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Internal Test Sets Studies in a Group of Antimalarials

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Abstract: Topological indices have been applied to build QSAR models for a set of 20 antimalarial cyclic peroxy acetals. In order to evaluate the reliability of the proposed linear models leave-*n*-out and Internal Test Sets (ITS) approaches have been considered. The proposed procedure resulted in a robust and consensued prediction equation and here it is shown why it is superior to the employed standard cross-validation algorithms involving multilinear regression models.

Keywords: Internal test sets method, topological indices, linear models, QSAR, statistical validation.

Introduction

The objective of the present work is to study true prediction possibilities in a congeneric group of antimalarials by using graph-theoretical indices as molecular descriptors. Malaria is one of the most concerning diseases in developing countries. The obtaining of an effective vaccine is a far expectative. The increasing of resistant strains to chloroquine has raised the search of new potential drugs [1] and artemisin-like substances are promising candidates in order to control this epidemic and intensive research is being made on cyclic peroxy compounds [1].

Graph-theoretical indices, also known as Topological Indices (TI), are non-empirical graph invariants calculated from the intuitive representation of the molecules [2-5]. They encode information on molecular size, shape and branching, the most important features of the molecular structure. The

computation of TI is very swift and they have the advantage of being true structural invariants. That is, their values are independent of molecular conformations. Their usefulness in the modeling of physical [6,7], chemical and biological [8] properties such as different therapeutical activities as well as toxicological properties [9], the drug-like character [10,11] and the molecular similarity/diversity [12-15], has been firmly established, even within structurally heterogeneous groups of compounds, making TI apt for their application in drug design [16-21]. Recent papers deal with the prediction of antimicrobial [22,23], specific anti-mycobacterial [24,25], anticonvulsant activities [26], drug-albumin binding affinity [27], brain-blood distribution [28], and antioxidant character [29], among others. Three-dimensional versions of the graph-theoretical indices have been also proposed [30-32]. But, in fact, it is very common to find studies in which the topostructural and topochemical indices explain the majority of the system variance, and that the inclusion of molecular geometry-dependent parameters does not result in significantly improved predictive models [33].

On the other hand, in the QSAR field oftentimes mathematical models are presented as a linear equation of some descriptors selected in some way with a good adjustment for the experimental data within the series. These models usually come accompanied by a test of validation of leave-one-out type in which the value of the property for each molecule is evaluated by an equation obtained with the whole rest of the population, in a manner that the selected variables remain fixed. To the apparent guarantees that supposes this validation method, when applying the equations to molecules that don't appear in the series of training, the results of prediction of the property are usually very poor. In part, this is due to the particular procedure which has been followed in order to perform the cross-validation. In this work, two related algorithmic designs are explored. First, a standard leave- n -out (L_nO) protocol normally considered when MLR models are searched. It will be seen how and why this procedure does not warrant reliable models, even in the cases for which sound statistical parameters are being obtained. The important thing is that in order to obtain reliable models, it is advisable to obtain acceptable results for test molecules *external* to the training group, although the predictions inside it were not so remarkable. This encompasses the second method explored here: as it will be seen, Internal Tests Sets (ITS) protocol constitutes a more severe L_nO procedure. Basically, this method internally generates external molecular test sets for which true predictions must be performed. Here, a leave-one-out variant will be presented. This means that, one at a time, each molecule in the original family is momentarily removed, a model is found using the data of the remaining molecules (even relying in an internal L_nO protocol) and a prediction is done for the hidden compound. In fact, this procedure is equivalent to an n -fold cross-validation test and constitutes an iterative and exhaustive process with reposition. The consequence is that a particular prediction equation is built for each removed structure and the selection of the relevant variables entering in models can vary among equations. Our experience reveals us that this method allows the automatic identification of outliers.

Calculations

Data

This study is made on the set of 20 cyclic peroxy acetals previously published by Posner et al. [34]. Table 1 shows the molecular structure of the studied compounds and their activities obtained experimentally by a reported method [35]. Activities are expressed as logarithm of IC_{50} (50%

inhibitory concentration, C , in nM units). Thus, the most active compounds show low values.

Table 1. Studied molecular structures and experimental activities.

Entry	Ar	R, R	Log(IC ₅₀ /nM)
1	Ph	Me, Me	3.041
2	Ph	cyclopentyl	2.279
3	Ph	cyclohexyl	2.447
4	Ph	cycloheptyl	2.342
5	4-MeOPh	cyclobutyl	2.204
6	4-MeOPh	cyclohexyl	2.255
7	4-MeOPh	cycloheptyl	2.322
8	3,4,5-(MeO) ₃ Ph	cycloheptyl	2.079
9	4-CF ₃ OPh	cycloheptyl	1.785
10	4-ClPh	cycloheptyl	1.763
11	4-FPh	cycloheptyl	1.929
12	4-MeSPh	cycloheptyl	1.892
13	4-MeS(O ₂)Ph	cycloheptyl	1.491
14	4-EtPh	cycloheptyl	2.255
15	4-MeSPh	cyclohexyl	2.204
16	4-MeS(O ₂)Ph	cyclohexyl	1.748
17	4-O ₂ NPh	cyclohexyl	1.663
18	4-ClPh	cyclohexyl	2.000
19	4-FPh	cyclohexyl	2.301
20	4-F ₃ CPh	cyclohexyl	2.146

Descriptors

Originally, 90 descriptors were computed for each structure. These graph-theoretical indices are briefly defined in table 2. Detailed definitions of these descriptors can be found in references 19, 36-41.

Table 2. Used descriptors.

Symbol	Name	Definition	Reference
N	Molecular size	Number of non-hydrogen atoms.	19
V_k $k=3,4$	Vertices of degree k	Number of atoms having k bonds, σ or π , to non-hydrogen atoms.	19
R	Ramification	Number of single structural branches.	19
W	Wiener path number	Sum of the distances between any two atoms in terms of bonds.	36
L	Length	Maximal distance between atoms in terms of bonds.	19
PR_k $k=0-3$	Pairs of ramifications at distance k	Number of pairs of single branches at distance k in terms of bonds.	19
${}^k\chi_t$ $k=0-4$ $t=p,c,pc$	Randić-like indices of order k and type path (p), cluster (c) and path-cluster (pc)	${}^k\chi_t = \sum_{j=1}^{n_t} \left(\prod_{i \in S_j} \delta_i \right)^{-1/2}$ δ_i , number of bonds, σ or π , of the atom i to non-hydrogen atoms. S_j , j th sub-structure of order k and type t .	37,38
${}^k\chi_t^v$ $k=0-4$ $t=p,c,pc$	Kier-Hall indices of order k and type path (p), cluster (c) and path-cluster (pc)	${}^k\chi_t^v = \sum_{j=1}^{n_t} \left(\prod_{i \in S_j} \delta_i^v \right)^{-1/2}$ δ_i^v , Kier-Hall valence of the atom i . S_j , j th sub-structure of order k and type t .	39
G_k $k=1-5$	Topological charge indices of order k	$G_k = \sum_{i=1}^{N-1} \sum_{j=i+1}^N M_{ij} - M_{ji} \delta(k, D_{ij})$ $M=AQ$, product of the adjacency and inverse squared distance matrices for the hydrogen-depleted molecular graph. D , distance matrix. δ , Kronecker delta	19,40
G_k^v $k=1-5$	Valence topological charge indices of order k	$G_k^v = \sum_{i=1}^{N-1} \sum_{j=i+1}^N M_{ij}^v - M_{ji}^v \delta(k, D_{ij})$ $M^v=A^vQ$, product of the electronegativity-modified adjacency and inverse squared distance matrices for the hydrogen-depleted molecular graph. D , distance matrix. δ , Kronecker delta	19,40
J_k $k=1-5$	Pondered topological charge indices of order k	$J_k = \frac{G_k}{N-1}$	19,40
J_k^v $k=1-5$	Pondered valence topological charge indices of order k	$J_k^v = \frac{G_k^v}{N-1}$	19,40
kD_t $k=0-4$ $t=p,c,pc$	Connectivity differences of order k and type path (p), cluster (c) and path-cluster (pc)	${}^kD_t = {}^k\chi_t - {}^k\chi_t^v$	19
E_k $k=1-5$	Topological charge differences of order k	$E_k = G_k^v - G_k$	41
F_k $k=1-5$	Pondered topological charge differences of order k	$F_k = J_k^v - J_k$	41
kC_t $k=0-4$ $t=p,c,pc$	Connectivity quotients of order k and type path (p), cluster (c) and path-cluster (pc)	${}^kC_t = \frac{{}^k\chi_t}{{}^k\chi_t^v}$	19
kQ_t $k=0-4$ $t=p,c,pc$	Inverse connectivity quotients of order k and type path (p), cluster (c) and path-cluster (pc)	${}^kQ_t = \frac{{}^k\chi_t^v}{{}^k\chi_t}$	41
CG_k $k=1-5$	Topological charge quotients of order k	$CG_k = \frac{G_k}{G_k^v}$	41
QG_k $k=1-5$	Inverse topological charge quotients of order k	$QG_k = \frac{G_k^v}{G_k}$	41

The original data matrix dimension was 20×90. The complexity of this primary set of indices was reduced resorting to the Unsupervised Forward Selection (UFS) algorithm due to Whitley et al. [42]. UFS procedure eliminates redundant vectors of descriptors according to the collinearities present in the data. In this way the original data matrix has been slightly reduced to dimension 20×84, avoiding the presence of descriptors that did not bring forward any information. Despite only a few vectors have been discarded, this prevents for the generation of linear dependences when constructing MLR models, especially in the cases where several compounds are iteratively eliminated during a L_nO or ITS procedure (see below).

Modeling

A first test of predictability was performed with the 20 molecules of table 1. It was a standard L_nO cross-validation, with n ranging from 0 to 2, using Multilinear Regression (MLR) of all the possible subsets of k independent variables, where k varies between 1 and 5. In order to select an optimal subset of variables for each n and each k , the following Algorithm A was used:

Algorithm A(N, n, m, k): Standard MLR- L_nO for N molecules for obtaining linear models involving k indices selected from a set of m .

1. Generate all the $M = \binom{m}{k}$ combinations of k descriptors taken from the group of m . For every combination:
2. Perform the L_nO test:
 - 2.1. Left it apart all the distinct $\binom{N}{n}$ sets of n molecules taken from the group of N . For each set, compute the MLR fitting equation involving the remaining $N-n$ ones. Apply the obtained linear model to the excluded molecules.
 - 2.2. Previous step furnishes with $\binom{N-1}{n-1}$ predictions by molecule. Evaluate the mean value. This constitutes the consensued set of N predictions attached to the k descriptors.
 - 2.3. Compute the correlation coefficient (R_{cv}) between the N experimental values and the consensued ones.
3. Final selected variables are those attached to the combination having the highest R_{cv} coefficient in step 2.3 and, additionally, an acceptable statistical significance (in this work, for each coefficient in the MLR model the probability to be zero is lesser than 1%).
4. Give as final model the MLR fitting equation obtained considering all the N molecules and the selected variables in the previous step.

It is well known that algorithm A overestimates the predictive capabilities of the selected models [24]. This is so because the final model arises from a selection within a very big pool of candidates (in general the external loop number 1 may generate millions of combinations) and the risk of overparametrization is evident as it is quite probable to find a combination of indices well correlated with the experimental property vector. Despite to this drawback, when considering MLR or other linear techniques this standard algorithm is widely used for its simplicity and execution speed, as steps 2.1 and 2.2 do not need to be explicitly reproduced for MLR models, as there is a general theorem that

allows obtaining the results in an even faster way [43]. Even more, the statistical parameters presented accompanying the results (such as the F one) were originally designed to evaluate a single model and not a model selected from a big pool of candidates [44]. This feature helps to optimistically interpret the obtained fittings.

A second and more robust test of predictability was also performed with the data, this time using the ITS method. It also constitutes a L_nO cross-validation, with n ranging from 0 to 2, and considering all the possible subsets of k independent variables, where k varies between 1 and 5. In order to select an optimal subset of variables for each n and each k values, the following Algorithm B implements a L1O-ITS protocol:

Algorithm B(N, n, m, k): MLR- L_nO /L1O-ITS method for N molecules for obtaining linear models involving k indices taken from a set of m .

1. Consider the N molecules with known activity and left apart one at a time (this is the L1O part in the ITS formalism). For each set of $N-1$ remaining molecules:
 - 1.1. Apply the $A(N-1, n, m, k)$ algorithm.
 - 1.2. Consider the MLR model obtained in previous step and apply it over the molecule excluded in step 1, obtaining in this way the property value prediction and the corresponding equation.

As it can be seen from the Algorithm B, for each particular value of k , it provides with a single model for each left out molecule. Therefore, in this case predictions are made without supervision and the process of selection of subset variables is performed without taking into account the information relative to the excluded structure (the data of the molecule left out are completely hidden to the system at every step) and obtaining in this way a *true prediction*. Evidently, algorithm B is much more severe than Algorithm A: first, because it is much more time consuming (approximately N times more as this is the number of required internal calls to Algorithm A) and, second, because it gives true external simulated predictions, which can be more unstable. Respect to this last point, the advantage relies in the fact that if consistent predictions are obtained, they have an extra value as they were obtained simulating external predictions. In this way, ITS procedures can be interpreted as a test for assessing the true predictive capabilities of the proposed models.

Results and Discussion

Results of prediction performance by using Algorithm A are shown in table 3. In order to compare models (despite the word of caution above) even in the case of involving distinct number of parameters, we revert to the clogPP term due to Pecka and Ponec [45]. This statistical parameter is the co-logarithm of the probability of finding a linear model involving a certain number of descriptors and objects and having an equal or greater value of the correlation coefficient. It has been recently demonstrated that this is equivalent to the computation of the statistical F term [46]. Higher values of clogPP imply greater model reliability. In general, it would be ideal to find a maximal value for clogPP, indicating how many descriptors must be taken in the model.

Table 3. Performance prediction by Algorithm A (MLR- L_n O).

Model		Number of descriptors (k)				
		1	2	3	4	5
Leave-0-out (MLR)	R^2	0.611	0.778	0.879	0.948	0.965
	clogPP	4.32	5.55	6.84	8.70	8.98
Leave-1-out	R^2	0.552	0.658	0.827	0.902	0.945
	clogPP	3.76	3.96	5.60	6.67	7.62
Leave-2-out	R^2	0.552	0.658	0.826	0.902	0.946
	clogPP	3.76	3.76	5.59	6.68	7.63

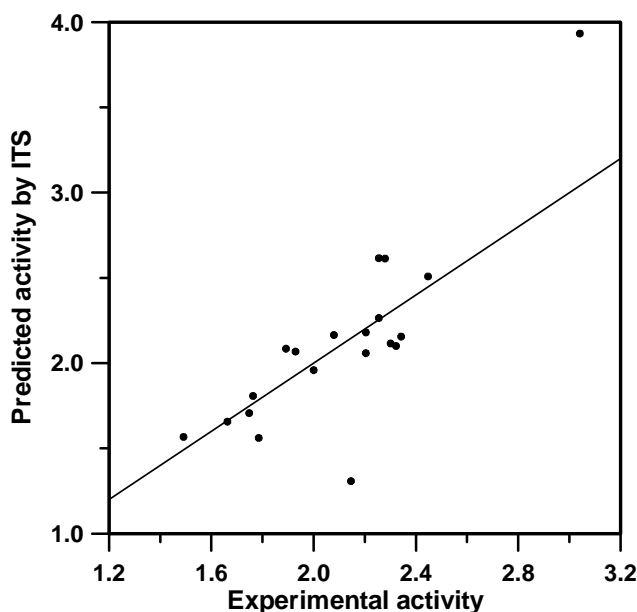
From Table 3, it seems that the most the number of variables increases, the best the equations obtained seems to be. This is a typical result and in some cases a maximum value of R^2 or clogPP can be achieved along a series in k or in n . In the particular case shown here such a maximum value is not found, but some L1O and L2O results are identical as the same final models are selected.

By contrast, when algorithm B is executed, the prediction performance varies irregularly, as it can be seen in Table 4. ITS results in Table 4 present a qualitative and quantitative improvement when 5 descriptors are being considered in the obtaining of internal models (combinations of 6 descriptors where not tested due to the big computation time required). This shows how ITS procedures are distinct in nature from simple overall fitting approaches. Authors interpret that the nature of the present QSAR problem needs the inclusion of at least 5 descriptors in order to deal with the molecular diversity and to achieve an acceptable molecule-property relationship description. This is revealed by the ITS procedure, as it forces to make individual and transparent predictions for each one of the compounds. The ITS algorithm can be refined implementing an overall L2O or higher protocols (making predictions for a couple or more molecules at a time) in step number 1. However, according to our experience the results of L2O are only slightly different from the results of L1O and they will not be shown here. Furthermore, in our case this was not necessary since at the L1O level a quantitative and instructive leap is already found when passing from $k = 3$ to 4 as presented in Table 4. The best models are the ones involving 5 descriptors considering internal L0O (ordinary MLR) or L2O models (L3O models are much more time consuming and have not been explored here).

Table 4. Performance prediction by algorithm B (MLR- L_n O/ITS-L1O).

Model		Number of descriptors (k)				
		1	2	3	4	5
Leave-0-out (MLR)	R^2	0.044	0.140	0.003	0.525	0.695
	clogPP	0.43	0.56	0.00	1.73	2.56
Leave-1-out	R^2	0.044	0.044	0.016	0.210	0.659
	clogPP	0.43	0.16	0.02	0.36	2.25
Leave-2-out	R^2	0.094	0.044	0.016	0.211	0.676
	clogPP	0.73	0.17	0.02	0.36	2.39

Figure 1. The predictions obtained from the model involving 5 descriptors for the ITS-L1O procedure. Internal models were obtained by ordinary MLR. $R^2=0.695$, $\text{clogPP}=2.56$.



For instance, the predictions obtained by the MLR models are displayed in Figure 1. Similar results are obtained by the L1O or L2O models. It must be noted that in Figure 1, each depicted point corresponds to a single prediction and is attached to a particular MLR equation model. Thus, in fact we are dealing with 20 distinct models. This constitutes another advantage of ITS methodologies: as it provides many equations, the possibility of performing a statistical study is open. For the particular case we are dealing with, predictions are reasonable except for two cases. The first one, the most diverging point, is attached to entry 1 in Table 1, which corresponds to the structure having not only an extreme value for the biological property, but also presenting the unique acyclic R,R structure (see Table 1). The second case corresponds to the entry number 20 in Table 1. Visually, there is no structural evidence to consider this molecule as a special case. This shows how the ITS protocol helps to detect outliers: the first one possibly due to structural reasons or to the fact of being an extrapolation, the second one due to non evident reasons related to descriptor or model deficiencies.

Table 5 shows the frequency with which every index appears in the final 20 models involving 5 descriptors each. As it can be seen in the table, only 12 descriptors appear in models more than once. In Table 5 the data are sorted according to the number of times the index was selected in models. The indices G_5 , J_3^v , 3C_c , QG_3 and $^3\chi_p$ are the most often used. Additionally, in all the cases in the table each index appears in models preserving the corresponding coefficient sign. This feature constitutes an indicator for model robustness and, additionally, permits to qualitatively correlate each index with the experimental property variation.

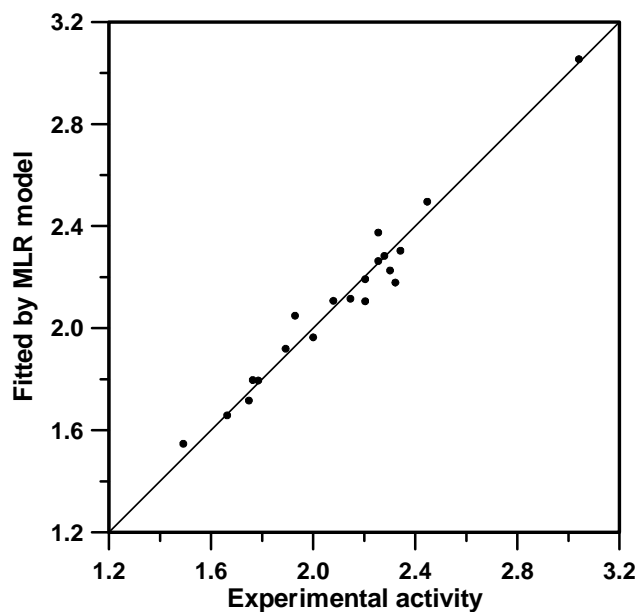
Actually, the model involving the 5 most voted descriptors in Table 5 coincides with the one presented in Table 3 for a L0O (MLR) procedure. Equation 1 below shows the explicit model formula and the attached statistical data. Figure 2 shows the corresponding adjusted predictions against the experimental ones. For this particular case, it is revealed that Equation 1 could be obtained in advance by the first numerical investigations which were carried out (Table 3), but ITS method allowed us to corroborate that the selected model bears extra value due to the coefficients sign stability and the popularity along all the individual models surveyed in Table 5.

Table 5. Frequency of use of the indices along the 20 MLR models of 5 descriptors selected by Algorithm B.

Cardinal	Index	Frequency of use	Coefficient sign
1	G ₅	16	–
2	J ₃ ^v	14	+
3	³ C _c	12	+
4	QG ₃	12	–
5	³ χ _p	10	+
6	E ₃	3	+
7	G ₄	3	–
8	J ₃	3	+
9	G ₃ ^v	3	+
10	⁴ C _{pc}	2	+
11	J ₅	2	+
12	⁴ Q _c	2	–

Equation 1. Global MLR model involving the 5 descriptors selected in the ITS-L10 procedure. Coefficient intervals are given at the 95% confidence level. All significance levels for coefficients are less than 0.3% (probability for each coefficient to be zero).

$$\begin{aligned} \text{Log(IC}_{50}/\text{nM}) = & 0.241563(\pm 0.077253) \text{ } ^3\chi_p - 2.23930(\pm 0.47090) G_5 + 35.3656(\pm 5.8769) J_3^v \\ & + 0.798064(\pm 0.165968) \text{ } ^3C_c - 0.825473(\pm 0.482103) QG_3 - 2.78771(\pm 1.25679) \\ & n=20, R^2=0.965 \text{ (clogPP}=8.98), F=77.59, p<0.00001. \end{aligned}$$

Figure 2. Fitting results obtained by using Equation 1.

Conclusion

A QSAR study of a set of antimalarial agents has been performed. It has been shown that the reliability of the resulting model is crucially influenced by its quality. Standard MLR Leave-*n*-out procedures with supervision have a much lower predictive power than allowing the process to be unsupervised. This is especially due to hidden overparametrization or instability problems. The last choice implemented in terms of Internal Tests Sets protocol, also prevents from these eventual problems and can be additionally useful for outlier detection. ITS models are more valuable because they can perform potentially well in interpolations and extrapolation predictions.

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