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QSAR study of structurally unrelated substrates of MDR efflux pump VmrA from *V. Parahaemolyticus*

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INTRODUCTION

Multidrug resistance (MDR) of microbes and parasites as well as of cancer cells to currently used drugs is becoming one of the major problems in combating infectious and parasitic diseases and cancer, respectively. Among major mechanisms of multidrug resistance in cellular microbes and cancer cells are efflux pumps, macromolecular systems that extrude drugs and a large variety of structurally dissimilar substances from cell into the outside medium. Bacterial MDR efflux pump VmrA exists in *V. parahaemolyticus*, a marine bacteria that causes frequently food poisoning in Japan and many other countries [1]. VmrA is being effective against several structurally unrelated drugs, organic dyes, detergents and xenobiotics. It is a Na+/drug antiporter that extrudes the substrates from the cytoplasm to the periplasmic space in a Gram-negative bacterial cell. Its functional form consists of a protomer placed in the inner membrane, with 448 [1] or 447 [2] residues that form twelve hydrophobic transmembrane segments of 21-24 residues. 3D structure of VmrA is not know yet. Substrates of this pump, although structurally very diverse, must share some common properties that are responsible for their efflux from *V. parahaemolyticus*.

METHODS

In this work, the relationships between drug molecular properties and efflux activity of VmrA (negative logarithm of Minimal Inhibitory Concentration) [1] have been studied by means of quantitative structure-activity relationships (QSAR). Twelve drugs were included in the study: DAPI (4',6-diamino-2-phenylindole), TPPCl (tetraphenylphosphonium chloride), acriflavine chloride, ethidium bromide, chloramphenicol, norfloxacin, rhodamine 6G chloride, tetracycline, erythromycin, streptomycin, sodium deoxycholate, and sodium dodecyl sulfate. Structures of the active organic parts of all drugs were modeled according to available experimental structures for these or the most similar drugs in the Cambridge Structural Database, taking into account the ionic state of these species at neutral pH. The geometry of the neutral and ionic species was optimized at PM3 semi-empirical level in Titan package. Molecular descriptors were calculated from the obtained structures by using Titan, MOPAC 6.0 and Chem3D programs. Other molecular descriptors, mainly of compositional and topological nature, were generated from two-dimensional chemical formula. The molecular descriptors data were autoscalled prior to chemometric analysis that was performed using programs Pirouette and Matlab. Cut-off 0.50 in correlation coefficients for descriptor-activity relationships was used in variable selection. PLS (Partial Least Squares) regression and PCR (Principal Component Regression) models were built and validated by leave-one-out crossvalidation.

RESULTS AND DISCUSSION

The obtained PLS and PCR regression models with one principal component (70% of the total variance) resulted in very similar and satisfactory statistics: correlation coefficient of validation $Q > 0.80$ and prediction $R > 0.88$, and standard error of validation $SEV < 0.73$ and prediction $SEP < 0.57$. This is reasonable to expect since the twelve drugs belong to twelve different classes of compounds. The first principal component discriminates the drugs according to their biological activities with respect to VmrA pump. DAPI, TPPCl, acriflavine and ethidium bromide behave as good substrates of this pump, what is in agreement with experimentally observed elevated resistance of *V. parahaemolyticus* to these drugs [1]. The other drugs form two groups that are partially mixed, as moderately good and poor substrates, to which there is a very modest and none MDR in the bacterial cells, respectively. Good substrates are characterized by rather rigid structures (planar fragments and rings) and predominant hydrophobic character, modest polarizability and dipole moment, and limited content of polar groups. Certain molecular properties exhibit parabolic relationships with the efflux activity of VmrA, thus indicating the optimal ranges of molecular descriptors that characterize the best and worst substrates of this pump. These observations agree with the pronounced hydrophobic character of this transmembrane pump.

CONCLUSIONS

Steric, eletronic and hydrophobicity molecular descriptors of twelve unrelated drugs are quantitatively related to the efflux rate of the drugs, as extruded by pump VmrA in cells of *V. parahaemolyticus*.

ACKNOWLEDGEMENT: FAPESP

LITERATURE

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[2] K. Makino *et al*., *Lancet*, **361** (2003) 743-749.