contact residues

Using ligandScout 3.0, the contact residues of the IDI structures with ligand and inhibitor were identified. Fig. 5 indicates that the selected inhibitor molecule can bind to 16 residues in the active site of the IDI_{Ps} while the natural substrate can bind only to 5 residues. Similar results were obtained for other bacterial IDIs used in this study. Based on the docking results and the number of contact residues, it may be assumed that the occupation of active site by the inhibitor molecule with high binding affinity can prevent the binding of natural substrate and inhibit the activity of IDIs.

4. Conclusion

In this study, a virtual screening approach was taken to find a potent inhibitor for isopentenyl diphosphate isomerase (IDI) as a target for blocking the isoprenoid biosynthesis in pathogenic bacteria. A search in CoCoCo databases resulted in finding a theoretically efficient inhibitor with high binding affinity to the bacterial IDIs particularly the type 2 IDIs of pathogenic *Streptococcus pneumonia* and *S. aureus*. Interestingly, the docking results suggest that the found inhibitor has no significant binding affinity to the human type 1 IDI. Previously, substrate analogs, alkyne 1-OPP and allene 2-OPP had been synthesized and introduced as inhibitors of *T. thermophilus* IDI-2 or *Escherichia coli* IDI-1 [33]. To the best of our knowledge, most of the inhibitors of IDIs, introduced so far, are substrate analogs functioning as mechanism-based inhibitors. So, they may affect both IDIs, i.e. type 1 and type 2, with little discrimination. In contrast, the found inhibitor in this study is a hexaose and may have less side effects on the human body due to its more specificity toward type 2 IDI. Beside the data we have achieved by in silico analysis in this study, additional data based on experimental studies are required to reveal the inhibitory effects of the inhibitor and to elucidate its mechanism of action. The current study taking advantage of several modeled and crystallography-solved structures of IDIs in an in silico approach may shed lights on designing new and potent inhibitors to be used as next generation drugs.

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