QSAR, Molecular Graphics and Modeling Study on b-Lactam Antibiotics as Substrates of the Multidrug Resistance Efflux AcrB Pump

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Introdução

AcrAB-TolC is the most important multidrug resistance efflux pump system of gram-negative bacteria.[1] This resistance includes β-lactam antibiotics, known as the most widely used antibacterial agents which primarily inhibit penicillin-binding proteins responsible for the construction and maintenance of the bacterial cell wall. AcrAB-TolC pump consists of transport protein AcrB, linker lipoprotein AcrA, and the channell-tunell TolC. It is supposed that drugs from cytoplasm or periplasm enter into AcrB, and then by proton motive force are transferred to TolC, from where they are excreted directly to the bacteria exterior. Bacterial resistance is initiated by high concentration of drugs and their metabolites which induce overexpression of efflux pumps. Besides pronounced lipophilic/amphiphilic character and the presence of multiple charges, there is no obvious structural similarity among exctreted drugs, including β-lactam antibiotics. The primary purpose of this work is to establish relationships between activity expressed as log of minimal inhibitor concentration (pMIC) elevated by three strains of *Salmonella typhimurium* (HN891, SH7616, SH5014)[1], and lipophilicity, electronic and hydrogen bond descriptors for 16 PM3 geometry optimized penicillins and cephalosporins at neutral pH. The next aim is to visualize pump – drug molecular recognition mechanism, using crystal structure of AcrB transporter from *Escherichia coli*.[2] These results can aid in explaining bacterial drug efflux mechanism, and design of novel β-lactams which would not be excreted from bacterial cells.

Resultados e Discussão

Chemometric analysis of pMICs. Hierachical Cluster Analysis (HCA) and Principal Component Analysis (PCA) revealed that lipophilicity and charges are important in excretion of β-lactams by bacterial strains. The presence of three charges in a molecule cause all strains to act the same way. β-Lactams were classified as good, moderately good to poor, and bad AcrB substrates.

Chemometric analysis of lipophilicity. Nine lipophilicity parameters, mostly logP values calculated by various methods, when analyzed by HCA and PCA, show heterogenicity described by the first three principal components and a few clusters of lipophilicity parameters.

QSAR studies. Partial Least Squares (PLS) regression models for calculation of pMICs included only two lipophilicity descriptors in parabolic form, and two electronic and hydrogen bond descriptors. Molecular dipole moment and its Y-component are essential for the drug action with respect to AcrB.

2D and 3D docking to vestibules. Three vestibules are the holes in AcrB through which drugs can come inside the transporter. Structural and electronic properties of a vestibule, especially of its 2D projection called BRAMLA (Brazil Map-Like Area), agree with conclusions from chemometric - QSAR results. Docking of 2D molecular images of selected β-lactams, rifampicin and erythromycin to BRAMLA, as well as docking of nafcillin to 3D vestibule structure, confirm the above findings.

3D docking to the pore. The pore is the AcrB channel connected to TolC. Docking of selected β-lactams, *n*-hexane and rifampicin to the pore recognition site is in accord to the above analyses. The pore – drug complexes optimized by molecular mechanics MMFF94 field, reveal that poor AcrB substrates bind to the pore recognition site *via* hydrogen bonds and do not enter the pore channel.

Conclusões

Lipophilicity parameters in parabolic form, electronic and hydrogen bonding properties of β-lactams determine their efflux elevated by AcrB pumps. Steric properties play an important role in pore-drug recognition mechanism.

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[1] Nikaido, H., Basina, M., Nguyen, V., Rosenberg, E. Y. *J. Bacteriol.* **1998**, 180, 4686.
[2] Koronakis, V., Sharff, A., Koronakis, E., Luisi, B., Hughes, C. *Nature*, **2000**, 405**,** 914.