Analysis of the interactions of a novel cephalosporin derivative with its potential targets penicillin-binding proteins from different sources using a covalent docking approac

Motivation. Cephalosporins are a class of beta-lactam antibiotics widely used in clinics for their antibacterial activity. Their mode of action, common to other beta lactam antibiotics such as penicillins, is the impairment of the synthesis of the peptidoglycan forming the bacterial cell wall. This polymer, essential for bacterium survival, is made by aminosugars connected by glycosidic bonds to form linear chains, and by short peptides forming cross-links between the linear chains. The enzymes catalyzing the creation of these cross-links are transpeptidases, also called penicillin binding proteins (PBPs) for their ability to interact with penicillins and other beta lactam antibiotics. These molecules mimic the D-Ala-D-Ala terminus of the peptides, therefore they competitively inactivate the PBPs by binding covalently to the Ser residue responsible for the catalysis and stopping the transpeptidation. This results in cell lysis and bacterial death. One of the main problems to face when using cephalosporins is the development of several mechanisms of resistance, either for the reduced affinity of PBPs to the beta lactams, or for the selection of new beta-lactam-insensitive PBPs, or for the production of beta lactamases, enzymes able to hydrolyze the beta lactam ring, thus deactivating the antibiotics. Additionally, most cephalosporins have a limited spectrum of action, against only Gram+ or Gram- bacteria. Therefore, during the time, many new beta lactam antibiotics have been synthesized with the aim of broadening the spectrum of action and/or overcoming the resistance. The prototype of a new group of cephalosporins is AMA-10, in which another beta lactam ring bound to a short alkyl chain has been linked to the aminocephalosporanic ring by means of an amidic bond. In order to develop other molecules, however, it is essential to understand how they interact with their target. Therefore, to apply a rational approach for the design of new derivatives, we have performed a computational study by simulating the binding of AMA-10 to selected PBPs of different species, whose crystallographic structures were available, using a particular approach, covalent docking, able to take into account the covalent bond formed between the antibiotic and the enzyme.

Methods. The structures of PBP3 and PBP4 from both Gram+ (S. aureus, B. subtilis) and Gram- (E. coli, P. aeruginosae) organisms were downloaded from Protein Data Bank (PDB) database, as well as the structures of beta-lactamase from S. aureus and from E. coli. The representative structures were selected on the basis of their quality. Then, covalent docking was made by using a modified version of the program AutoDock 4.2, using the flexible side chain method [Bianco et al, 2016]. [Abstract truncated at 3,000 characters - the full version is available in the pdf file].

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ABSTRACT BOOK

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Analysis of the interactions of a novel cephalosporin derivative with its potential targets penicillin-binding proteins from different sources using a covalent docking approach

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Methods

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To test the performances of the method, the ligands present in the crystallographic structures of selected PBPs were first extracted and then covalently redocked into the structure. After that, the same ligands were also downloaded from PubChem and/or ZINC databases in .mol2 format, the beta lactam ring was opened and the covalent docking strategy was applied. After this preliminary study, the structure of AMA-10 was built up by using ChemDraw, with one of the two beta lactam rings alternately open. Moreover, the two diastereoisomers obtainable from the synthesis were separately designed. The structures were saved in .mol2 format and used to perform covalent docking against the selected PBPs. For all docking simulations, polar hydrogens were added to the proteins and ligands, and charges were assigned. A grid map focused on the pool of residues identifying the active site of each protein was used to set up the calculation. For each complex, 100 docking runs were performed using the AutoDock Lamarckian genetic algorithm. The conformations representative of the best energetic and/or of the most populated cluster of poses were selected and analyzed for their interactions with the enzyme by using the tools available in Discovery Studio, and LigPlot+.

Results

AMA-10 binds to all PBPs with a predicted binding energy always lower than -10 kcal/mol, indicating a potential binding affinity in the low nanomolar range. This energy is similar to the one predicted for known beta lactam antibiotics, indicating that this new compound interacts very well with its target proteins. Additionally, it is possible to note that the predicted binding affinities are generally higher when the beta lactam ring belonging to the aminocephalosporanic moiety is bound to the enzyme. The detailed analysis of the complexes obtained shows the residues involved in different kind of interactions with the chemical groups of the antibiotic, therefore suggesting which groups might be useful for increasing its affinity, or broadening its selectivity.

References:

Bianco G, Forli S, Goodsell DS, Olson AJ. Covalent docking using autodock: Two-point attractor and flexible side chain methods. Protein Sci. 2016;25(1):295-301.

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