Inhibition of polyamine biosynthesis for toxicity control in *Serratia marcescens* strain WW4 by targeting ornithine decarboxylase: a structure-based virtual screening study

Kalyani Dhusia, Pramod K. Yadav and Rohit Farmer

Department of Computational Biology and Bioinformatics, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, 211007, India
Email: kalyanidhusia.bhu@gmail.com
Email: pramod.yadav@shiats.edu.in
Email: rohit.farmer@shiats.edu.in

Pramod W. Ramteke*

Department of Biological Sciences, Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, Uttar Pradesh, 211007, India
Email: pramod.ramteke@shiats.edu.in
*Corresponding author

Abstract: Ornithine decarboxylase (ODC) enzyme, catalyses the decarboxylation of ornithine to form spermidine which is a committed step in the biosynthesis of polyamines. Here, in the present work, structure of ODC was modelled and studied for its active site. The stability of modelled structure was revalidated by the molecular dynamics simulation at 50 nano second time scale. 142 Natural products of Indofine Herbal Ingredient library from ZINC database were screened using Autodock Vina for the identification of potential leading herbal inhibitors. The results obtained from docking showed that Conessine is best inhibiting candidate with docking affinity of –9.7 Kcal/mol. Conessine is an alkaloid which proves its immense importance as metabolites. Thus, polyamine being harmful when synthesised in excess are necessary to be controlled at their genesis. Therefore, conessine might be a potential inhibitor for toxicity control in plant growth promoting rhizobacteria.

Keywords: ornithine decarboxylase; herbal inhibitor; molecular dynamics simulation; docking; virtual screening.


Copyright © 2018 Inderscience Enterprises Ltd.
Biographical notes: Kalyani Dhusia is currently enrolled for Doctoral degree at Sam Higginbottom University of Agriculture, Technology and Sciences (Formerly Allahabad Agriculture Institute), India with Prof. (Dr.) Pramod Wasudeo Ramteke’s lab. She graduated as a first ranking student in Master’s degree with Bioinformatics and Securing a Silver Medal in her university; she is an awardee of Rajiv Gandhi National Fellowship by University Grants Commission, India. Currently, her work emphasises on ‘Control of bacterial pathogenesis by designing novel siderophore production pathway using computational approaches’. Her scholarly publications include three research papers which are published in the peer-reviewed journals.

Pramod K. Yadav is an Assistant Professor in the Department of Computational Biology and Bioinformatics at Sam Higginbottom University of Agriculture, Technology and Sciences, Allahabad, India. He has an experience of 11 years in Teaching and Research. He has published 21 research papers in peer-reviewed journals, and written one chapter in a book. He is Editor and Reviewer of several peer-reviewed international journals such as PLoS ONE, Computational Biology and Chemistry (Elsevier) IJSS: CLS (Springer), CCAD (Bentham). His areas of research interest includes, drug target identification, molecular modelling, docking, structure-based drug design, molecular dynamics simulation, and In-silico epitope prediction.

Rohit Farmer is currently working as an Assistant Professor in the Department of Computational Biology and Bioinformatics at Sam Higginbottom University of Agriculture, Technology and Sciences and is computational structural biologist by training with over seven-year experience in protein structure/complex prediction, modelling protein-protein interactions, parametrising force fields to represent novel molecular species in molecular dynamics simulations, performing virtual screening for lead identification and using machine learning for sequence analysis.

Pramod W. Ramteke working as Head and Professor in the Department of Biological Sciences at Sam Higginbottom University of Agriculture, Technology and Sciences, India. He has over 36 years of Research and Teaching experience. He has over 127 research publications in reputed journals like Biotechnological Advances, Critical Reviews in Microbiology and Scientific Reports. He is a Fellow of National Academy of Agricultural Sciences, The Linnean Society of London and National Environmental Science Academy. He has been facilitated Prof. K.S. Bilgramy Memorial Award in 2012, Er. V.S. Chauhan Gold Medal in 2013 and Life Time Achievement Award in 2015.

This paper is a revised and expanded version of a paper entitled ‘Inhibition of Polyamine biosynthesis for toxicity control in Serratia marcescens strain WW4 by targeting ornithine decarboxylase: a structure-based virtual screening study’ presented at The International Conference on Intelligent Biology and Medicine, Houston, TX, USA, 8–10 December, 2016.

1 Introduction

Serratia marcescens strain WW4 are antagonists of Pseudomonas aeruginosa WW5 showing biofilm formation and significantly compromised mortality rate when
co-cultured under phosphate-limited conditions with *Pseudomonas aeruginosa* (Chung et al., 2013; De Queiroz and De Melo, 2006; Kuo et al., 2013). *Serratia species* are gram-negative bacteria, categorised in the large family of *Enterobacteriaceae* (Benevides-Matos and Biville, 2010; Moradigavarav et al., 2016; Zaheer et al., 2016). Secondary metabolites of bacterial origin embrace several pigments, antibiotics, enzymes etc., which could be of importance to human kind in many ways (Mukherjee et al., 2012; Phadke and Jacob, 2016; Reino et al., 2007). The polyamine is a multifaceted secondary metabolite (Michael, 2016). In greenhouse trials, *Serratia marscens strain WW4* is reported to suppress more than 50% of the disease (Kim et al., 2015) and promote the root and shoot growth in plants (Tabor and Tabor, 1985; Mounce et al., 2016). It is therefore used in Citrus plants as PGPB to control pathogens, induce systemic resistance, promote growth and show synergistic results with controlled polyamine production (Anwar et al., 2014; Wang and Liu, 2016).

Polyamines play important role in growth as well as stress and disease resistance in plants (Minois et al., 2011; Minocha et al., 2014; Michael, 2016). On the other hand being regulators for cell growth and death, it is likely that excess production of polyamines affects the severity and process of diseases. Putrescine, Spermidine and spermine are polyamines mainly found in microorganisms (Aliou et al., 2014; Miller-Fleming et al., 2015; Saha et al., 2016). Putrescine is deduced to Spermidine (Kraus et al., 2014) and finally down to spermine.

Polyamines are synthesised from the amino acids either methionine, arginine (Fuhrmann et al., 2015; Gong et al., 2016) or ornithine by different life forms (Gong et al., 2016; Li et al., 2015; Miller-Fleming et al., 2015). The leading step in the biosynthesis pathway of ornithine is the production of ornithine from Glutamate by the mitochondrial enzyme acetylglutamate kinase (ArgEF) (Daidone et al., 2012; Mounce et al., 2016; Vitali et al., 1999). Ornithine is then consequently decarboxylated by ornithine decarboxylase (Kandiah et al., 2016; Kanjee et al., 2011) (ODC) to produce Spermidine via putrescine (Gong et al., 2016; Zhu et al., 2015) in the cytosol of *Serratia marscens strain WW4* (as shown in Figure 1). Hence for the controlled production of polyamines, conversion of ornithine into polyamine shall cease (Badieyan, 2013; Kurian et al., 2011). Thus, originates the need for the development of specific ODC inhibitors with herbal ingredients to control polyamine accumulation (Badieyan, 2013). ODC inhibitors (herbal inhibitor in this case) directly curtail down ODC activity and is also responsible for facilitating ODC degradation.

2 Methods

2.1 Sequence retrieval and characterisation

The 722 amino acid long sequence of ODC from *Serratia marscens strain WW4* was obtained from NCBI (WP_043148234.1) with Uniprot_id (L7ZQJ6_SERMA) and blast search was performed against Protein Data Bank (PDB)(www.rcsb.org/pdb) (Berman, 2008; Rose et al., 2015) entries to find similar structures.

Physiochemical properties such as molecular weight, theoretical pI, the total number of negatively (Asp + Glu) and positively (Arg + Lys) charged residues, extinction
coefficients, instability index, aliphatic index and grand average of hydropathicity (GRAVY) of the mature protein were computed using Expasy’s ProtParam Proteomics server.

**Figure 1** Schematic view of the biosynthesis mechanism of polyamine via ornithine, arginine, and glutamate in *S. marcescens* strain WW4 (see online version for colours)

ArgEF: acetylglutamate kinase; ArgB: ornithine transcarbamoyl transferase; AmcA: mitochondrial ornithine exporter; ODC: ornithine decarboxylase; SidA: L-ornithine N5-oxygenase.

### 2.2 Structure prediction and validation

The amino acid sequence of target protein Ornithine decarboxylase *Serratia marcescens* WW4 taken from the UniProtKB database (Uniprot accession no: L7ZQJ6) (www.uniprot.org) (Wu, 2006). On the basis of sequence similarity search for the target protein against the PDB database using the BLASTp program (Rose et al., 2015; White and Matise, 2016) two templates protein structures were identified. These were: One template (pdb id: 1c4k) with (55.31%) similarity and second template (pdb id: 1ord) with identity (56.27%). They were selected subsequently for multiple sequence alignment between the target and template sequences using the clustalW2 program (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (Larkin et al., 2007).

To generate a reliable 3D model of ODC by homology modelling, MODELLER 9.15 software (http://www.salilab.org/modeller) using those target-template alignments was used (Biasini et al., 2014; Kiefer et al., 2009; Eswar et al., 2006). To evaluate and identify any anomalies in the predicted model of ODC, it was submitted to the Structural Analysis and Verification Server (http://nihserver.mbi.ucla.edu/SAVES/). SAVES is a metaserver for analysing and validating protein structures which integrates five modules i.e., Procheck, What_check, Errat, Verify_3D and Prove.

To improve the quality of predicted model of ODC, energy minimisation was performed with the GROMOS 96 force field (Hess et al., 2008). This force field permits to evaluate the energy of the modelled structure as well as overhaul distorted geometries through energy minimisation. All computations during energy minimisation were done in
a vacuum, without reaction field. The predicted 3D structure of ODC was visualised by UCSF chimera Viewer (http://www.cgl.ucsf.edu/chimera/).

The stereochemical quality and accuracy of the predicted model was evaluated with PROCHECK by Ramachandran plot analysis. The best model was selected on the basis of overall number of residues in core, allowed, generously allowed and disallowed regions. The selected model was further put to analysis by VERIFY 3D.

2.3 Molecular dynamics simulation

The molecular dynamics simulation of modelled 3D structure of the Ornithine decarboxylase was performed using the Gromacs ver 5.1.2 program (Hess et al., 2008; Van Der Spoel et al., 2005) to track the motion of individual atoms. A folded enzyme was solvated in SPC (a simple three-point water model) water with surrounding counter ions and then energy-minimised for 1000 steps of steepest-descent minimisation to remove close van der Waals contacts using Gromos 43a1 force field (Oostenbrink et al., 2004; Schuler et al., 2001). There system was heated—in 20 distinct, independent, unrestricted, unbiased, and classical MD simulations with a periodic boundary condition and unique seed numbers for initial velocities—from where the water molecules are at 350 K and the protein is at 200 K of 10K/ps under constant temperature and constant volume, then equilibrated with a periodic boundary condition for 106 time steps under constant temperature and constant pressure of 1atm employing triclinic molecule-based scaling, and lastly simulated under the NPT condition at 1atm and a constant temperature of 310 K using the Parrinello-Rahman method to couple pressure isotropically to a value of 1.0 bar.

2.4 Virtual screening

Structure-based virtual screening (SBVS) (Huang et al., 2015; Zhang et al., 2012) was used to exploit the molecular recognition between the ligands and ODC to select chemical entities that bind strongly to the active sites (Daidone et al., 2012; Pérez-Regidor et al., 2016). This approach used docking and scoring to sort the candidates in a virtual library. The docking algorithms with the prediction of ligand conformation and orientation within the targeted active site of the receptor (Yadav Madhu, 2011). The scoring methods evaluate the binding interactions between the target and the small molecule and aimed to predict the biological activity of the natural compound based on the computed binding interactions.

2.4.1 Ligand preparation

Autodock Vina recognises and executes only pdbqt file format. Hence to perform virtual screening all 142 ligands Natural products of Indofine library as targeted ligands from ZINC database were converted from mol2 to pdbqt file format using PERL script by MGLTools-1.5.7rc1. These conversions altered their conformations and orientations to give the best binding with receptor molecule.
Inhibition of polyamine biosynthesis for toxicity control

2.4.2 Receptor molecule preparation

3D structure of ODC in pdb format was converted to pdbqt file and was then optimised and minimised for more stable configurations.

2.5 Calculation of grid size and centre of molecule

In order to perform the virtual screening for Ornithine decarboxylase (ODC), the active site was predicted in the modelled structure using the metaPocket 2.0 server (http://projects.biotec.tu-dresden.de/pocket/). This pocket was having 12 residues in which Gly200, Thr201, Ser202, Asp319, Ala321, Trp322 and Lys357 have been reported to be conserved in the active site. Active site on the target molecule calculated earlier was cross-validated from the literature of the template structure. Using the know coordinates, the Grid size and centre were defined in prepared 3D structure of ODC.

2.6 Inhibition and binding prediction via docking

Molecular docking was performed to predict ligand conformation and orientation at binding site (Ramírez, 2016; Trott and Olson, 2010). The algorithm used by Vina (Trott and Olson, 2010) for finding favourable positions of ligands in the active site of proteins does not count hydrogen atoms into its calculation; however, the protonation state of active site residues needs to be evaluated and assigned correctly before docking (Dhusia et al., 2016). Scoring was noted to evaluate the interaction energy between the target and the ligand (Rodriguez et al., 1998). Visual inspection using Pymol was performed to select molecules showing good interactions with binding site residues.

3 Results and discussions

3.1 Prediction of tertiary structure of ODC and its validation

All the statistics about a protein cannot be established by measley expressing about its primary sequence or the secondary structure. Consequently, it becomes necessary to identify its tertiary structure. Additionally, the 3D structure of ODC of Serratia marscens strain WW4 has not been reported in RCSB PDB Data bank. Therefore, the automated 3D structure of ODC from Serratia marscens strain WW4 was predicted based on the sequence-to-structure paradigm using Modeller 9.15 (Figure 2(A)). For template selection Swiss-Model was used as the sequence identity is measley 56%. A template (PDB ID: 1ORD_A) having 56% sequence identity and 0.0 E-value was selected for generating the 3D model of the ORD. The sequence identities and Expected-values are essential factors to be considered in the course of the template(s) selection. The templates which are having high alignment score(S), high sequence identity and low E-value are often selected for the reliable model generation of the target protein. The expected (E) value is a parameter that measures the significance of similarity between sequences during the database searching.

Raw QUALITY_SCORE of the multiple alignment 664.3 and QUALITY_SCORE of 91.2% was given by meodeller9.15. 1ord with 706 amino acids length and 1c4k with 708 amino acid length showed query coverage of 97% with the query sequence.
Figure 2  (A) Predicted structure by homology modelling for ornithine decarboxylase (ODC) showing Helix, Beta sheets and turns in red, yellow and green colour respectively and (B) the Ramachandran plot of modelled structure validated by PROCHECK program (see online version for colours)
The DOPE potential in MODELLER is used to evaluate the fold of the selected model. The Dope scores for both template and target structures per residues were in same range as shown in Figure 3.

**Figure 3** A comparison of the pseudo-energy profiles of the model (green) and the template (purple) structures (see online version for colours)

The structure was then validated using SAVES server. The stereochemical quality of ODC structure was checked by PROCHECK and the Ramachandran plot for ODC has been illustrated in Figure 2(B). 93% of the predicted structure i.e., 672 amino acid residues of all the 722 amino acid long enzyme fall in most favoured regions; 6.3% i.e., 45 amino acids were additional allowed regions and 0.6% equating to 4 amino acids were in the generously allowed region after the energy minimisation.

The active binding site was predicted using Meta pocket active site prediction Server and to check the authentication of results literature (Malet et al., 2014) was referred (Table 1). On the basis of these observations, centre and grid size of molecules were calculated. These were found to be located at the given axis in 3D space (Table 2).

ODC and its ligand interaction (Figure 4) shows that inhibition and binding features of the models were directed towards key amino acids like Gly200, His226, Gln291, Asp319, Ser354 and Lys357 which play a major role in ODC inhibition activity.

<table>
<thead>
<tr>
<th>Residue</th>
<th>Amino acid</th>
<th>Contact</th>
<th>AV distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>200</td>
<td>GLY</td>
<td>19</td>
<td>0.04</td>
</tr>
<tr>
<td>201</td>
<td>THR</td>
<td>19</td>
<td>0.04</td>
</tr>
<tr>
<td>202</td>
<td>SER</td>
<td>19</td>
<td>0.00</td>
</tr>
<tr>
<td>226</td>
<td>HIS</td>
<td>12</td>
<td>0.04</td>
</tr>
<tr>
<td>291</td>
<td>GLN</td>
<td>10</td>
<td>0.50</td>
</tr>
<tr>
<td>293</td>
<td>GLY</td>
<td>9</td>
<td>0.29</td>
</tr>
</tbody>
</table>
Table 1  Predicted binding site (continued)

<table>
<thead>
<tr>
<th>Residue</th>
<th>Amino acid</th>
<th>Contact</th>
<th>AV distance</th>
</tr>
</thead>
<tbody>
<tr>
<td>319</td>
<td>ASP</td>
<td>21</td>
<td>0.24</td>
</tr>
<tr>
<td>321</td>
<td>ALA</td>
<td>21</td>
<td>0.00</td>
</tr>
<tr>
<td>322</td>
<td>TRP</td>
<td>20</td>
<td>0.00</td>
</tr>
<tr>
<td>354</td>
<td>SER</td>
<td>14</td>
<td>0.06</td>
</tr>
<tr>
<td>356</td>
<td>HIS</td>
<td>21</td>
<td>0.21</td>
</tr>
<tr>
<td>357</td>
<td>LYS</td>
<td>21</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Table 2  Grid size and coordinate for central positions of active binding site

<table>
<thead>
<tr>
<th>Axis</th>
<th>Centre</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>81.242</td>
<td>48</td>
</tr>
<tr>
<td>Y</td>
<td>20.359</td>
<td>36</td>
</tr>
<tr>
<td>Z</td>
<td>31.887</td>
<td>56</td>
</tr>
</tbody>
</table>

Figure 4  Chemical structures of selected compounds from the first virtual screening (see online version for colours)

3.2 Molecular dynamics simulation

The protein dynamics (Henzler and Kern, 2007) of modelled 3D structure of Ornithine decarboxylase (Vitali et al., 1999) of Serratia spp. was studied using the Gromacs v5.1.2 tool (Jurèus and Langel, 1996). Before performing molecular dynamics simulation (MDS) (Henzler-Wildman and Kern, 2007; Liu et al., 2016a), the protein was solvated in a water box followed by equilibration using Newton’s laws of motion. Steepest Descents converged to Fmax < 800 in 1545 steps, the energy of the structure was minimised at
100 ps time scale to obtain the preferred conformation. The average potential energy of
the predicted structure was calculated using the GROMOS 96 force field which was
found to \(-1.70884e+06\) KJ/Mole. The starting structure was having potential energy of\( -1.455e+01\) KJ/Mole and the minimisation were converging at \(-4.670e + 05\) KJ/Mole. The
energies of predicted model and template (1ord) show similar pattern at each point on
time scale (ps) (Figure 5(A)) which proves the authenticity and stability of the protein
structure. Since our model has a net charge of \(+1.00\), therefore one chloride ion was
added to neutralise the net charge. Before starting the actual MDS (Faraldo-Gómez et al.,
2003; Pang, 2016; Yadav et al., 2013), position-restrained molecular dynamics was
performed. In this process, the atom positions of the protein molecule were restrained
(partially freezeed) while solvent was allowed to move. This was done to soak the water
molecule into the protein molecule. The relaxation time of 100 ps was given to water
molecule.

The molecular dynamics simulation of the modelled structure was performed using
the 50,000 ps time scale and 2500001 steps (iterations) at 300°K temperature and 1 atm
pressure. The trajectory of the MDS was analysed using the ‘Gnuplot’ program
(Wagenaar, 2014) (http://www.gnuplot.info/).

The trajectory of the radius of gyration (Rg) of the modelled structure was analysed
which gives a measure of the mass of the atom (s) relative to the centre of mass of the
molecule. This quantity gives a measure of the compactness of the structure. The average
of Rg of the protein was found to be 2.95 nm which reveals that the modelled structure is
compact (Figure 5(B)).

Figure 5  (A) Compared graph for potential energies of template (1ORD) and target structures;
(B) radius of gyration; (C) RMSD of protein from backbone and (D) RMSF of amino
acid residues of target protein (see online version for colours)
Figure 5  (A) Compared graph for potential energies of template (1ORD) and target structures;  
(B) radius of gyration; (C) RMSD of protein from backbone and (D) RMSF of amino acid residues of target protein (see online version for colours) (continued)
One of the most important criteria to analyse the stability of the protein structure is to measure the root mean square deviations (RMSD). The deviations from the original starting structure have been measured over the course of the simulation. RMSD increases pretty rapidly in the beginning of the simulation, but stabilises around 35 nanoseconds (Figure 5(C)).

The trajectory of root mean square fluctuation (RMSF) of individual amino acid residues of protein was also analysed, which computes the fluctuations of atomic positions in the structure. Except very few amino acid residues, the RMSF (i.e., standard deviation) of almost all amino acid residues was found between the 0.05 nm to 0.21 nm, which indicates that the structure is stable (Figure 5(D)).

3.2.1 First virtual screening

With the prepared executable files virtual screening (Tomar and Peddinti, 2016) was performed in Autodock Vina and 10 ligands were sorted out to be the best inhibitors on the basis of docking scores and inhibition profile. The first virtual screening took Lipinski rule of five into its screening parameter against the target molecule. For each natural compound, the chemical structure is reported in Figure 5 and their physical and chemical features are noted in Table 3. These ligands were namely; Conessine (Santora et al., 2008), Sumaresinolic acid (3-6-dihydroxy olean-12-en-28-oice acid) (Yang et al., 2016), DNC (tetrandrine) (Choi et al., 2000), Enoxolone (Cai et al., 2016; Chen et al., 2016), Naringenin (Katsumata et al., 2016; Stohs and Badmaev, 2016), Hesperidin (Hemanth Kumar et al., 2016; Peterson et al., 2006; Stohs and Badmaev, 2016), Baicailin (Liu et al., 2016b; Tarragó et al., 2008). Amongst the above noted natural inhibitors for polyamine biosynthesis (Guerra et al., 2016), most of them had anomers (tautomers to be specific) hence the second run of screening was required.

3.2.2 Second virtual screening

The second run for virtual screening (Tomar and Peddinti, 2016) took tautomerism of the screened molecules into account. It is because of the change in single hydrogen (-H) bond orientation called tautomerism that led to the rise in affinity energies from –9.7 (Kcal/mol) (Kcal/mol) to –9.5 (Kcal/mol) then to –8.9 (Kcal/mol) in Conessine and from –9.2 (Kcal/mol) to –8.8 (Kcal/mol) in Sumaresinolic acid. These gradual changes can be easily made out in Table 3.

3.3 Docking settings and analysis

The natural compounds retrieved from the ZINC database after virtual screening were docked into the active site of ODC. The active site comprised of 12 residues as predicted by Meta pocket server and literature verification bound to the ligands with hydrophobic bonds.

The obtained complex was visually inspected in order to verify the absence of steric clashes between ligands and the residues at the active site. To this end, an energy grid with 48x36x56 (numbers refer to the number of grid points in xyz), centred on the e-amino group of the active site Gly293 of Serratia marscens strain WW4 ODC was used.
<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand name</th>
<th>Zinc code</th>
<th>Energy score Kcal/mol</th>
<th>H-Bond acceptor</th>
<th>Rotatable bond</th>
<th>H-bond donor</th>
<th>xlogP value</th>
<th>M. P. (°C)</th>
<th>Molecular weight</th>
<th>Inhibition profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ornithine decarboxylase of <em>Serratia marcescens</em></td>
<td>CONESSINE</td>
<td>Zinc5752284</td>
<td>−9.7</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>4.79</td>
<td>127</td>
<td>358.614</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>CONESSINE</td>
<td>Zinc4577167</td>
<td>−9.5</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>4.79</td>
<td>127</td>
<td>358.614</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>Sumaresinolic acid (3-6-Dihydroxy oleic-12-en-28-oic acid)</td>
<td>Zinc38140519</td>
<td>−9.2</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>5.81</td>
<td>285</td>
<td>471.702</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>DNC</td>
<td>Zinc38146181</td>
<td>−9.0</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>6.55</td>
<td>221</td>
<td>622.762</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>ENOXOLONE</td>
<td>Zinc19203134</td>
<td>−8.9</td>
<td>4</td>
<td>1</td>
<td>1</td>
<td>5.62</td>
<td>279</td>
<td>469.686</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>CONESSINE</td>
<td>Zinc3977747</td>
<td>−8.9</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>4.79</td>
<td>127</td>
<td>358.614</td>
<td>Competitive</td>
</tr>
<tr>
<td></td>
<td>SUMARESINOLIC ACID</td>
<td>Zinc38140520</td>
<td>−8.8</td>
<td>4</td>
<td>1</td>
<td>2</td>
<td>5.81</td>
<td>285</td>
<td>471.702</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Naringenin*</td>
<td>Zinc1785</td>
<td>−8.8</td>
<td>5</td>
<td>1</td>
<td>3</td>
<td>2.12</td>
<td>222</td>
<td>272.256</td>
<td>Non-competitive</td>
</tr>
<tr>
<td></td>
<td>Hesperidin*</td>
<td>Zinc8680006</td>
<td>−8.8</td>
<td>15</td>
<td>7</td>
<td>8</td>
<td>-0.55</td>
<td>240</td>
<td>610.565</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>Baicailin*</td>
<td>Zinc4349038</td>
<td>−8.2</td>
<td>11</td>
<td>4</td>
<td>5</td>
<td>0.55</td>
<td>225</td>
<td>445.356</td>
<td>Competitive</td>
</tr>
</tbody>
</table>

The table summarises, for each compound, the ZINC-code, Hydrogen bond acceptor and donor, number of rotatable bonds, the molecular weight, melting point, the xlogP values, and the inhibition profile. n.d., not determined. (*) number of aromatic rings.
During each simulation, grid dimension and centre values were systematically maintained with other parameters. The selected values for grid dimension and centre were respectively $48 \times 36 \times 56$. However, the coordinates $x = 81.242$, $y = 20.359$, $z = 31.887$ were also verified from literature and put into default for all the ligands. Water molecules were excluded from the docking calculations.

All the docking and screening studies in this work were conducted using AutoDock-Vina (Kim et al., 2016a). Three Vina replicates were conducted for each ligand in the cross-docking and re-docking steps, while a single run was performed for screening. In Autodock Vina the exhaustiveness option was set to 6, and all other options were kept at their default values.

During the post-filtering phase, the poses of all docked natural (Stohs and Badmaev, 2016) compounds obtained in the previous docking-based virtual screening step were ranked according to their score, obtained with the scoring function implemented in Autodock Vina.

Binding affinity data alone does not determine the overall potency of a drug. Potency is a result of the complex interplay of both the binding and ligand efficacy. Ligand efficacy refers to the ability of the ligand to produce a biological response upon binding to the target receptor and the quantitative magnitude of this response. This response may be as an agonist, antagonist depending on the physiological response produced. The analysis of the inhibition profile against predicted ODC model of *Serratia marcescens strain WW4* reveals that natural compounds Conessine, Sumaresinolic acid (Patil et al., 2015), DNC, Exolone, Naringenin (Orhan et al., 2015; Patel et al., 2014), Hesperidin (Peterson et al., 2006) and Baicailin (Liu et al., 2016b) were the top most inhibiting candidates with Docking Affinity $-9.7$ (Kcal/mol), $-9.2$ (Kcal/mol), $-9.0$ (Kcal/mol), $-8.9$ (Kcal/mol), $-8.8$ (Kcal/mol), $-8.8$ (Kcal/mol) and $-8.2$ (Kcal/mol) behave as inhibitors of polyamine biosynthesis in *Serratia marcescens strain WW4*. The residues in active site compactly bind with the ligands (Figure 6).

Conessine (Kim et al., 2016b; Santora et al., 2008) is a naturally occurring alkaloid and generally high selectivity against other sites, including histamine receptors H1, H2, and H4 (Morais-Silva et al., 2016). Baicalin (De Oliveira et al., 2015; Liu et al., 2016b) is a known prolyl endopeptidase inhibitor and is a flavone, type of flavonoid (Peterson et al., 2006). It is found in several species in the genus Scutellaria, including Scutellaria baicalensis and Scutellaria lateriflora (Tarragó et al., 2008). Baicalin is the glucuronide of baicalein, and it is one of the chemical ingredients of Sho-Saiko-To which is a herbal supplement (Awad et al., 2003).

DNC (Delchev et al., 2014) or tetrandra is obtained from the herbaceous perennial vine of the Menispermaceae family native to China and Taiwan. It grows from a short, woody caudex, climbing to a height of around three meters. The leaves are arranged spirally on the stem, and are peltate, i.e., with the leaf petiole attached near the centre of the leaf. Its root is used in traditional Chinese medicine (Moradigaravand et al., 2016).

The results from Ligplot (Laskowski and Swindells, 2011) showed the active site binding residues such as Val645 and Leu644 to form hydrogen bonds with C13 and aromatics ring of the Baicalin. In case of Conessine, Ligand forms three hydrogen bonds with Gln680 of 2.90 Å, Ala650 of 3.33 Å and Gln649 of 3.06 Å. Bonds are seen with Val647 and are termed as Ligand bonds (Figure 7).
Figure 6  This represents different possess of all three enantiomers of Conessine Docked with the ODC enzyme (see online version for colours)

Hydrogen bonds are indicated in green and hydrophobic bonds in purple.

Figure 7  Enzyme-ligand interaction of docked complex (ODC + Conessine) (see online version for colours)
4 Conclusion

The 3D structure of ornithine decarboxylase (ODC) was predicted and validated. The stability of modelled structure was revalidated by the molecular dynamics simulation at 50 nano second-time scale. The inhibition of polyamine biosynthesis precursor is a great idea, and the natural products playing a significant role in the inhibition is even more exciting, as it will be in favour of plant health and its environment. According to our findings, Conessine (N, N-dimethylcon-5-enin-3β-amine) was found to be the best inhibitor which is an alkaloid.

In future, the Conessine molecule can be procured from the ZINC and its inhibitory property can further be validated in a wet laboratory.

References


Inhibition of polyamine biosynthesis for toxicity control


Inhibition of polyamine biosynthesis for toxicity control


