In-Silico Testing Of Nutraceutical Against The MurD Enzymes From Mycobacterium Tuberculosis

Mohammad Teimouri, Hamidreza Kamrani

Abstract: In spite of availability of moderately protective vaccine and antibiotics, new antibacterial agents are urgently needed to decrease the global incidence of Mycobacterium tuberculosis infections. Mur family is an important target for the development of new drugs as they are involved in the biosynthesis of bacterial cell wall. MurC-MurF ligases catalyze a series of irreversible steps in the biosynthesis of peptidoglycan precursor, i.e. MurD catalyzes the ligation of D-acetate to the nucleotide precursor UMA. Here, we developed a homology model of MurD from M. Tuberculosis and was validated by using rampage, Errat and ProSA online servers. Different nutraceuticals were tested and reported for their activity. Among the 14 nutraceuticals, Diosgenin, Xanthohumol, Capsaicin, 1'-acetoxychavicol acetate and [6]-Gingerol have best docking score. The best of all was Diosgenin with the docking score -14.22988, Xanthohumol with -13.923555, Capsaicin with -12.880404, 1'-acetoxychavicol acetate with -12.573502 and [6]-Gingerol -12.349156 which will play a guiding role in the experimental design and development of mycobacterium tuberculosis MurD.

Index Terms: Nutraceutical, MurD, Mycobacterium Tuberculosis, homology modeling, Molecular Docking, I-Tasser Server, Modeler v9.14

1 INTRODUCTION

Mycobacterium tuberculosis is among the major diseases throughout the world. It is a serious health problem and has been reemerged as MDR in the world [1]. Around 40 years early new drugs were developed for this [2]. To date 9 million new and 1.7 million death cases are reported annually [3]. So, chemotherapy against the bacterial infections also remained a major obstacle and lead to the development of urgent drugs using pathogen specific targets [4]. Peptidoglycan biosynthesis machinery is among the best targets for drug development. Bacterial cell wall, its shape, stability and functioning is totally dependent on peptidoglycans. So due to the unique feature of prokaryotes targeting the peptidoglycan will lead to the discovery of new potential candidates [5]. A two stage complex peptidoglycans biosynthetic pathway is catalyzed by a group of Mur enzymes from MurA to MurF. All the steps are catalyzed by each enzyme. All of these from MurA to MurF are conserved in almost all bacterias. Effort are made to explore and develop new inhibitors but MurC, MurD and MurF are still the best targets for the development of new drugs[6]. In this study MurD from Mycobacterium tuberculosis was targeted which is a D-glutamic acid adding enzymes. Phosphinate inhibitors are known for MurD. Several substrate analogues were used for MurD enzymes from E. coli but no promising results were obtained from those. Here we have docked nutraceuticals against the MurD enzymes from M. Tuberculosis. MurD is d-Glutamic acid-adding enzyme (MurD ligase) which catalysis the addition of d-glutamic acid to UDP-N-acetylmuramoyl-l-alanine, an essential cytoplasmic step in the pathway for bacterial cell-wall peptidoglycan synthesis as shown in the figure [6]. As such, it represents an important antibacterial drug-discovery target enzyme. Till now a number of compounds are synthesized and tested against MurD from Escherichia coli [7].

In the present work, we have tested different nutraceuticals including [6]-Gingerol, Anethol analogues, Capsaicinoids, Curcumin, Dibenzoylmethane, Diosgenin, Eugenol, Gambogic acid, Thymoquinone, Ursolic acid, Xanthohumol and Zerumbone against the MurD enzymes from Mycobacterium tuberculosis. Despite the fact that nutraceuticals are using in food, it also shows great potential for modulating multiple diseases such as Alzheimer's disease (AD), Antioxidants, cardiovascular diseases, Parkinson's disease, Obesity, Diabetes, Osteoarthritis and Adrenal Dysfunction. Here we used the molecular modeling technique and docking approach to test the effect of different nutraceuticals against the bacterial infections.

Figure 1. The pathway of peptidoglycan biosynthesis. Each step is catalyzed by a specific enzyme from Mur enzyme family.
2 MATERIAL AND METHODS
In this work a homology modeling followed by a molecular docking was carried out using different softwares including Modeler, MOE, Pymol and DS visualizer 4.5. Initial validation of the model was carried out by using Rampage, Errat, ProSA and Qmean.

2.1 Software and hardware
To get a good quality model of MurD, I-Tasser [8], Pyre 2 [9], Lomets [10], Geno3D [11], MOE [12] and Modeler v 9.14[13] was used to generate a good homology model. The evaluation of the model was carried out using online servers including ERRAT [14], RAMPAGE [15], Qmean and ProSA[16]. For docking MOE was used.

2.2 Templates selection and Sequence alignment
The complete amino acid sequence of MurD was retrieved from Uniprot [17]. The length of MurD is 214 Amino acids. Using the primary sequence of MurD as query a PSI-BLAST[18]was carried out to select suitable templates against the query. The PDB codes with the highest homology and coverage were retrieved from RCSB. Initially two templates were used for manual model building. The alignment of the query sequence with the selected templates was carried out using t-coffee online server [19].

2.3 Homology modeling
Using I-Tasser[20], Phyre 2 [21] and Modeler v 9.14[22] were used to construct the final model. Initially 100 different models were constructed but due to low quality they were discarded. The final model was generated by using Modeler v 9.14. A PSI-BLAST revealed only two templates with good homology and coverage. A combine model was generated by using Modeler v 9.14.

2.4 Ligand Molecules Selection
The 3D structures of different active nutraceuticals were retrieved from chemspider (http://www.chemspider.com/) and drugbank (http://www.drugbank.ca/). These nutraceuticals were then clustered into a single .mdb file using MOE 2014. Before the preparation of database protonation and energy minimization of the selected compounds was also conducted. The ligands selected for docking include [6]-Gingerol, Anethol analogues, Capsaicinoids, Curcumin, Dibenzoylemethane, Diosgenin, Eugenol, Gambogic acid, Thymoquinone, Ursolic acid, Xanthohumol and Zerumbone.

2.5 Molecular Docking
After modeling and energy minimization removal of water molecules addition of hydrogen atoms was carried out. Optimization of receptor molecule was also optimized using energy minimization and protonation. After this the site finder tool of MOE was used to find actual binding site which was marked as dummy and those residues were used for docking. Around 30 conformations were allowed to each ligand and the results were analyzed by using MOE and DS Visualizer 4.5 client.

3 RESULTS AND DISCUSSION
The primary sequence of MurD was submitted to different online servers for the 3D structure including I-Tasser[20] and Phyre2[21]. The structure was finally built on Modeler v 9.14. A PSI-BLAST reported templates with accession numbers 1EOD having coverage 94%, and identity 32% and 3LK7 with coverage 94% and identity 29% are the best templates to be used to model the structure of MurD. Using MOE, the energy minimization function and protonation was carried out. Using t-coffee for multiple sequence alignment showed conserved residues among the query sequence and the selected templates. The alignment is shown in the figure 2.

Figure 2. The alignment between the query sequence and the templates is shown in the figure. The alignment can be interpreted by using the colors as bad, average and good.

Construction of the model was followed by validation using different servers. The final generated model along with its surface is shown in the figure 3a. Rampage[23], Errat[14] and ProSA [16] servers were used as validation for the model. The results of Rampage showed that 86.8% amino acids lie in the favored region, 9.4% amino acids plotted in allowed region while 3.8% amino acids lie in the outlier region. The initial results of Errat showed that the overall quality factor was 79.8% and the Z-score of the ProSA was -8.7 which is showing the best quality of the model. The plot for Errat is shown in the figure 3b while the plot for ProSA is shown in the figure 4.
Figure 4: The figure is showing the ProSA validation of the MurD enzymes. The Z-score was -8.7 which is showing the best quality of the model.

Confirmation of the quality of the model was followed by the identification of active site which was done by using the site finder tool of MOE. Before the molecular docking a total of 14 nutraceuticals collected from different databases were prepared by energy minimization and protonation using MOE. Illustration of the active site is shown in the figure 5.

Figure 5. The figure is showing the identified active site for MurD using MOE. The red circle is showing the docked ligand present in the cavity.

Of the total allowed 30 conformations for each ligand the best were only selected for further visualization and hydrogen bond analysis. The total results only revealed 5 compounds with the best docking results. The best of all was Diosgenin which showed a very good docking score followed by Xanthohumol, Capsaicin, 1'-acetoxychavicol acetate and then [6]-Gingerol. The resulted docking score was Diosgenin with -14.22988 docking score, Xanthohumol with -13.923555, Capsaicin with -12.880404, 1'-acetoxychavicol acetate with -12.573502 and [6]-Gingerol -12.349156 docking score was found. The table 1 is showing the docking score of the best ligands with the number of interactions and interacting residues. The 2D interaction of the best ligands is shown in the figure 6. Thus using Diosgenin could be a base for identifying further ligands in the database using pharmacophore and virtual screening approach. These results not only reporting Diosgenin as the only but the results of other ligands are also promising.

3 Conclusion
Homology modeling is always the best alternative to x-ray crystallography and NMR. Here the 3D homology model of MurD from Mycobacterium Tuberculosis revealed further insight into the Mur family. The developed model showed good overall structural quality and was confirmed using several different validation tools. Our study provided structural insight about the interaction of different nutraceuticals against the MurD. Our docking results showed that using nutraceuticals is also a better choice of chemotheraphy. The results of Diosgenin is showed the promising results and this proved to be a natural potential candidate. This study revealed the best 5 nutraceuticals pre-clinical analysis of these nutraceuticals is necessary to accurately understand its molecular mechanism of action and pharmacological efficiency to conclusively state it as an anti-bacterial analogue.

<table>
<thead>
<tr>
<th>S.NO</th>
<th>Compound Name</th>
<th>Docking Score</th>
<th>Interacting Residues</th>
<th>No of HBDs</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Diosgenin</td>
<td>-14.22988</td>
<td>Phe120, Ser119</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>Xanthohumol</td>
<td>-13.923555</td>
<td>Phe156, Ser119, Ser56</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>Capsaicin</td>
<td>-12.880404</td>
<td>Phe156, Ser119, Arg65,Glu116</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>1'-acetoxychavicol acetate</td>
<td>-12.573502</td>
<td>Ser64, Asn97</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>[6]-Gingerol</td>
<td>-12.349156</td>
<td>Lys156, Ser119</td>
<td>2</td>
</tr>
</tbody>
</table>

Table 1 showing the best ligands among the docked. Three docking score, interacting residues and number of hydrogen bonds formed are shown in the table.

Figure 6. The figure is Showing the 2D interactions of the best ligands with MurD. (A) [6]-Gingerol (B) Capsaicin (C) Diosgenin (D) 1'-acetoxychavicol acetate and (E) Xanthohumol.
4 REFERENCES


