Penicillins are valuable β-lactam antibiotics and useful starting materials for the preparation of semi-synthetic penicillins and cephalosporins. Penicillins are natural products of fungi such as Penicillium chrysogenum. The theoretical investigation of biological activity of penicillins has been studied systematically in the recent years and provided valuable insights. The diastereoisomer of (3S,5S,6S)-6-acetylamidopenicillanic acid has not yet been investigated. In the current investigation we used FRED (Fast Rigid Exhaustive Docking) to dock the conformers of (3S,5S,6S)-6-acetylamidopenicillanic acid into the crystal structure of Isopenicillin N Acyltransferase in complex with 6-aminopenicillanic acid (PDB code 2X1E). The influence of conformational expansion parameters and energy levels on the docking performances was investigated. The conformers of (3S,5S,6S)-6-acetylamidopenicillanic acid docked into the binding pocket suggested some biological activity on the basis of hydrogen bonding interactions (ARG 310, ASP121 similar to 2X1E ligand, and GLY311) and RMSD values.

**Keywords:** (3S,5S,6S)-6-acetylamidopenicillanic acid, conformational sampling, docking

Penicillins are the most studied β-lactams from all drug classes of the 20th century [1]. β-Lactam antibiotics are among the most used antibacterial agents on account of their broad spectrum and low toxicity [1]. The central molecular feature of penicillins consists of two fused rings (a four-member β-lactamic ring and a five-member thiazolidinic ring) including three chiral centers, [2] marked with an asterisk in figure 1. These chiral centers generate 23=8 diastereoisomers: 3R,5R,6R, 3S,5R,6R, 3R,5S,6R, 3S,5R,6R, 3R,5S,6R, 3S,5S,6S, 3S,5S,6S.*

![Fig. 1. Atom numbering in (3S,5S,6S)-6-acetylamidopenicillanic acid (* denotes chirality)](image)

Meanwhile, theoretical research has been oriented only on the natural diastereoisomer (3S,5R,6R) and the other seven classes of diastereoisomers have been completely ignored [4]. Although the chemical synthesis of penicillins which contain natural diastereoisomer was performed more that 50 years ago [4], the penicillins based on diastereoisomer (3S,5S,6S) have not yet been synthesized [5-8]. As for the natural diastereoisomer (3S,5R,6R) we shall admit the hypothesis that the antibacteria action of diastereoisomer (3S,5R,6R) will be subject to the same mechanism based on Strominger’s structural similarities, because this diastereoisomer contains also the D-aladala sequence [9].

Previous theoretical studies have been based on molecular mechanics (MMFF) [10], semiempirical (AM1 [11], PM3 [12]) and ab initio methods. Ab initio methods may be preferred if the system under investigation is relatively small or if the interest is to model only the active site of the system [13].

In the current work the conformational sampling and docking ability of (3S,5S,6S)-6-acetylamidopenicillanic acid in the active binding site of Isopenicillin N Acyltransferase (PDB code 2X1E) [14] is investigated by means of OpenEye package [15]. The conformational expansion of the (3S,5S,6S)-6-acetylamidopenicillanic acid has been performed employing intrinsic parameter variation in order to get the best representation of the conformational space. Docking was performed using FRED (Fast Exhaustive Rigid Docking) [15] from OpenEye package.

**Experimental part**

**Materials and methods**

**Conformer generation**

Conformer generation of (3S,5S,6S)-6-acetylamidopenicillanic acid was performed with Omega version 2.3.2 from OpenEye software [15]. The isomeric smiles code of the ligand was prepared using Marvin, Calculator Plugin and Chemical Terms module of online variant of Chemaxon software [16] and introduced in the conformer generator Omega. Omega split the ligand into fragments that are afterwards put together according to energetic criteria [15]. The conformations that conform to the specified energy window and heavy atom root mean square (RMS) distance are saved [15].

In the present paper, conformer generation was carried out using by default settings (RMSD = 0.8 Å, an energy window of 10 kcal, and maximum output conformers 400) provided only six conformers for (3S,5S,6S)-6-acetylamidopenicillanic acid, a very small number of conformers to consider a good representation of the conformational space. Therefore, we lowered the RMS (root mean square) distance for conformer detection at 0.3 Å, which resulted in 16 conformers. The resulting two groups of ligand conformers were used as input for docking and denoted in this paper as group 1 (RMS=0.8 Å) and group 2 (RMS=0.3 Å) conformers.

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Protein preparation

The crystal structure of *Isopenicillin N Acyltransferase* (PDB code: 2XIE) in complex with 6-aminoopenicillanic acid (PDB ID: X1E) was downloaded from Brookhaven Protein Database (PDB) [14]. The active site of the enzyme has been prepared for docking using FRED_RECEPTOR [15] facility from OpenEye package by supplying the X-ray crystallographic structure of the protein as input. The receptor file is provided by the program as a specialized *oeb* (OpenEye’s molecular format) file, which is used by FRED software. The active site box volume generated by means of smooth Gaussian functions is of 2281Å³, the inner and outer contours of the active site are of 125Å³ and respectively 885Å³. All the crystallographic water molecules were deleted from the protein.

Docking

Docking investigations were carried out in the ligand binding site of 2XIE using FRED version 2.2.5. FRED builds the pose ensemble by executing solid rotations and translations of every conformer inside the binding site [15]. The translations and rotations are made taking into account the atomic displacements that need to be less than a certain translational and rotational relocation threshold [15]. By default FRED returns a single docked structure for each molecule in the input database. In our study all docked poses of (3S,5S,6S)-6-acetylamidopenicillanic acid were saved using -conftest none option available in FRED software and seven classical scoring functions Chemscore (CS), Chemgauss-2 (CG2), Chemgauss-3 (CG3), Shapegauss (SG), Screenscore (SC), OEChemscore (OECS), Piecewise Linear Potential (PLP) were employed. No constraints were imposed on any of the active site of protein atoms vis-à-vis to their interactions with ligand atoms. For all the generated docked poses the RMSD values were calculated.

RMSD calculation

RMSD values were calculated in order to quantify the difference between the X-ray crystal ligand coordinates and generated poses coordinates with the help of Maestro [17] software. The ligand was extracted from protein structure and was designated as the reference structure that was used for the calculation of RMSD values. In this study, the docking accuracy has been classified according to the following criteria: (i) good RMSD < 2 Å; (ii) acceptable RMSD >2 and <3 Å; (iii) bad RMSD >3 Å when docking result is in an inverted or incorrect position.

Results and discussions

The results provided by the conformational analysis, using by default options (0.8 Å) and the conformers which were generated employing a RMS cutoff of 0.3 Å, six

Table 1

<table>
<thead>
<tr>
<th>Conformers</th>
<th>Energy (kcal/mol)</th>
<th>RMSD (Å)</th>
<th>CSS</th>
<th>CG2</th>
<th>CG3</th>
<th>CS</th>
<th>OECS</th>
<th>SC</th>
<th>PLP</th>
<th>SG</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>53.69</td>
<td>1.903</td>
<td>6</td>
<td>-35.47</td>
<td>-52.39</td>
<td>-10.462</td>
<td>-20.216</td>
<td>-60.991</td>
<td>-32.537</td>
<td>-254.326</td>
</tr>
<tr>
<td>2</td>
<td>54.01</td>
<td>3.131</td>
<td>4</td>
<td>-36.075</td>
<td>-42.158</td>
<td>-10.853</td>
<td>-26.595</td>
<td>-93.672</td>
<td>-41.926</td>
<td>-291.237</td>
</tr>
<tr>
<td>5</td>
<td>56.26</td>
<td>2.892</td>
<td>2</td>
<td>-42.856</td>
<td>-47.714</td>
<td>-2.952</td>
<td>-22.629</td>
<td>-81.875</td>
<td>-39.734</td>
<td>-293.428</td>
</tr>
</tbody>
</table>

- CSS - Consensus order
- ΔE1 - the difference between the lowest and the highest free energy of binding;
- ΔE2 - correspond to the difference among the most negative values of the estimated free energy of binding;
- ΔE3 - the difference between the free energy of binding of the best docked pose and the pose with the lowest

RMSDs:

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respectively sixteen different conformers were analyzed. The two groups of conformers were docked into the binding site of Isopenicillin N Acyltransferase (PDB code: 2X1E). The RMSD values between the X-ray ligand and all docked conformers are used to assess whether a correct docking position was obtained by the docking simulation. The RMSD value gives the average deviation between the corresponding atoms of two structures according to equation (1).

\[ \text{RMSD} = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (x_i - x_{i_0})^2 + (y_i - y_{i_0})^2 + (z_i - z_{i_0})^2} \]

(1)

The smaller the RMSD between two structures, the closer is the spatial arrangement of these structures (see table 1 and fig. 2).

Conformational sampling is an important step in docking studies [18] because the low energy conformations are most likely to interact with the biological receptor, assuming that these interactions take place at a local energy minimum [19]. The selected conformer of the ligand for binding to the receptor is probably not the lowest-energy one in the free state [20]. Previous investigations revealed that the ligands bind to the receptor in a conformation that is about 4.5 kcal/mol higher in energy than the lowest-energy conformer [21]. In our investigation, the highest affinity predicted conformer by any of the seven scoring functions is not the lowest-energy one. The most accurate pose is provided by the conformer 11 that is higher in energy by 4.27 kcal/mol than the lowest energy conformer (fig. 3b and table 1).

The conformer energy gap \( \Delta E_{\text{conf}} \) and free energy gap \( \Delta E_{\text{1}} \) (the difference between the lowest and the highest free energy of binding) calculated by means of scoring functions shift to more negative values for the conformers with the lowest RMS, except in the case of screenscore and shapegauss. RMSD range is higher for the conformers generated with 0.3 RMS, but among them there are the best docked conformers. There is a negative difference between the estimated free energy of binding of the best docked pose for conformers group 1 and 2, showing better docking abilities of the second group conformers. The lowest free energy of binding corresponds to the best RMSD for the CG3 scoring function demonstrating the appropriate description of the binding site interactions by Chemgauss 3 for the current target. \( \Delta E_{\text{2}} \) corresponds to the difference among the most negative values of estimated free energy of binding and these values are lower for the second group of conformers, showing that there are more interactions accounted by the scoring functions between these conformers and the target protein.

The \( \Delta E_{\text{3}} \) denotes the difference between the free binding energy of binding between the best docked pose and the pose with the lowest RMSD. This is always favorable to the best scoring conformer probably due to the accounting of the less important interactions by the scoring functions. The lowest energy conformer is of -53.69 kcal/mol, and this is for the first conformer that is common for both conformer groups included in the current study. The hydrogen bonding interactions of this conformer were detected with ARG310 (2.65 Å) similar to X-ray ligand and with GLY311 (3.09 Å) and are shown in figure 3a.

The lowest RMSD value is of 1.418 Å that corresponds to the eleventh conformer belonging to the second group of conformers (RMS of 0.3) and therefore we suggested possible biological activity on the basis of hydrogen bonding interactions depicted in figure 3b, with ASP121 (4.56Å) and ARG310 (2.9Å) similar to X1E ligand and additionally with ARG302 (2.9Å).

Fig. 2. RMSD versus conformer energy; four common conformer belonging to both groups are depicted in blue circles

Fig. 3a. Hydrogen bonding interactions for the lowest energy conformer

Fig. 3b. Hydrogen bonding interactions for the conformer that display the lowest RMSD (conformer depicted in grey; ligand display in brown)
The conformers of \((3S,5S,6S)-6\text{-acetylamidopenicillanic acid}\) interact with ARG310 the same way as observed experimentally and show supplementary hydrogen bonding interactions with GLY311, ARG302. Thus, based on the above considerations we can assume that conformational sampling play a major role in providing good quality docked poses, especially when structural pattern of the ligand displays particular features. Therefore, in order to get the best docking results a higher number of poses have to be retained, in our case the number of poses that include the conformer 11 vary between 2-13. The consensus scheme implemented in FRED selected as the best docked conformer according to consensus score the sixth conformer from the first group and the sixteenth conformer from the second group, but these conformers display lower quality RMSD values (higher than 3Å). The conformers that show the best RMSD are placed on the sixth position in the both groups according to the scores. Anyway, there is nothing new at this point since docking versus scoring paradigm is noteworthy. Based on this evidence, in our case we can establish the number of minimum alternative poses to be retained to six to improve the quality of docking outcomes for the case of \((3S,5S,6S)-6\text{-acetylamidopenicillanic acid}\) conformers and the Isopenicillin N Acyltransferase binding site.

Conformer-protein interactions are best reproduced when the RMSD is small, and when the minimal numbers of interaction are observed; respectively a RMSD less or equal to 2Å is considered suitable. We found that four of the six conformers generated by default method are also present among the conformers generated with a RMS 0.3Å. The two methods allowed to obtain eighteen distinct conformers and protein-conformer interactions resulted from docking experiments reproduce a portion of the crystallographic pattern observed experimentally (ARG310) and display several additional hydrogen bonding interactions with GLY311, ARG302. The best docked conformer is 4.27kcal/mol higher in energy than the lowest energy conformer. Better representation of the conformational space by increasing the number of low energy conformers leads to lower values for the free energy of binding and better RMS values for the docked poses. In the current work according to energetic and geometric criteria we set up the number of minimum alternative poses to be retained to six to improve the quality of docking outcomes for the case of \((3S,5S,6S)-6\text{-acetylamidopenicillanic acid}\) conformers and the Isopenicillin N Acyltransferase.

Conclusions

In this work we considered the interaction between the conformers of \((3S,5S,6S)-6\text{-acetylamidopenicillanic acid}\) and Isopenicillin N Acyltransferase by means of seven scoring functions Chemscore (CS), Chemgauss-2 (CG2), Chemgauss-3 (CG3), Shapegauss (SG), Screenscore (SC), OEChemscore (OECs) and Piecewise Linear Potential (PLP). Docking accuracy was evaluated by means of RMSD and hydrogen bonding. Lowering the RMS leads to a better repartition of energy levels of conformers, respectively the number of low energy levels that increased than higher energy levels. As the low energy conformers are prone to bind to the receptor, this leads to better conformational sampling that influence positively docking and scoring.

Protein-conformer interactions resulted from docking experiments reproduce a portion of the crystallographic pattern observed experimentally (ARG310) and display several additional hydrogen bonding interactions with GLY311, ARG302. The best docked conformer is 4.27kcal/mol higher in energy than the lowest energy conformer. Better representation of the conformational space by increasing the number of low energy conformers leads to lower values for the free energy of binding and better RMS values for the docked poses. In the current work according to energetic and geometric criteria we set up the number of minimum alternative poses to be retained to six to improve the quality of docking outcomes for the case of \((3S,5S,6S)-6\text{-acetylamidopenicillanic acid}\) conformers and the Isopenicillin N Acyltransferase.

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Fig. 4. The docked conformer displaying the highest RMSD value (depicted in grey) overlaid upon the X-ray ligand.