Theoretical Study on Some β-Lactams as Substrates of the Bacterial Multidrug Resistance AcrB Pump

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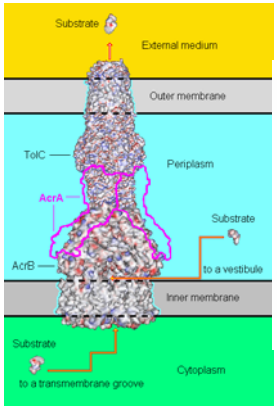
G E N E R A L

Problem

AcrAB-TolC (Figure 1) is the most important efflux pump system of gram negative bacteria, responsible for their resistance to a large variety of lipophilic and amphiphilic drugs like β-lactam antibiotics.



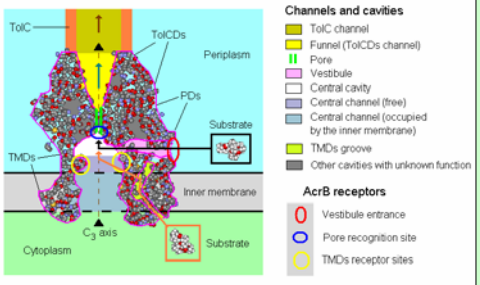


Fig. 1. External (left) and internal (right) structure, position and function of the bacterial AcrAB-TolC multidrug resistance efflux pump.

The primary purpose of this work

- **To establish relationships between activity expressed as log of minimal inhibitor concentration (pMIC) elevated by three strains of *Salmonella typhimurium* (HN891, SH7616, SH5014), and lipophilicity, electronic and hydrogen bond descriptors for 16 PM3 geometry optimized penicillins and cephalosporins at neutral pH.**
- **To visualize pump – drug molecular recognition mechanism, using crystal structure of AcrB transporter from *Escherichia coli*.**
- **These results can aid in explaining bacterial drug efflux mechanism, and design of novel β-lactams which would not be excreted from bacterial cells.**

Further information on chemometric analysis, molecular graphic and modeling as well as on literature data (biological activities, crystal structures) is contained in works: R. Kiralj, M. M. C. Ferreira, *J. Chemometr.* and *J. Mol. Graph. Mod.*, submitted.

Conclusions visible from chemometrics and molecular graphics & modeling

PLS models of good quality were obtained using lipophilic, electronic and hydrogen bond descriptors for 16 β-lactams.

Proposed efflux mechanism based on chemometrics and molecular graphics and modeling methods:

1) a drug molecule comes from periplasmic space and interacts with a vestibule through a mechanism of molecular recognition → large and highly hydrophilic molecules hardly enter the vestibule and come to the central cavity of AcrB protein.

2) a drug molecule from the central cavity comes to the pore recognition site and through a mechanism of molecular recognition enters the pore channel → again large and highly hydrophilic molecules hardly enter the pore channel to be excreted from the cell.

Acknowledgement: FAPESP

CHEMOMETRICS

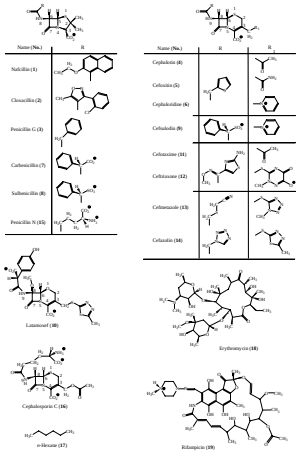


Fig. 2. β-Lactams (1-16) and other (17-19) substrates of the AcrAB-TolC efflux pump studied in this work.

Table 1. PLS regression models for the 3 pMIC scales with lipophilicity, electronic and hydrogen bond molecular descriptors.

pMIC	Parameters[a]	SEP[b]	Q^2	R^2	PCs (%)
HN891	w_C, S_T, GlogK_{OW}, logP_n, SlogP_n, logK_{WH}, SlogK_{WH}, logP_{IA}, logP_X	0.467	0.912	0.967	3 (82%)
	logP_{IA}, GlogK_{OW}, SlogP_n, H_S A_{HB}, D_y, M_{NS}	0.221	0.980	0.992	3 (85%)
SH5014	w_C, S_T, GlogK_{OW}, logP_n, SlogP_n, logK_{WH}, SlogK_{WH}, logP_{IA}, logP_X	0.391	0.942	0.975	2 (82%)
	logP_{IA}, GlogK_{OW}, SlogP_n, H_S A_{HB}, D_y, M_{NS}	0.305	0.964	0.983	3 (85%)
SH7616	w_C, S_T, GlogK_{OW}, logP_n, SlogP_n, logK_{WH}, SlogK_{WH}, logP_{IA}, logP_X	0.792	0.645	0.886	2 (76%)
	logP_{IA}, GlogK_{OW}, SlogK_{WH}, N_{CH}, H_S, M_{NS}	0.446	0.862	0.935	3 (84%)

[a]Glog and Slog stand for Gaussian transformation and square term, respectively.
[b]Standar error of validation. [c]Correlation coefficient from validation.
[d]Correlation coefficient from prediction.

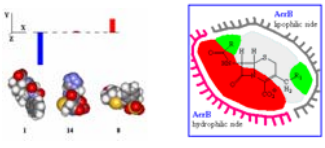


Fig. 6. Size and position of the Y-component of the dipole moment of representative substrates (left) and PLS-based pump-substrate molecular recognition.

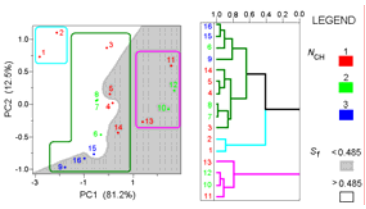


Fig. 3. PCA (left) and HCA (right) on the 3 pMICs scales for β-lactams (1-16). No. of charged groups N_{CH} and lipophilicity parameter S_T are also presented. Samples are classified as good moderately good and poor susbstrates (light blue, dark green and pink cluster, respectively).

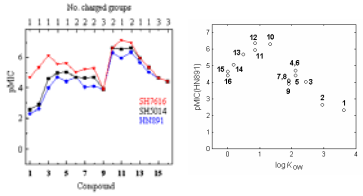


Fig. 4. Variation of the 3 pMICs scales for substrates 1-16 (left) and an example of non-linear pMIC - logK_{OW} relationship (right).

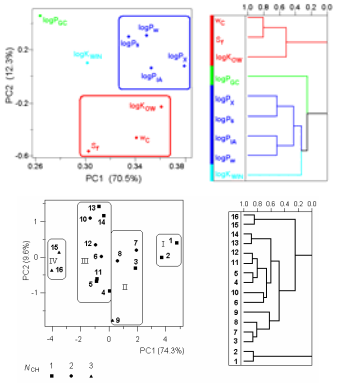


Fig 5. Results of the PCA (left) and HCA (right) analysis on lipophilicity parameters calculated by various methods for 1-16: the parameters show tendency to form more than one cluster (top), and the samples also (bottom). This means that various calculation methods can result in quite different lipophilicity parameters for β-lactams.

MOLECULAR GRAPHICS & MODELING

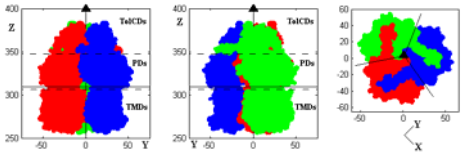


Fig. 7. Three views on external structure of the trimeric AcrB protein and the definition of the XYZ coordinate system and the AcrB domains: TolC-docked domains (TolCDs), periplasmic domains (PDs) and transmembrane domains (TMDs). See also Fig. 1 for further details.

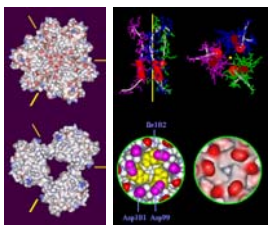


Fig. 8. The pore structure.

Left: two views on AcrB cross-section through PDs-TMDs border plane (dashed line, Fig. 7). Yellow lines mark the position of vestibules.

Right: The structure of the pore channel represented by 3 α-helix+random coil chains (top), and the pore recognition site consisting of highly polar and hydrophobic residues around the C_3 axis.

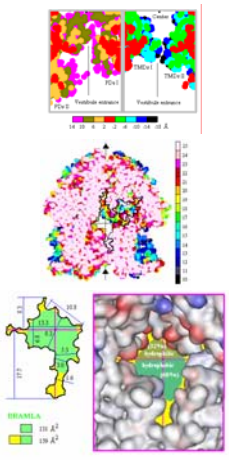


Fig. 9. Slicing a vestibule in directions as in Fig. 8 left (top) and in a perpendicular direction (middle), and the vestibule structure with dimensions and hydrophobic character (bottom). Green area (bottom, left) is BRAMLA (BRAzil Map-Like Area).

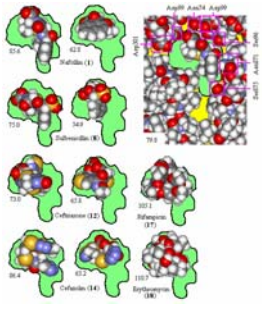


Fig. 10. 2D image-to-2D image docking of some substrates to a vestibule, and 3D docking of 1 (top right) by satisfying steric (maximal and minimal) and electronic fitting without geometry optimization. Percentage counts for occupied BRAMLA area by a drug image.

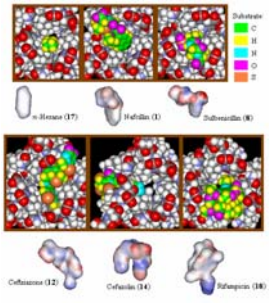


Fig. 11. Some MMFF94 optimized drug – pore complexes and electrostatic potential surface of the drug molecules. The pore is from Fig. 8 right, and the viewing is towards the pore recognition site.

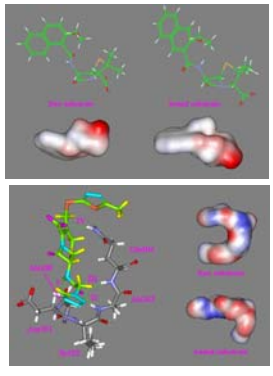


Fig. 12. Comparison of free and pore bound substrate geometries for 1 (top) and 14 (bottom). Hydrogen bonding of 14 and a pore side chain is presented also. Binding to pore provokes molecular elongation and redirection of polar and hydrophobic groups.