QSAR Study of β-Lactam Antibiotic Efflux by the Bacterial Multidrug Resistance Pump AcrB

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ABSTRACT

Principal Component (PCA) and Hierarchical Cluster (HCA) Analysis and Partial Least Squares (PLS) regression have been applied in QSAR study of 16 β-lactam antibiotics (penicillins and cephalosporins) as substrates of multidrug resistance efflux membrane pump AcrAB-TolC in three strains of Gram-negative bacteria *Salmonella typhimurium*. Electronic and hydrogen bonding molecular descriptors were obtained by using quantum chemical methods or structure fragment counts, while lipophilicity descriptors were generated in various calculation methods. PCA - HCA analysis on three activities (pMICs) resulted in classification of samples as good, moderately good and poor pump substrates, and in clear evidence that the efflux activity is determined also by bacterial strain. The analogous analysis on lipophilicity descriptors discovered significant differences among them and clustering of samples in accordance with the type of β-lactam side chains. The parsimonius cross-validated PLS models with three principal components (Q=0.85-0.98, R=0.93-0.99, SEP=0.21-0.46) included linear and non-linear terms of some lipophilicity descriptors, hydrogen bonding and electronic descriptors.

THE PROBLEM

One of the main problems that comtemporary medicinal chemisty confronts are infectious and parasitic diseases. They have been human companions since pre-historic times, causing deaths that can be numbered in billions, influencing human health and way of life, human mind and philosophy. Many historical events, processes and trends were considerably affected by activity of microbes and parasites. Since the middle of the last century, medicinal chemistry confronted a new threat called multidrug resistance (MDR), developed by all types of microbes and parasites and even tumor/cancer cells. One of the major mechanisms of MDR are multidrug efflux pumps present in all cellular microbes, parasites, plants, animals and humans, including tumor/cancer cells.

Understanding the efflux mechanism of these pumps is important because it can enable: 1) development of new drugs that can be more effective against diseases and cannot be extruded from pathogen organisms or cells; 2) modeling efflux pump blockers or reversal agents; 3) application of more effective anti-MDR measures; 4) help in understanding other similar or different efflux pumps in similar or different cells and organisms. The same pump can exist in species of similar or different organisms with or without small variations in its sequence.

AIM OF THIS WORK

✔To establish relationships between activities (pMICs) for 16 β-lactams excrected by three strains of *Salmonella typhimurium* (HN891, SH7616, SH5014). The efflux pump is AcrAB-TolC from the RND family.

✔To establish relationships between various lipophilicities calculated by using different methodologies.

✔To establish quantitative relationships between pMICs and lipophilicities, electronic and hydrogen bond descriptors based on PM3 geometry-optimized structures of the β-lactams at neutral pH.

✔These results can aid in explaining bacterial drug efflux mechanism, and design of novel β-lactams which would not be excreted from bacterial cells.

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Conclusions

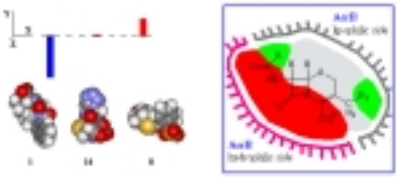
Figures and tables at the right side of this poster confirm the conclusions that can be made in this work. It can be said that:

-the biological activities (pMICS, drug efflux rates) strongly depend both on properties of bacterial strains and drug molecules; classes of good, moderately good and poor substrates were observed;

-the molecular descriptors quantitatively related to pMICs are lipophilicity parameters, electronic and hydrogen bond descriptors; different lipophilicity parameters behave differently with respent to pMICs, and also do not always correlate highly with each others;

-the obtained PLS models describe MDR QSAR quite satisfactorily, representing one among very few similar studies treating bacterial MDR phenomena;

-selected molecular descriptors and the results of chemometric analyses suggest pump-drug molecular recognition schemes as shown right below.

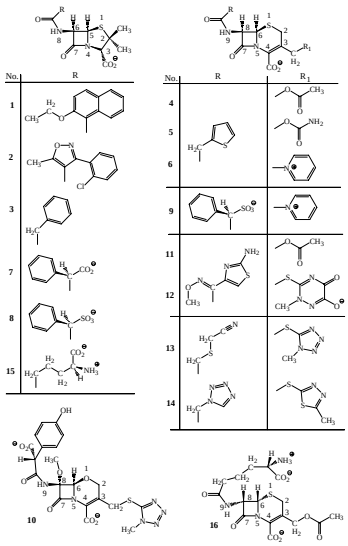


Left: β-Lactam representatives with the most negative (1), zero (14) and the most positive (8) Y-component of the dipole moment. This descriptor shows that dipole-dipole and probably other intermolecular interactions including charges and dipoles, are important in pump-drug recognition and the efflux process.

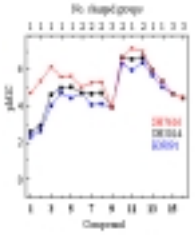
Right: A simplified 2D representation of possible stereoelectronic pump-drug interactions, based on known rule that similar interacts with similar (favorable are lipophilic-lipophilic and polar-polar group interactions). The represented hole is an AcrB vestibule through which a drug molecule enters the pump. Similar interactions may occur between the drug and other parts of the pump.

ACKNOWLEDGEMENT

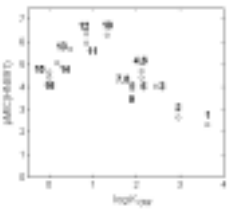
FAPESP



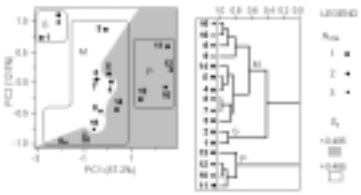
β-Lactams (1-16) as substrates of the AcrAB-TolC efflux pump at neutral pH, with atomic numbering for penicillines and cephalosporins.



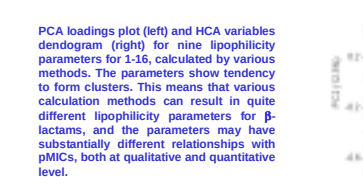
Variation of the 3 pMICs for substrates 1-16. Strains HN891 (the parent) and SH5014 (AcrAB-TolC overproducer) actively efflux β-lactams, while in strain SH76716 passive efflux is predominant (AcrAB-TolC practically inactive).



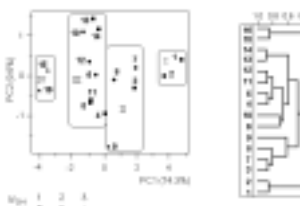
An example of non-linear activity-lipophilicity (pMIC-logK_{OW}) relationship. This lipophilicity variable was transformed into a linear descriptor.



PCA scores plot (left) and HCA samples dendogram (right) for the 3 pMICs of β-lactams 1-16. No. of charged groups N_{CH} and lipophilicity parameter S_t are also presented. Samples are classified as good (G), moderately good (M) and poor (P) substrates. Qualitative relationship between sample classification and variables N_{CH} and S_t can be observed similarly in PCA and HCA. In general, more lipophilic and less charged β-lactams are better pump substrates.



PCA loadings plot (left) and HCA variables dendogram (right) for nine lipophilicity parameters for 1-16, calculated by various methods. The parameters show tendency to form clusters. This means that various calculation methods can result in quite different lipophilicity parameters for β-lactams, and the parameters may have substantially different relationships with pMICs, both at qualitative and quantitative level.



PCA scores plot (left) and HCA samples dendogram (right) for seven lipophilicity parameters for 1-16. Two structure-based parameters (S_t and w_C) were excluded from the previous analysis. The samples tend to cluster with respect to chemical character of side-chains at position 8 in penicillins and position 9 in cephalosporins. The corresponding side-chains can be named in following decreasing order of lipophilic character: 1 – aromatic two-ring chains; 2 – aromatic phenyl-containing chains; 3 – heteroaromatic (various π-systems) containing chains; 4 – aliphatic chains.

PLS regression models for the 3 pMICs

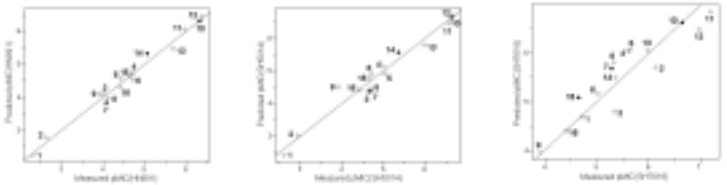
pMIC	Selected parameters[a]	SEP[b]	Q^2	R^2	PCs (%)
HN891	w_C, S_t, GlogK$_{OW}$, logP_n, SlogP$_n$, logK$_{WIN}$, SlogK$_{WIN}$, logP_{1A}, logP_X	0.467	0.912	0.967	3 (82%)
	logP_{1A}, GlogK$_{OW}$, SlogP$_n$, H_t, A_{HB}, D_y, N_{NS}	0.209	0.982	0.993	3 (85%)
SH5014	w_C, S_t, GlogK$_{OW}$, logP_n, SlogP$_n$, logK$_{WIN}$, SlogK$_{WIN}$, logP_{1A}, logP_X	0.391	0.942	0.975	2 (82%)
	logP_{1A}, GlogK$_{OW}$, SlogP$_n$, H_t, A_{HB}, D_y, N_{NS}	0.316	0.962	0.982	3 (85%)
SH7616	w_C, S_t, GlogK$_{OW}$, logP_n, SlogP$_n$, logK$_{WIN}$, SlogK$_{WIN}$, logP_{1A}, logP_X	0.792	0.645	0.886	2 (76%)
	logP_{1A}, GlogK$_{OW}$, SlogK$_{WIN}$, N_{CH}, H_t, N_{NS}	0.461	0.851	0.930	3 (83%)

[a]Original lipophilicity descriptors calculated by various methods: w_C, S_t, logK$_{OW}$, logP_n, logK$_{WIN}$, logP_{1A}, logP_X. Transformation of lipophilicity descriptors: GlogK$_{OW}$ = exp[–(logK$_{OW}$ – 1.1)^2]; SlogP$_n$ = (logP_n)^2; SlogK$_{WIN}$ = (logK$_{WIN}$)^2. Electronic and hydrogen bond descriptors: H_t - number fraction of heteroatoms; A_{HB} - the number of hydrogen bond acceptors; D_y - Y-component of the molecular dipole moment; N_{NS} - number of nitrogen and sulfur atoms; N_{CH} - the number of charged groups. [b]Standard error of validation. [c]Correlation coefficient from validation. [d]Correlation coefficient from prediction.

Validation of the PLS models.

pMIC	No.[a]	Exp[b]	Pred[c]	% Error[d]
HN891	T	4.873	5.041	3.5
	80	6.316	6.303	0.3
	84	5.855	5.523	5.3
SH5014	T	4.875	4.766	6.6
	80	6.637	6.642	0.1
	84	5.357	5.573	4.0
SH7616	T	5.277	5.693	7.9
	80	6.637	6.656	0.82
	85	4.652	5.083	9.3
pMIC	SEP[e]	Q^2	R^2	PCs(%)
HN891	0.245	0.975	0.994	3 (88%)
SH5014	0.379	0.945	0.981	3 (88%)
SH7616	0.529	0.797	0.924	3 (86%)

[a]Compounds from the external validation set. [b]Experimental and predicted pMICs and their difference expressed as % error. [e]Standard error of validation. [d]Correlation coefficient from validation. [e]Correlation coefficient from prediction.



Measured against predicted pMICs as obtained from the validation of the PLS models. White and black samples are from the training and external validation set, respectively.